Vitamin D Status in Critically Ill Patients

90 ECTS Credits

Rúnar Bragi Kvaran

Thesis for the degree of Master of Science

University of Iceland
Faculty of Medicine
Department of Biomedical Sciences
School of Health Sciences

HÁSKÓLI ÍSLANDS

D-vítamínbúskapur bráðveikra sjúklinga

Rúnar Bragi Kvaran

Ritgerð til meistaragráðu í læknisfræði

Umsjónarkennari: Gísli H. Sigurðsson

Meistaranámsnefnd: Gísli H. Sigurðsson, Tómas Guðbjartsson, Martin I. Sigurðsson

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Ágrip

Bakgrunnur

D-vítamín er nauðsynlegt til viðhalds góðrar stoðkerfisheilsu. D-vítamínskortur getur valdið beinkröm og aukið hættu á vöðvaslappleika, byltum og beinbrotum. Undanfarin ár hefur þekking á D-vítamínbúskap aukist og D-vítamínskortur einnig verið tengdur við aukna hættu á hjarta- og æðasjúkdómum, krabbameinum og fleiri sjúkdómum. D-vítamínskortur er algengur á Íslandi þótt hefð sé fyrir því hjá Íslendingum að taka D-vítamín í formi bætiefna. Rannsóknir í suðlægum löndum hafa sýnt lág D-vítamíngildi í blóði gjörgæslusjúklinga og tengt D-vítamínskort við meðal annars lengri spítalalegu. Rannsóknir á D-vítamínbúskap gjörgæslusjúklinga á norðurslóðum hefur vantað. Markmið rannsóknarinnar voru að kanna D-vítamínbúskap bráðveikra sjúklinga á gjörgæslu Landspítala í Reykjavík, Íslandi, að meta umfang D-vítamínskorts hjá gjörgæslusjúklingum og kanna hvort D-vítamínskortur hefði áhrif á spítalalegu og dánartíðni þessara sjúklinga.

Efni og aðferðir

Rannsóknin var framsýn athugunarrannsókn á 122 sjúklingum sem lögðust inn á gjörgæslu Landspítala árin 2014-2015. D-vítamín (25(OH)D) í sermi var mælt hjá öllum sjúklingum í tvígang (á fyrsta og öðrum degi legu). Algengi D-vítamínskorts og áhrif hans á spítalalegu og dánartíðni voru könnuð.

Niðurstöður

11/122 (9%) sjúklinga mældust með D-vítamíngildi > 75 nmól/L sem mælt er með til viðhalds góðrar heilsu. 84/122 (69%) höfðu D-vítamíngildi < 50 nmól/L sem er skilgreint sem D-vítamínskortur og þar af höfðu 52/122 (43%) gildi < 25 nmól/L sem er skilgreint sem alvarlegur D-vítamínskortur. Meðaltal fyrstu D-vítamínmælingar var 40,9 nmól/L og meðaltal annarrar mælingar var 38,1 nmól/L (meðalmunur 2,8 nmól/L). 46/122 (38%) sjúklinga dvöldu lengur en fjóra daga á gjörgæslu. 23/52 (43%) sjúklinga með D-vítamíngildi < 25 nmól/L dvöldu lengur en fjóra daga á gjörgæslu borið saman við 23/70 (19%) sjúklinga með hærri gildi (p = 0,196). 90-daga dánartíðni í sjúklingahópnum var 31/122 (25%), 14/50 (27%) hjá sjúklingum með D-vítamíngildi < 25 nmól/L borið saman við 17/72 (24%) hjá sjúklingum með hærri gildi (p = 0,741).

Ályktun

D-vítamínskortur reyndist vera algengur hjá bráðveikum sjúklingum á Landspítala. Næstum helmingur gjörgæslusjúklinga höfðu D-vítamíngildi sem samræmast alvarlegum D-vítamínskorti sem hefur í för með sér aukna hættu á beinkröm. Ekki var tölfræðilega marktækur munur á legulengd eða dánartíðni sjúklinga með alvarlegan D-vítamínskort borið saman sjúklinga með hærri D-vítamíngildi en sjúklingar sem mælast með alvarlegan D-vítamínskort gætu haft tilhneigingu til lengri gjörgæslulegu. Ekki var munur á meðaltali fyrstu og annarrar mælingar D-vítamíns hjá sjúklingahópnum. Ástæða er til þess að kanna D-vítamínbúskap gjörgæslusjúklinga nánar og hvort D-vítamíngjöf geti bætt spítalalegu og horfur þessa sjúklingahóps.

Abstract

Introduction

Vitamin D is important for maintaining musculoskeletal health. Vitamin D deficiency can cause osteomalacia and increase the risk for muscle weakness, falls and fractures. Recent studies have revealed that vitamin D deficiency is associated with many chronic diseases like cardiovascular diseases, cancers and more. Vitamin D deficiency is common in Iceland. Critically ill patients at southern latitudes have been shown to have low vitamin D levels that were associated with prolonged hospital stay. To our knowledge no studies have been conducted on vitamin D status amongst critically ill patients at high northern latitudes. The aims of the study were to characterize the vitamin D status of the critically ill patients in Reykjavík, Iceland, to evaluate the extent of vitamin D deficiency and its effect on hospital stay and mortality of these critically ill patients.

Methods

This was a prospective observational study on 122 patients admitted to Landspitali – The National University Hospital of Iceland (LUH) intensive care unit (ICU) during the years 2014-2015. Serum vitamin D (25(OH)D) was measured in all patients on two occasions (first and second day). The prevalence of vitamin D deficiency and its effect on hospital stay and mortality was calculated.

Results

11/122 (9%) patients had serum vitamin D values > 75 nmol/L which is recommended for good health. 84/122 (69%) had serum vitamin D values < 50 nmol/L which is defined as vitamin D deficiency and 52/122 (43%) of these patients had serum vitamin D values < 25 nmol/L which is defined as severe vitamin D deficiency. The average serum vitamin D value in the first measurement was 40.9 nmol/L and 38.1 nmol/L in the second measurement (average difference 2.8 nmol/L). 46/122 (38%) patients stayed longer than four days in the ICU. 23/52 (43%) patients with serum vitamin D values < 25 nmol/L stayed longer than four days in the ICU compared with 23/70 (19%) patients with higher values ($p = 0.196$). 90-day mortality in the study group was 31/122 (25%). For patients with serum vitamin D values < 25 nmol/L it was 14/50 (27%) compared with 17/72 (24%) for patients with higher values ($p = 0.741$).

Conclusion

Vitamin D deficiency was common in the critically ill patients at Landspitali – the National University Hospital of Iceland. Almost half of the ICU patients had serum vitamin D values defined as severe vitamin D deficiency which is associated with osteomalacia. A statistically significant difference was not observed when comparing hospital stay and mortality for patients with severe vitamin D deficiency with patients with higher serum vitamin D values but patients with severe vitamin D deficiency might have trend towards longer ICU stay. There was no difference in the average of the first serum vitamin D concentration and the second concentration suggesting that a single spot test of vitamin D is sufficient to get a broad idea about the vitamin D status in critically ill patients. The results of the study warrant further studies on the role of vitamin D in the critically ill and possible effects of vitamin D supplementation on hospital stay and outcome.

Acknowledgements

I wish to thank my supervisor professor Gísli H. Sigurðsson MD, PhD and the members of my master's committee professor Tómas Guðbjartsson MD, PhD and Martin I. Sigurðsson MD, PhD for their guidance during the last years. Special thanks to my co-researcher Sigurbjörg J. Skarphéðinsdóttir, MD for her input in the research. I also want to thank the nurses and physicians of Landspitali – The National University Hospital of Iceland Intensive Care Unit and the biomedical scientists at the laboratory for clinical biochemistry for their assistance in collecting and measuring blood samples. In addition, I thank my family for their help and support during research and writing of the thesis.

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List of Abbreviations

25(OH)D	25-hydroxyvitamin D
1,25(OH)2D	1-25-dihydroxyvitamin D
DBP	Vitamin D binding protein
PTH	Parathyroid hormone
PTG	Parathyroid gland
BMI	Body mass index
LUH	Landspitali – The National University Hospital of Iceland
ICU	Intensive care unit
AKI	Acute kidney injury

1 Introduction

Vitamin D and its metabolites, the circulating 25-hydroxyvitamin D (25(OH)D), calcidiol, and the active 1-25-dihydroxyvitamin D (1,25(OH)2D), calcitriol, are fat soluble vitamins or pro-hormones. Their essential role in bone metabolism and musculoskeletal health has long been known. In later years their more complex role in many different metabolic pathways in the human body has been described (Holick, 2007).

The diseases caused by various vitamin deficiencies have been known for centuries although their cause was unknown. It was first in the early 20th century that the existence of vitamins, essential dietary factors not produced in sufficient amount in the human body for maintaining life, was suggested. A few years later, vitamin A, B and C were discovered as the factors preventing xeropthalmia, beri-beri and scurvy respectively (Deluca, 2014).

Rickets is a disease caused by defective mineralization or calcification of bones in children and is most often caused by vitamin D deficiency. It presents as variety of skeletal deformities since there is low concentrations of mineral in children's skeleton and the epiphyseal plates are still open. In adults, this disease is known as osteomalacia which often goes undetected because the epiphyseal plates are closed and there is enough mineral to avoid skeletal deformities (Holick et al., 2011).

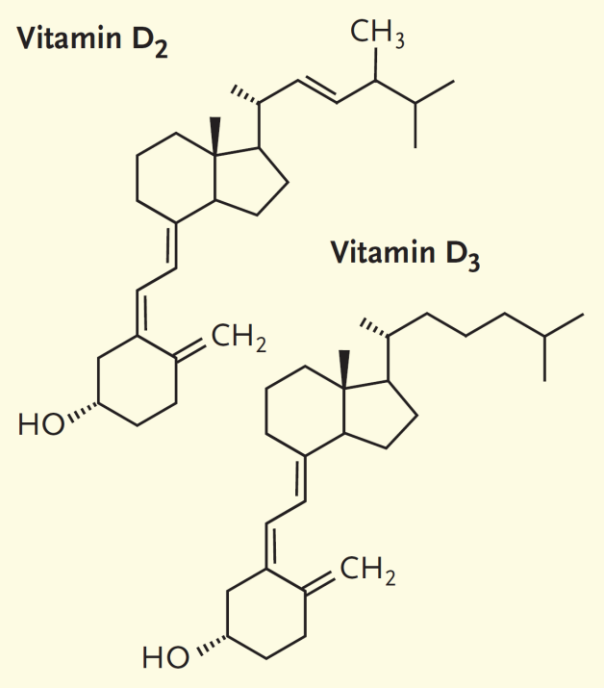

Figure 1. Chemical structure of vitamin D_2 and vitamin D_3 (Holick, 2007).

McCollum and Davis performed rat studies at the University of Wisconsin that demonstrated that cod liver oil included a factor, vitamin A, able to prevent xeropthalmia. Sir Edward Mellanby in Great Britain followed the work of McCollum and Davis and believed that rickets, which had high incidence in the United Kingdom at the time, was also caused by a dietary deficiency. He cured dogs suffering from

rickets by feeding them with cod liver oil and assumed that vitamin A cured rickets. It was then McCollum that was able to show that cod liver oil could still prevent rickets after the earlier discovered vitamin A had been eliminated from the oil. This other factor in cod liver oil able to prevent and heal rickets was named vitamin D (McCollum, 1967). At the same time, Steenbock at the University of Wisconsin revealed that ultraviolet light exposure also prevented rickets which lead to the conclusion that vitamin D could be produced from a substance in the skin during exposure to sunlight (Schneider, 1973). This suggested that the recently discovered vitamin D was in fact not an essential dietary factor. The main forms of vitamin D, vitamin D_2, ergocalciferol, and vitamin D_3, cholecalciferol, were then chemically characterized in the 1930s but it was not until 1978 that vitamin D_3 production in skin was finally proven (Figure 1). Following these discoveries around vitamin D, rickets was almost eliminated as a major global health problem (Deluca, 2014).

The interest in vitamin D research decreased as the incidence of rickets decreased but the interest of both scientists and the general public in vitamin D has increased again in recent years. During the years 1995-2015 the publication of peer-reviewed articles on vitamin D and vitamin D-related topics has quadrupled (Deluca, 2014; Quraishi, Camargo, & Manson, 2016).

1.1 Vitamin D sources

Vitamin D is now mainly found in two forms, vitamin D2 and vitamin D3. Vitamin D2 is found in some fish and plants while vitamin D3 is synthesized in the skin. Humans' predominant source of vitamin D is therefore from exposure to sunlight but this synthesis depends highly on the season, latitude and time of day. Around 3000 IU of vitamin D3 can be produced in the skin during and after 5-10 minutes of exposure of arms and legs to direct sunlight in optimal conditions. The ultraviolet B photons from the sunlight produce vitamin D in the skin and the number of them reaching the surface of the earth depends on the solar zenith angle. When the angle becomes more oblique, fewer ultraviolet B photons reach the earth and humans skin eventually. The angle is more oblique at latitudes further from the equator. The vitamin D synthesis is therefore very limited or absent during the winter above and below latitudes 33°. Due to the solar zenith angle, it is possible to get tanned and even sunburned without producing vitamin D, all depending on which photons reach the skin.

Humans also receive vitamin D in both major forms from their diet but there are not many products that naturally contain vitamin D and only few countries fortify for example milk, cereal, bread or margarine with vitamin D (Figure 2). The best diet to prevent vitamin D deficiency is a diet with high ratio of oily fish. There are around 600-1000 IU of vitamin D3 in 100 g of fresh wild salmon and 250 IU in the same amount of mackerel. An egg yolk contains around 20 IU of vitamin D2 or D3. Fortified foods only contain small amounts of vitamin D. Dietary supplements can include both vitamin D2 and vitamin D3 in different amounts, most often ranging from 400 to 2000 IU. Together, these sources of vitamin D are often insufficient to maintain normal vitamin D levels despite regular and daily sunlight exposure, unremarkable diet history and active supplementation (Binkley et al., 2007; Holick et al., 2011; Kennel, Drake, & Hurley, 2010; Kulie, Groff, Redmer, Hounshell, & Schrager, 2009; Levis et al., 2005).

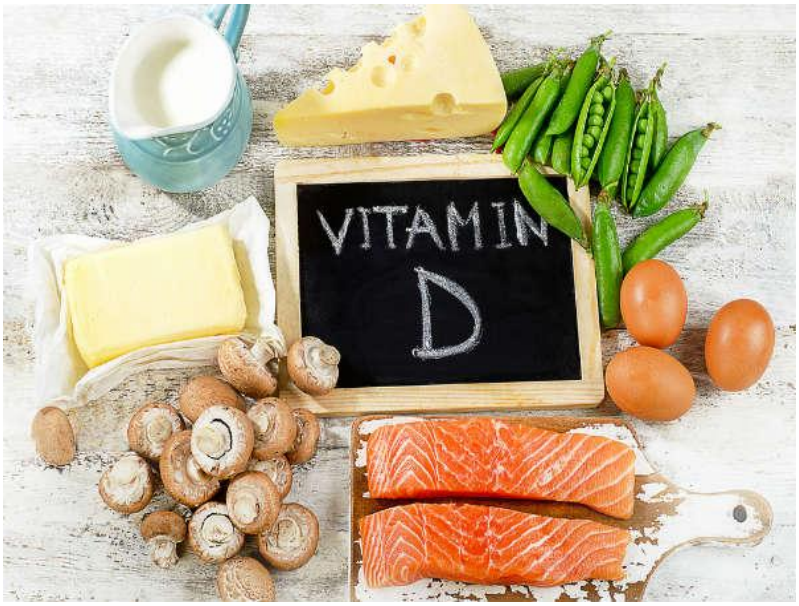

Figure 2. Foods rich in vitamin D ("Foods rich in vitamin D," n.d.).

1.2 Vitamin D synthesis, metabolism and function

When solar ultraviolet B radiation of the wavelength 290–315 nm penetrates the skin 7-dehydrocholesterol is converted to previtamin D_3 which converts into vitamin D_3 in a heat-dependent process. Vitamin D_3 is then released into the venous circulation. The vitamin D_2 and D_3 from diet are absorbed and incorporated in chylomicrons which transport them to the venous circulation via the lymphatic system. Vitamin D can then be stored in the fat cells of the body and released on demand. In the circulation, it is bound to vitamin D binding protein (DBP), and then it is transported to the liver. In the liver, it undergoes hydroxylation to 25(OH)D, the major circulating form of vitamin D, by vitamin D-25 hydroxylase. 25(OH)D is the vitamin D form measured in blood to determine vitamin D status. 25(OH)D is however biologically inactive and it is the kidneys where 25(OH)D is further hydroxylated to the biologically active form, 1,25(OH)2D, by 25-hydroxyvitamin D-1α-hydroxylase. The synthesis and of the biologically active form of vitamin D is seen in Figure 3. 1,25(OH)2D has a half-life under 4 hours and 25(OH)D has a half-life around 2–3 weeks and vitamin D supplies of the body must therefore be continuously replenished (Brannon, Yetley, Bailey, & Picciano, 2008; Holick, 2007; Kulie et al., 2009).

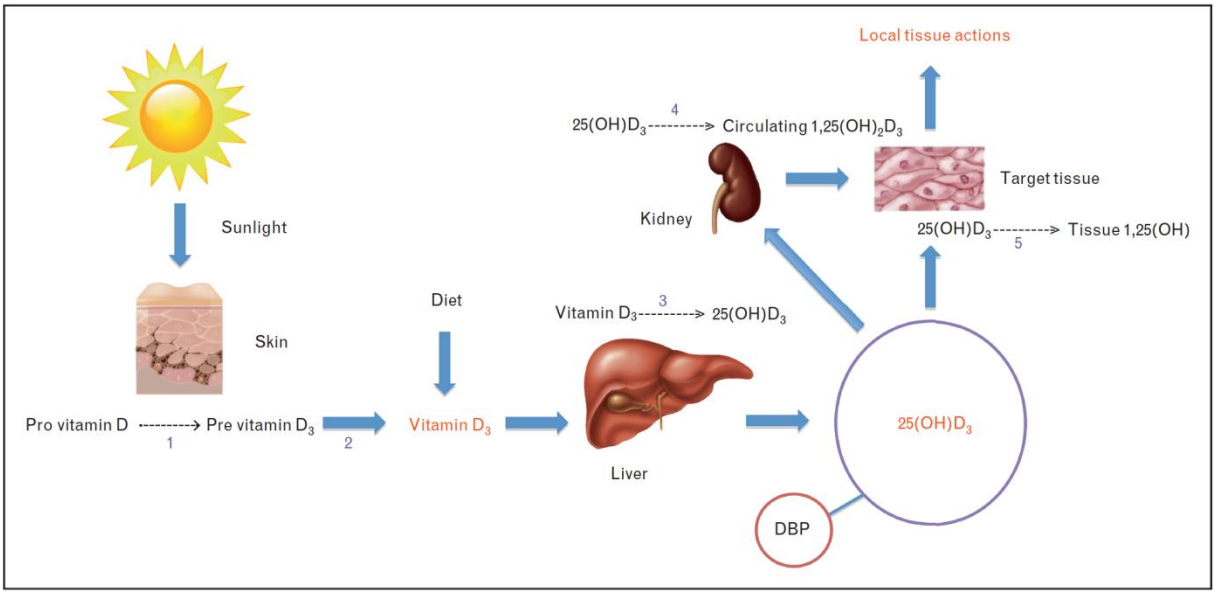

Figure 3. The synthesis of vitamin D_3 and other vitamin D metabolites. First is the reaction in the skin catalyzed by ultraviolet B radiation. Second is an isomerization reaction which is catalyzed by heat, third is the hydroxylation by vitamin D-25 hydroxylase and the fourth step is the hydroxylation in the kidneys by 25-hydroxyvitamin D-1α-hydroxylase. Number five is the reaction in other tissues expressing the 25-hydroxyvitamin D-1α-hydroxylase. 25(OH)D is bound to DBP in the circulation (Quraishi & Camargo, 2012).

Vitamin D has a major role in bone and calcium metabolism in the body. It controls blood calcium levels through three different mechanisms. Firstly, it induces the proteins that are involved in active absorption of calcium in the small intestines and stimulates the active absorption of phosphate. Secondly, in the absence of dietary calcium, vitamin D activates resting osteoclasts, through osteoblasts, for bone resorption which makes mobilization of calcium from bones possible. Thirdly, it stimulates the reabsorption of calcium in the distal renal tubule of the kidneys. In the second and third process, parathyroid hormone (PTH) is also needed. The parathyroid glands (PTGs) sense lowered calcium concentrations in the blood and then secrete PTH which proceeds to osteoblasts and the kidneys. In the proximal convoluted tubule of the kidneys, PTH then stimulates activation of vitamin D. Primarily this effect of PTH results in increased intestinal absorption of calcium. If intestinal absorption does not meet the calcium demands of the body, mobilization from bones and renal reabsorption is stimulated. This explains why vitamin D deficiency might lead to osteoporosis. This system includes a negative feedback mechanism. If blood calcium levels are too high, the C cells of the thyroid gland secrete calcitonin which blocks calcium mobilization from bones. Presence of sufficient amounts of 1,25(OH)2D also sends negative feedback to the PTG, decreasing secretion of the PTH. This vitamin D-based endocrine system is demonstrated in Figure 4 (DeLuca, 2004; Kulie et al., 2009).

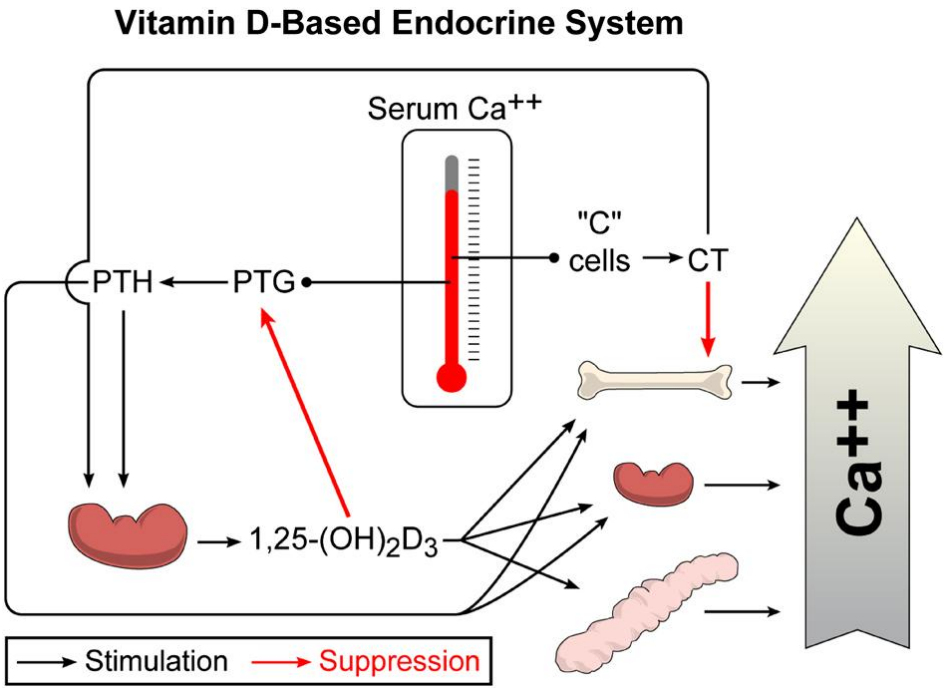

Figure 4. The vitamin D-based endocrine system. The thermometer represents calcium-sensing proteins in the PTGs and C cells of the thyroid. Hypocalcemia causes secretion of PTH leading to synthesis of 1,25(OH)2D that causes calcium mobilization in the intestines, kidneys and bones. Sufficient 1,25(OH)2D in the circulation then causes a feedback suppression of PTH synthesis and secretion. Calcitonin from C cells suppresses bone resorption (DeLuca, 2004).

Vitamin D has its functions in the bone and calcium metabolism described through the vitamin D receptor found in enterocytes, osteoblasts and distal renal tubule. In recent years numerous studies on the role of vitamin D have revealed that 25-hydroxyvitamin D-1α-hydroxylase is also expressed in many extrarenal tissues including osteoclasts, skin, macrophages, placenta, colon, brain, prostate, endothelium and PTGs (Brannon et al., 2008). Furthermore, studies have also revealed that a vitamin D receptor was found in over 30 different tissues in the human body. This knowledge points out that vitamin D has a larger role in the human body besides its well-known role in calcium metabolism. Vitamin D receptor is present in both T and B lymphocytes and vitamin D has an immunoregulatory role in the adaptive immune system and an antimicrobial role in the innate immune system. Its role in the development of diabetes through its function in the pancreas, brain development and heart function and blood pressure regulation has also been revealed (Brannon et al., 2008; Norman, 2008).

1.3 Vitamin D deficiency

Vitamin D deficiency is a state in which the body lacks vitamin D to meet physiologic needs. Two different forms of vitamin D have most often through history been measured in blood to establish the diagnosis. The Endocrine Society recommends measuring 25(OH)D levels with a reliable assay. 25(OH)D levels are given in either ng/mL or nmol/L (ratio around 1:2.5). The Endocrine Society recommends against measuring 1,25(OH)2D unless for monitoring acquired and inherited disorders in

25(OH)D and phosphate metabolism such as in chronic kidney disease. Levels of 1,25(OH)2D are around 1,000 times lower in blood than 25(OH)D levels, are tightly regulated by PTH, calcium and phosphate and do not reflect vitamin D reserves (Holick et al., 2011).

The definition of vitamin D deficiency has been debated and different criteria used. Although there is still some controversy concerning what serum 25(OH)D value should be considered sufficient, the Endocrine Society has defined vitamin D deficiency as a 25(OH)D value of 50 nmol/L or less, vitamin D insufficiency as 50–75 nmol/L and vitamin D sufficiency as 75 nmol/L or greater for children and adults. More studies on patient outcomes at certain serum levels of 25(OH)D are needed to erase controversy and strengthen these recommendations (Holick et al., 2011). Moreover, has severe vitamin D deficiency frequently been described as 25(OH)D value below 25 nmol/L. Screening is recommended in those at risk for vitamin D deficiency (Rosen, 2011). The Endocrine Society recommends 600 IU/d of vitamin D for healthy adults to maintain musculoskeletal health but also states that 1500-2000 IU/d might be needed for serum 25(OH)D value of 75 nmol/L or greater. The tolerable upper limit for adults is at least 4000 IU/d (Holick et al., 2011).

There are multiple causes to vitamin D deficiency. Since sunlight exposure is the most important source of vitamin D it is the lack of skin exposure to sunlight which is the major cause of vitamin D deficiency. Lack of skin exposure to sunlight can be further divided into more specific causes: Inadequate outdoor stay, living at latitude with few sun hours, wearing strong sunscreen and having dark skin (Holick et al., 2011; Wacker & Holick, 2013). Another cause is low intake, not as important since not many foods contain vitamin D. Causes can also include decreased bioavailability because of malabsorption and obesity, reduced skin synthesis for example caused by aging, decreased conversion to the active form because of liver or renal failure, increased urinary loss and then other various heritable and acquired disorders leading to deficiency (Holick, 2007; Holick et al., 2011). In a large study by Melamed et al. from 2008 on the risks and consequences for vitamin D deficiency in the general adult population, increasing age, female sex, non-white ethnicity, diabetes, smoking and higher body mass index (BMI) were all independently associated with higher odds of vitamin D deficiency (Melamed, Michos, Post, & Astor, 2008).

Vitamin D deficiency causes abnormal calcium, phosphate and bone metabolism. It can precipitate or exacerbate osteopenia and osteoporosis by decreasing bone mineral density. Besides this vitamin D deficiency causes rickets in children and osteomalacia in adults. Vitamin D deficiency also causes muscle weakness and by that increases the risk of falls and bone fractures (Holick, 2007; Holick et al., 2011).

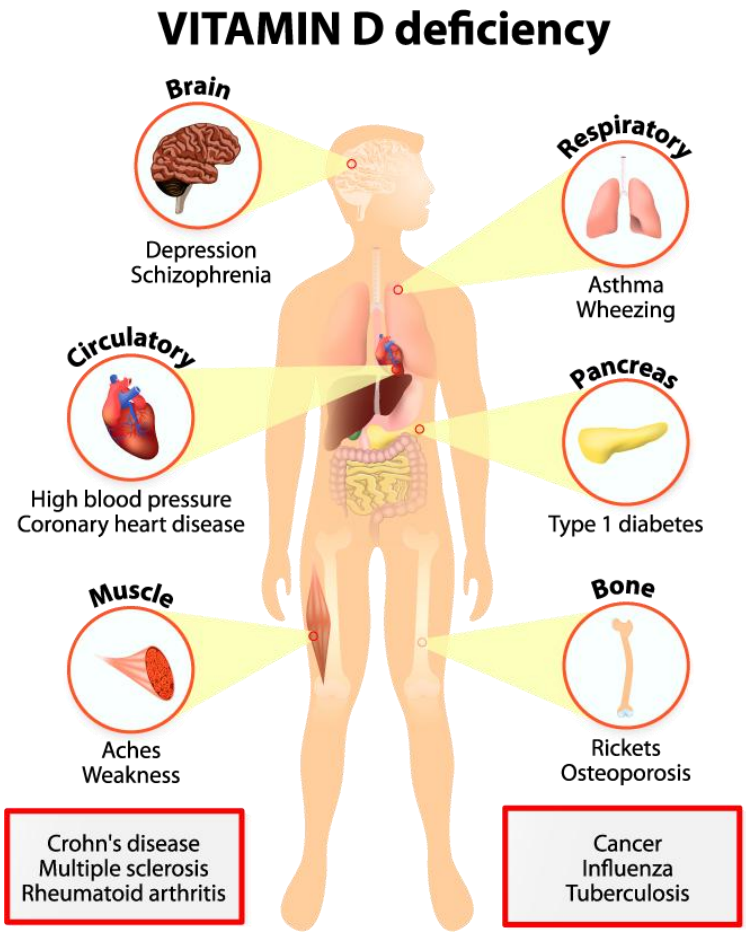

Figure 5. Symptoms and diseases related to vitamin D deficiency ("Vitamin D deficiency. Symptoms and diseases caused by insufficient vitamin D.," n.d.).

Despite the increased public awareness of the importance of vitamin D the prevalence of vitamin D insufficiency has been increasing in the general population. Plausible explanations include campaigns to control sun exposure by sun avoidance and protection and the decrease in outdoor activities. Large evaluation in the US population, comparing 1988-1994 and 2001-2004, revealed an average 15 nmol/L decrease in serum 25(OH)D. Vitamin D deficiency is now also highly prevalent worldwide and has been recognized as a pandemic with prevalence ranging from 20 to 100% depending on the population studied (Ginde, Liu, & Camargo, 2009; Holick et al., 2011; Hossein-nezhad & Holick, 2013). Data leading to this declaration of pandemic has been of variable quality. Cashman et al. addressed this problem in a study published in 2016. The authors pooled data from 14 European studies with a total of 55,844 patients. Serum 25(OH)D levels from the studies were standardized according to protocols from an international vitamin D standardization program. According to these standardized levels a new estimate on the prevalence on vitamin D deficiency was done. Vitamin D deficiency was defined as 25(OH)D < 50 nmol/L and the prevalence of vitamin D deficiency was estimated to be 40,4%. This recent confirmation of the high prevalence of vitamin D deficiency in Europe warrants the attention of public health authorities (Cashman et al., 2016).

This pandemic of vitamin D deficiency combined with more understanding of the complex role of vitamin D has led to vitamin D deficiency being associated with many non-musculoskeletal chronic

diseases like cancer, cardiovascular diseases, autoimmune diseases and diabetes mellitus (Holick, 2007; Hossein-nezhad & Holick, 2013). The relationship of vitamin D deficiency and various symptoms and diseases are illustrated in summary in Figure 5 and possible consequences of vitamin D deficiency along with metabolism of vitamin D and biological responses of the body to vitamin D leading to the related diseases are summarized in Figure 6.

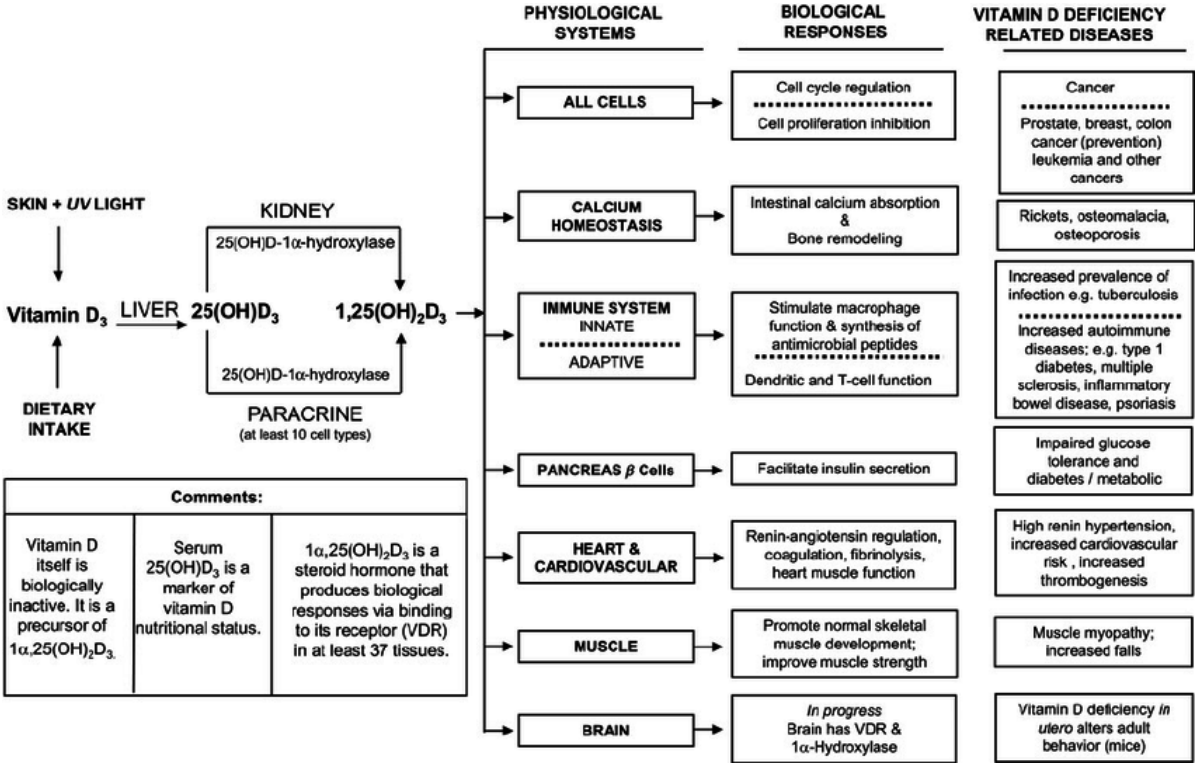

Figure 6. Metabolism and function of vitamin D including possible consequences of vitamin D deficiency (Norman & Bouillon, 2010).

The risk for various cancers, including colon, ovarian, prostate and breast cancer and cancer mortality is increased among individuals living at high latitudes in comparison with people that reside at lower latitudes (Garland, Mohr, Gorham, Grant, & Garland, 2006; Giovannucci et al., 2006; Hanchette & Schwartz, 1992; Holick, 2007; Kricker & Armstrong, 2006). Studies have also revealed that serum 25(OH)D levels below 50 nmol/L are related to 30-50% increased risk for some of the aforementioned cancers but also increased mortality from these cancers (Gorham et al., 2005; Grant, 2002). Other studies have showed slower progress of prostate cancer in men working outdoors compared with men working indoors and lower risk of breast cancer in women with the highest vitamin D intake compared with women with less intake (Garland, Garland, et al., 2006; Luscombe et al., 2001). Possible explanation is that 1,25(OH)2D controls genes locally that prevent cancer by regulating cell proliferation and differentiation and might be able to induce apoptosis and prevent angiogenesis in a cell that has become malignant (Holick, 2007). This link between cancer and 25(OH)D is plausible but the relationship is likely more complex and more research is needed before

vitamin D supplementation in cancer can be encouraged (Freedman, Looker, Abnet, Linet, & Graubard, 2010; Lamberg-Allardt, Brustad, Meyer, & Steingrimsdottir, 2013).

Studies have showed that cardiovascular diseases and hypertension are more common in individuals who live at high latitudes compared to the ones living at lower latitudes (Holick, 2007). A systematic review also shows that lower levels of serum 25(OH)D may be associated with increased risk of cardiovascular disease and hypertension (Lamberg-Allardt et al., 2013). In a study on 18 patients with mild untreated hypertension Krause et al. could show the normalization of blood pressure in people with hypertension when exposed to ultraviolet B radiation regularly for three months. Furthermore, their 25(OH)D levels were raised by 180% (Krause, Bühring, Hopfenmüller, Holick, & Sharma, 1998). Although promising, a randomized controlled trial was not able to show the same positive effects on blood pressure (Lamberg-Allardt et al., 2013). Furthermore, in a recent randomized controlled trial, a high-dose monthly vitamin D supplementation was not shown to decrease the risk for cardiovascular diseases but the authors leave the possible protective effects of daily and weekly supplementation for future studies (Scragg et al., 2017).

Crohn's disease, type 1 diabetes mellitus and multiple sclerosis also occur more often in people living at higher latitudes compared with others. High serum levels of 25(OH)D and vitamin D supplementation have been shown to decrease the risk of multiple sclerosis and rheumatoid arthritis for example (Holick, 2007).

Vitamin D supplementation in children has been shown to reduce the risk of developing type 1 diabetes mellitus but larger randomized controlled trials are needed to confirm causality. Also, Vitamin D supplementation combined with calcium in adults might reduce the risk of type 2 diabetes mellitus but evidence is still scarce (Holick, 2007; Lamberg-Allardt et al., 2013).

Studies on the role of vitamin D in cognitive function are ongoing. Low serum levels of 25(OH)D have been associated with more rapid cognitive decline but studies on vitamin D supplementation and cognitive function are needed to show if supplementation can slow down the rate of cognitive decline (Miller et al., 2015).

Besides the studies on the relationship of vitamin D deficiency with different diseases, multiple studies have researched the effect of vitamin D deficiency or low serum 25(OH)D levels on mortality. Vitamin D deficiency has been shown to increase all-cause mortality by 26% in the general population (Melamed et al., 2008). Another study by Dobnig et al. revealed a hazard ratio of 2.08 for all-cause mortality in the severely vitamin D deficient compared with those who had sufficient levels of 25(OH)D. Similar results were also seen when using 1,25(OH)2D to measure vitamin D status (Dobnig et al., 2008). Results of a meta-analysis published in 2012 showed a possible nonlinear decrease in mortality with increasing serum 25(OH)D levels with the optimal levels being 75–87.5 nmol/L (Zittermann et al., 2012). In a recent meta-analysis using standardized levels of 25(OH)D it was shown that low serum 25(OH)D levels were associated with higher risk of all-cause mortality (Gaksch et al., 2017). Similar results are seen in more earlier studies but still further studies are needed to clarify the causality and whether vitamin D supplementation can improve survival (Zittermann, Gummert, & Börgermann, 2009). Supplementation is most likely to benefit the vitamin D deficient. A recent systematic review on the health effects of vitamin D supplementation suggested that the lack of

studies with positive results might be partly explained by the fact that the focus has been on populations without low serum 25(OH)D levels (Rejnmark et al., 2017).

The number of studies on vitamin D and the relationship of vitamin D deficiency with various symptoms and diseases have not gone unnoticed by the general public. Results of a study on trends in dietary supplementation among US adults from 1999-2012 published in 2016 showed that vitamin D supplementation from other sources than multivitamins increased from 5% to 19% during the period (Kantor, Rehm, Du, White, & Giovannucci, 2016).

1.4 Vitamin D in the critically ill

The critically ill are patients which have been or are at risk of developing acute and life-threatening organ dysfunction. These patients are warded in an intensive care unit (ICU) which is a department within a hospital where the most severely ill patients are treated. Intensive care is the special medical treatment these patients are given with the aim of preventing or correcting organ dysfunction (Marshall et al., 2017). The often complex treatment provided in the ICU at Landspitali – The National University Hospital of Iceland (LUH) is depicted in Figure 7. The ICU at LUH is divided into two units, 10 beds in each and another ICU of five beds, is located in Akureyri Hospital, a secondary care teaching hospital.

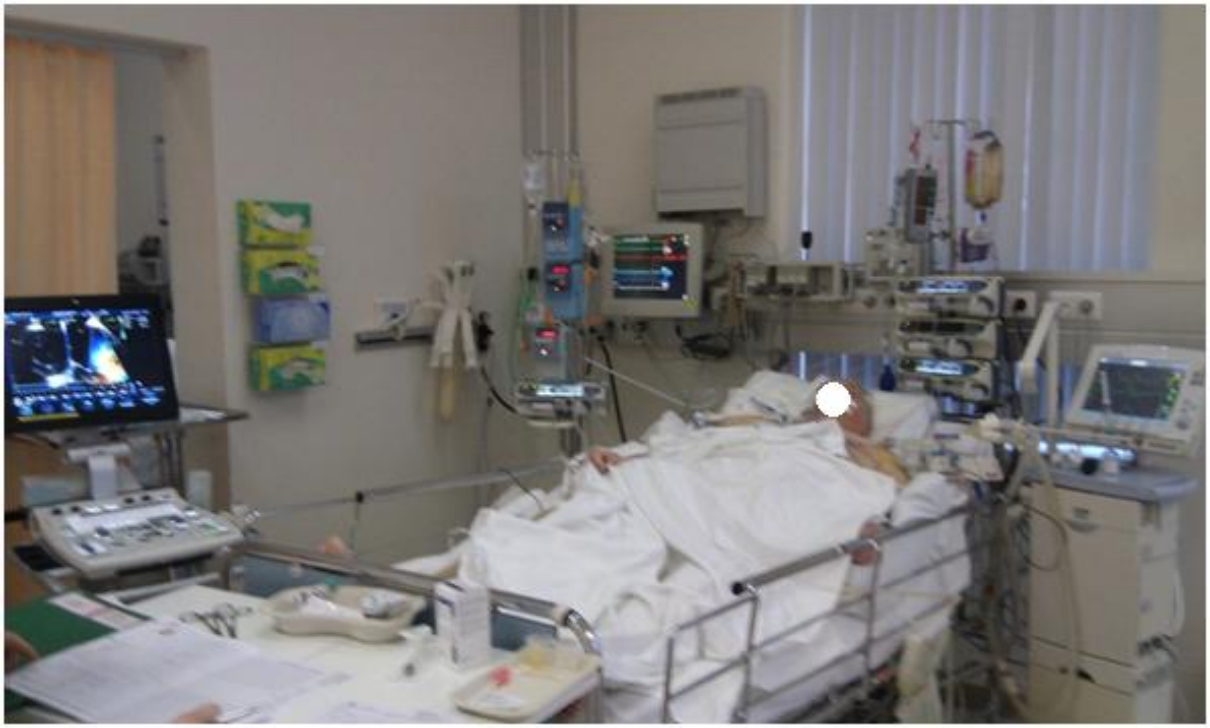

Figure 7. A photograph showing an ICU patient in LUH ICU. The patient is sedated and intubated, connected to an invasive mechanical ventilator, a temporary pacemaker and multiple infusion pumps while various vital signs are being continuously monitored (Photographer: Gísli H. Sigurðsson).

Serum levels of 25(OH)D have been shown to be inversely associated with all-cause, cardiovascular, cancer and respiratory disease mortality, however the role of vitamin D in acute critical illness is less well studied and understood. Recent studies have suggested association between serum 25 (OH)D levels and acute respiratory infections and acute myocardial infarction (Berry, Hesketh, Power, & Hyppönen, 2011; J. H. Lee, Gadi, Spertus, Tang, & O'Keefe, 2011).

Vitamin D deficiency is frequently not considered or treated in intensive care units. A prospective study in Sydney on 42 ICU patients referred to endocrinology revealed high prevalence of vitamin D insufficiency and deficiency within the group. All three patients that died had undetectable levels of 25(OH)D and 25(OH)D was an independent predictor of severity of organ dysfunction. Moreover, two recent studies from countries at southern latitudes showed that low levels of vitamin D are common in ICU patients and low levels were associated with prolonged hospital stay and increased mortality. In all studies only a single serum 25(OH)D level measurement was performed (Arnson, Gringauz, Itzhaky, & Amital, 2012; P. Lee, Eisman, & Center, 2009; Matthews, Ahmed, Wilson, Griggs, & Danner, 2012). In one of these two studies, by Arnson et al. from Israel including severely ill mechanically ventilated patients, the average 25(OH)D in serum was around 35 nmol/L and 82% of the patients were vitamin D deficient. 60-day mortality for all patients was 44%. Mean survival was about 15 days for the vitamin D deficient patients compared with 24 days in the vitamin D sufficient (Arnson et al., 2012). Matthews et al. did a study on vitamin D status among 258 surgical ICU patients (Matthews et al., 2012). In total 53.5% of the patients had severe vitamin D deficiency. Patients with severe vitamin D deficiency had an average ICU stay of around 13 days compared with around 7 and 5 days in the moderately and mildly deficient respectively. The cost of treatment was also higher in the severely vitamin D deficient group compared with moderately deficient (Matthews et al., 2012).

Vitamin D deficiency and sufficiency in critical illness might be seen as a balance between supply and demand. A functional model of vitamin D deficiency in critical illness has been described and is illustrated in Figure 8. It describes that the consequences of vitamin D deficiency are not entirely dependent on vitamin D depletion but also the requirements for vitamin D in the body tissues. The circulating 25(OH)D functions as a reservoir from which the tissues can convert 25(OH)D into the active 1,25(OH)2D. Vitamin D deficiency in critical illness might therefore describe a mismatch between supply and demand when the stimulation of 25-hydroxyvitamin D-1α-hydroxylase by PTH does not fulfill the requirements for 1,25(OH)2D. The differences in how each patient responds in critical illness might depend on severity of vitamin D deficiency and the magnitude of the tissue requirements (Quraishi & Camargo, 2012).

Prevalence of vitamin D deficiency in critical illness has inspired intervention trials. The VITdAL-ICU trial by Amrein et al. was a randomized, double-blind, placebo-controlled, single-center trial in a medical and surgical ICU (Amrein et al., 2014). A group of 237 patients were given vitamin D supplementation, 540,000 IU of vitamin D_3 as a loading dose followed by 90,000 IU monthly for five months. Pilot data from 25 patients showed that vitamin D deficiency was corrected within 48 hours from the beginning of this type of supplementation without any adverse effects. A group of 238 patients were given placebo. The primary end point of the study was length of hospital stay. Mortality and length of ICU stay were among the secondary outcomes. Vitamin D administration did not reduce

length of hospital stay, length of ICU stay or mortality. It was only in a subgroup of patients with severe vitamin D deficiency that lower hospital mortality was observed. These results produce hypothesis for further intervention studies (Amrein et al., 2014).

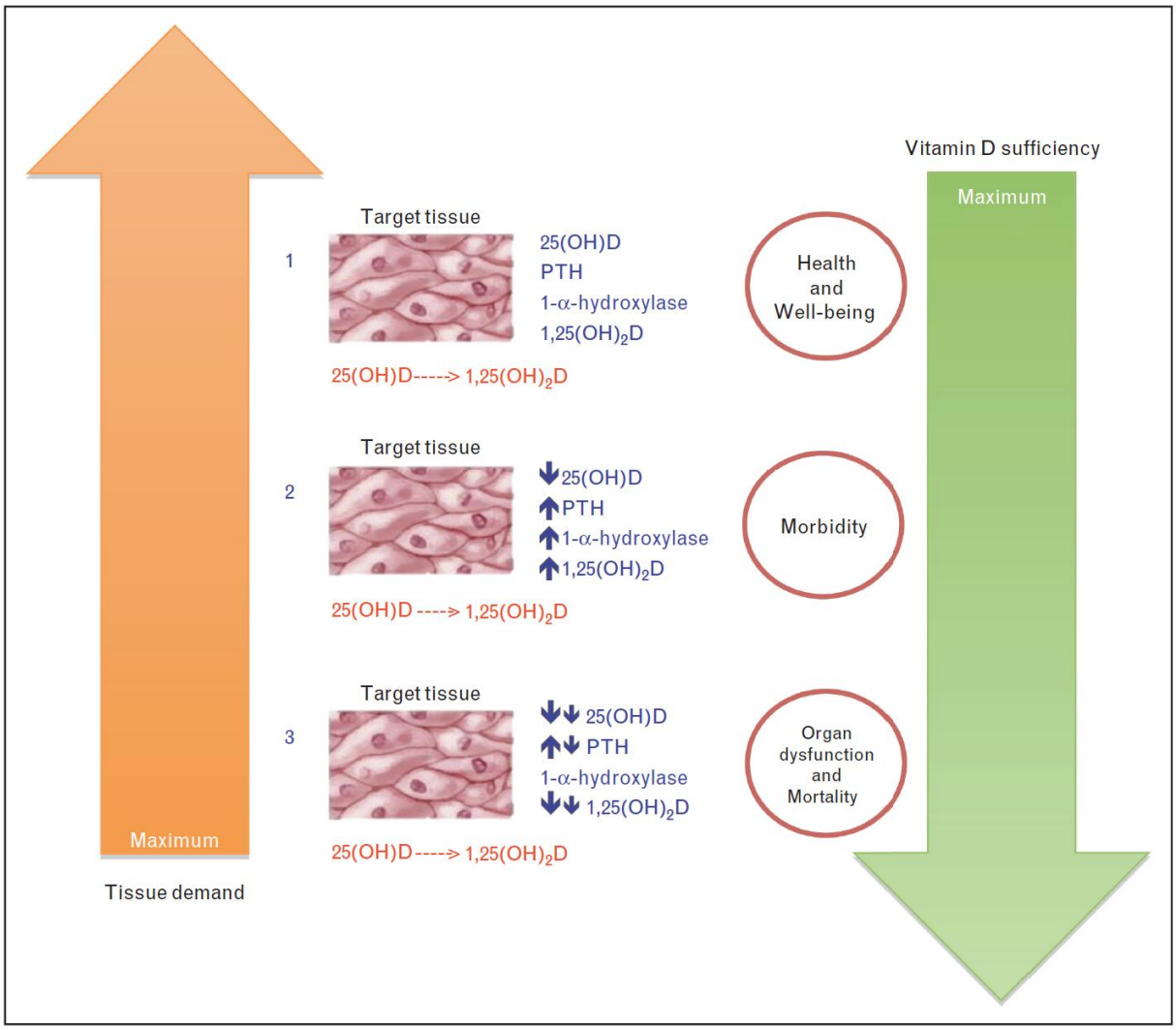

Figure 8. Functional model of vitamin D deficiency. (1) Sufficient vitamin D levels and low tissue requirements lead to health and well-being. (2) Insufficient vitamin D levels and moderate tissue requirements. The activation of 25-hydroxyvitamin D-1α-hydroxylase by PTH generates 1,25(OH)2D from 25(OH)D and preserves health initially but this state leads to morbidity when the 25(OH)D reservoir is depleted. (3) Vitamin D deficiency and maximal tissue requirement result in organ dysfunction and mortality (Quraishi & Camargo, 2012).

In a recently published preliminary study on 14 ICU patients it was shown that serum 25(OH)D levels vary when measured every hour over 24 hours suggesting that a single random measurement not necessarily reflects the correct vitamin D status of critically ill patients. The variability was reduced when any two or three measurements were used within the 24 hours (Venkatesh et al., 2012).

1.5 Vitamin D in Iceland

LUH is located in Reykjavik, in the southwest of Iceland at 64° north, a high latitude location where the winter season is long. Patients seek ward from the whole country but the majority seeking ward resides at around 64° north. For over six months of the year ultraviolet B photons are blocked by the atmosphere so they cannot influence the synthesis of vitamin D in the skin. Even in the summer the large solar zenith angle causes inhibition of ultraviolet B radiation so that hardly any vitamin D can be produced in the skin before 10 am and after 3 pm. This contributes to vitamin D deficiency with seasonal variation at northern latitudes (Wacker & Holick, 2013). Five to 30 minutes exposure to sunlight during noon in the summer twice a week might adequately reduce the risk for vitamin D deficiency (Holick, 2007).

For Icelanders, cod liver oil has traditionally been the major source of vitamin D from diet, supplying them with 48% of vitamin D coming from nutrition according to National Nutrition Survey 2010-2011. According to Steingrimsdottir et al. the intake of vitamin D increased between the years 2002 and 2010-2011. The increase was mostly explained by the increased intake of cod liver oil but also that the concentration of vitamin D in cod liver oil had been increased again by the producer after a decrease in concentration the year 2002 (Lamberg-Allardt et al., 2013; Steingrimsdottir et al., 2014). Other important sources include fatty fish, including salmon and herring as well as eggs, liver and fats fortified with vitamin D. Vitamin D supplements are of major importance for Icelanders. Mean serum 25(OH)D levels in February-March are around 28 nmol/L in the Icelanders not taking supplements compared to 48 nmol/L in the ones taking supplements. Despite good public awareness of vitamin D importance for health, Icelandic food and nutrition recommendations advising the use of vitamin D supplements and the good availability of inexpensive supplements, vitamin D deficiency is common (Gunnarsson, Indridason, Franzson, & Sigurdsson, 2009; Gunnarsson, Indriðason, Franzson, Halldórsdóttir, & Sigurðsson, 2004; Lamberg-Allardt et al., 2013; Ramel, Jonsson, Bjornsson, & Thorsdottir, 2009; Steingrimsdottir, Gunnarsson, Indridason, Franzson, & Sigurdsson, 2005). No national government controlled vitamin D fortification program exists in Iceland.

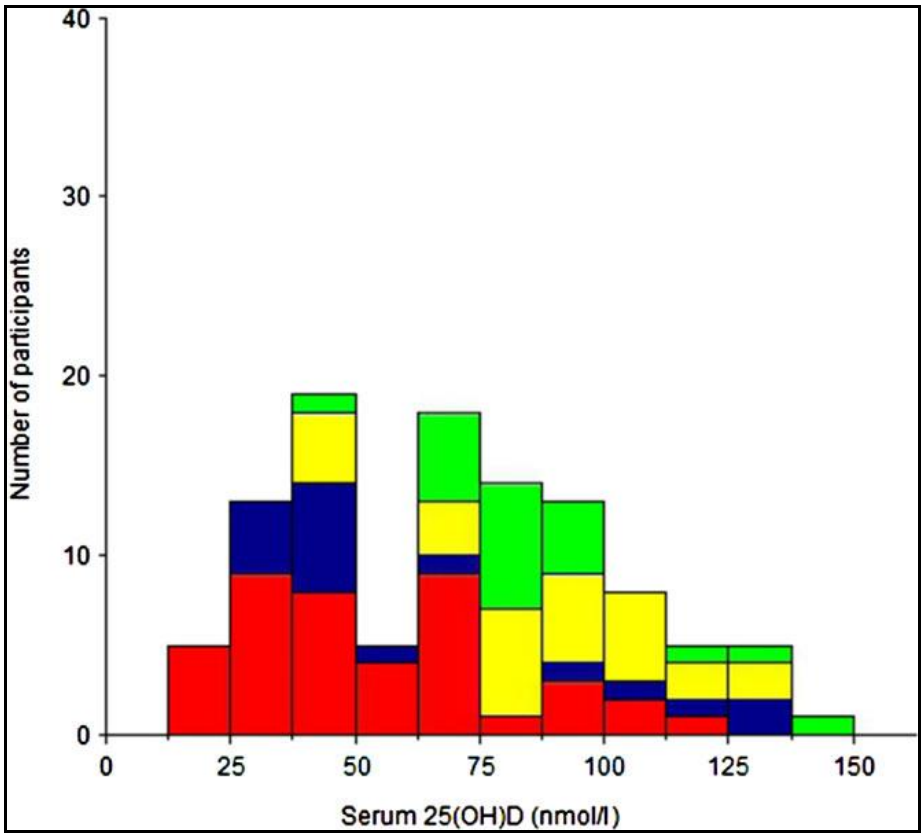

Figure 9. 25(OH)D levels (nmol/l) of anesthesia department caregivers in Iceland. Red: No vitamin D supplement. Blue: Daily multivitamin. Yellow: Daily vitamin D supplement. Green: Daily multivitamin and vitamin D supplement (Skarphedinsdottir et al., 2014).

Skarphedinsdottir et al. compared vitamin D status of anesthesia department caregivers at high northern latitude, in Iceland and Wisconsin USA, at the end of winter (Skarphedinsdottir et al., 2014). The results of the study in Iceland showed that the caregivers are at a high risk for low serum levels of 25(OH)D. The results also showed that vitamin D supplementation practiced by these caregivers, although helpful, was not able to raise serum 25(OH)D to sufficient levels during long winters without exposure to sun (Figure 9).

2 Objective

The aim of this study was to characterize the vitamin D status of critically ill patients at a Nordic university hospital. The primary hypothesis was that the critically ill patients in the ICU at LUH in Iceland had on average low vitamin D levels and the secondary hypothesis was that low vitamin D levels were associated with longer hospital stay and higher mortality.

To this date vitamin D in critically ill patients has not been prospectively measured at similar high northern latitude.

3 Materials and methods

3.1 Patients

The study was a prospective observational study. Adult patients, 18 years or older, admitted to the ICU of LUH for 12 hours or longer were prospectively included between February 2014 and September 2015. Patients admitted after elective major surgery with planned overnight stay in the ICU were excluded from the study. The patients were evenly distributed over the different seasons of the year, but otherwise not selected.

An approval for the study was obtained from the National Bioethics Committee (Hafnarhusid, Tryggvagata 17, 101 Reykjavik, Iceland; Protocol no. VSN 13-042-S1) and the Data Protection Authority (Raudararstig 10, 105 Reykjavik, Iceland; Protocol no. 2013020322VEL/-). A written informed consent was obtained from every participant or a close relative when patient was unable to give his/her consent.

The ICU serves both medical and surgical patients in the only tertiary care hospital in Iceland. The following clinical data were collected from patients' charts during hospital stay: Gender, age, height, weight, indication for ICU admission, major underlying diseases, ICU length of stay, readmission to the ICU, duration of mechanical ventilation, APACHE-II score, hospital length of stay, vitamin D (25(OH)D) serum concentrations and routine blood tests (hemoglobin values, white blood cell count, sodium, potassium, creatinine, ionized calcium, C-reactive protein).

Patients' vital status was followed throughout the hospital stay and up to 90 days from admission to the ICU.

3.2 Definition of vitamin D deficiency

Patients' vitamin D status was classified according to the classification of the Endocrine Society and previous studies as sufficient (25(OH)D > 75 nmol/l), insufficient (25(OH)D 50–75 nmol/l), deficient (25(OH)D 25–50 nmol/l) and severely deficient (25(OH)D 25 nmol/L) (Holick et al., 2011; Rosen, 2011).

3.3 Laboratory measurements

For measurement of 25(OH)D, blood samples were drawn from every patient within 24 hours following admission to the ICU and then again during the following 48 hours. An average of the two measurements was used to define patients' vitamin D status. The use of two consecutive measurements in every patient was to reduce a possible inaccuracy due to variability of serum 25(OH)D in patients over time. Additionally, a third sample was drawn from a subgroup of 20% of all patients within a time frame of three to 90 days following the first sample. This was done to collect a sample after the early acute phase of illness had passed and able a screen for a possible lack of correlation with the samples drawn in the early acute phase of illness. The third sample may reflect

deviation from baseline 25(OH)D levels possibly altered due to the acute illness. 25(OH)D was measured in the serum of every participating patient, using Elecsys® Vitamin D total assay by Roche Diagnostics, an electro-chemiluminescence binding assay, as previously described (Emmen, Wielders, Boer, van den Ouweland, & Vader, 2012).

3.4 Statistical analysis

The primary outcome parameter was serum 25(OH)D concentrations and secondary outcomes included serum 25(OH)D concentrations below 25 nmol/L, ICU length of stay (in days) and hospital length of stay (in days). Based on pilot data 120 subjects were considered sufficient to describe the topic and answer the main question of the study. Data registration, analysis and preparation of graphical figures were performed using Microsoft® Office Excel® 2007 and R version 3.2.2 statistical software. Descriptive statistics were used to describe patients' baseline characteristics. Descriptive statistics were also used to describe the primary outcome, results of 25(OH)D measurements and classifications of vitamin D deficiency. Baseline characteristics and secondary outcomes were analyzed separately for the severely vitamin D deficient patients and others. Difference in baseline characteristics and secondary outcomes between the two groups was evaluated with multiple t-tests, chi-square tests and Fisher's exact tests. Paired t-test was used to compare repeated measurements of 25(OH)D. In all analysis *P* values, less than 0.05 were considered statistically significant.

4 Results

During the 20 months study period, 122 patients admitted to the ICU of LUH were included in the study. Table 1 shows the baseline characteristics of the study population. Most of the patients were males (64%), mean age 65 years and mean BMI of 27 kg/m^2. APACHE-II score ranged from 4–38 with a mean score of 20 which predicts hospital mortality of 35% (Knaus, Draper, Wagner, & Zimmerman, 1985).

Table 1. Baseline characteristics.

Characteristics	Total study group (n = 122)
Age, mean (SD), years	66.0 (14.4)
Gender, count (ratio), M/F	78/44 (1.8)
BMI, mean (SD), m^2/kg	26.7 (6.1)
APACHE II, mean (SD), count (%)	20 (8)
<15	37 (30)
15–23	44 (36)
>23	41 (34)
Reason for admission, count (%)	
Acute abdominal surgery	8 (7)
Bleeding	18 (15)
Cardiac arrest	16 (13)
Heart failure	4 (3)
Myocardial infarction	5 (4)
Renal failure	6 (5)
Respiratory failure	22 (18)
Sepsis	24 (20)
Other	19 (16)
Comorbidity, count (%)	
Coronary heart disease	43 (35)
Hypertension	73 (60)
Insulin dependant diabetes mellitus	9 (7)
Non-insulin dependant diabetes mellitus	13 (11)
Heart failure	31 (25)
Liver disease	8 (7)
Metastatic cancer	20 (16)
Lung disease	33 (27)
Renal disease	18 (15)
Peripheral vascular disease	8 (7)
Cerebrovascular disease	12 (10)
Smoking	62 (51)
Alcohol abuse	17 (14)

In Figure 10 the primary outcome, 25(OH)D values of the study population, are shown. Nine percent of all included ICU patients had 25(OH)D > 75 nmol/L. 69% of the patients were classified as vitamin D deficient and 43% severely deficient. Mean 25(OH)D value in all three separate measurements were similar, ranged from 38–41 (Figure 11).

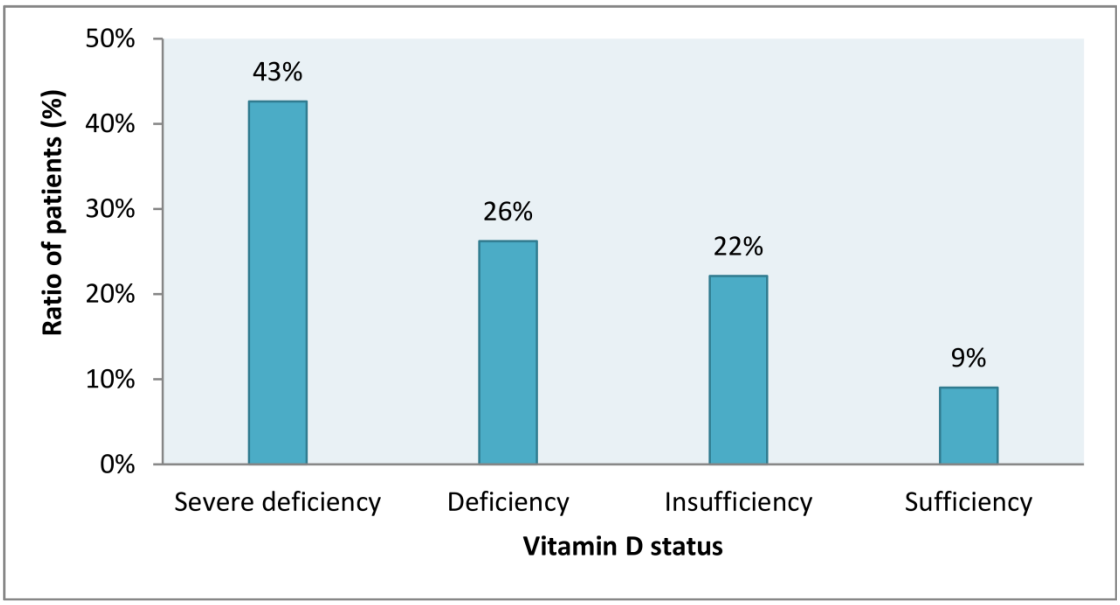

Figure 10. Primary outcome. Classification of vitamin D status according to mean of two measurements of 25(OH)D in 122 ICU patients.

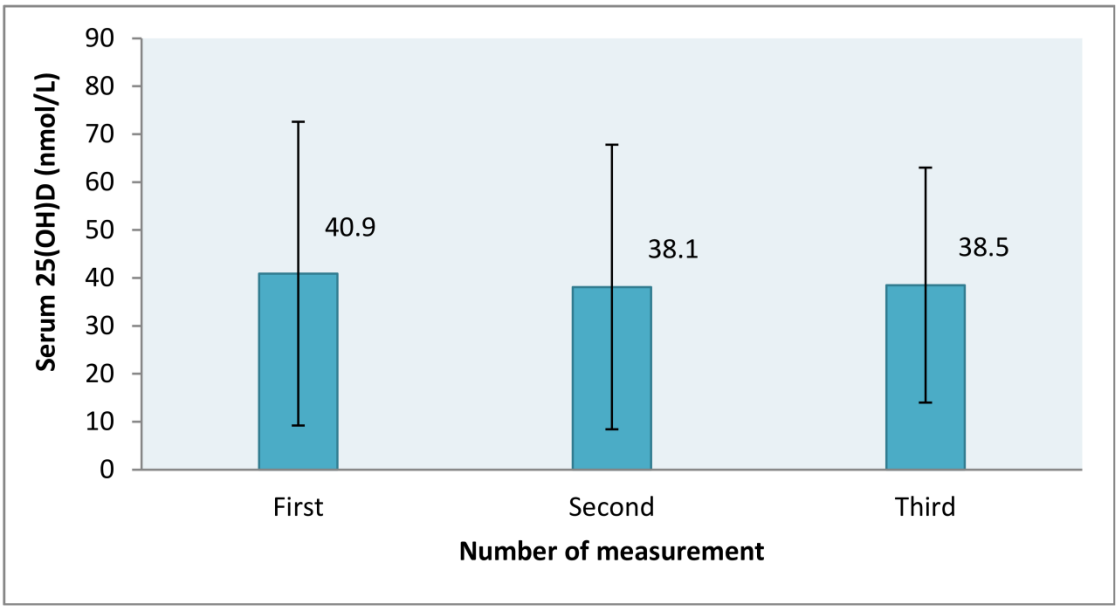

Figure 11. Mean 25(OH)D on three separate occasions, first 24 hours, following 48 hours and between three to 90 days following admission to the ICU. One hundred and twenty-two patients were measured in the first and second measurement and a subgroup of 23 patients were measured in the third measurement. Error bars show the standard deviation.

The most common reason for ICU admission was severe sepsis in 20% of patients, followed by respiratory failure (18%), severe bleedings (15%) and cardiac arrest (13%) (Figure 12).

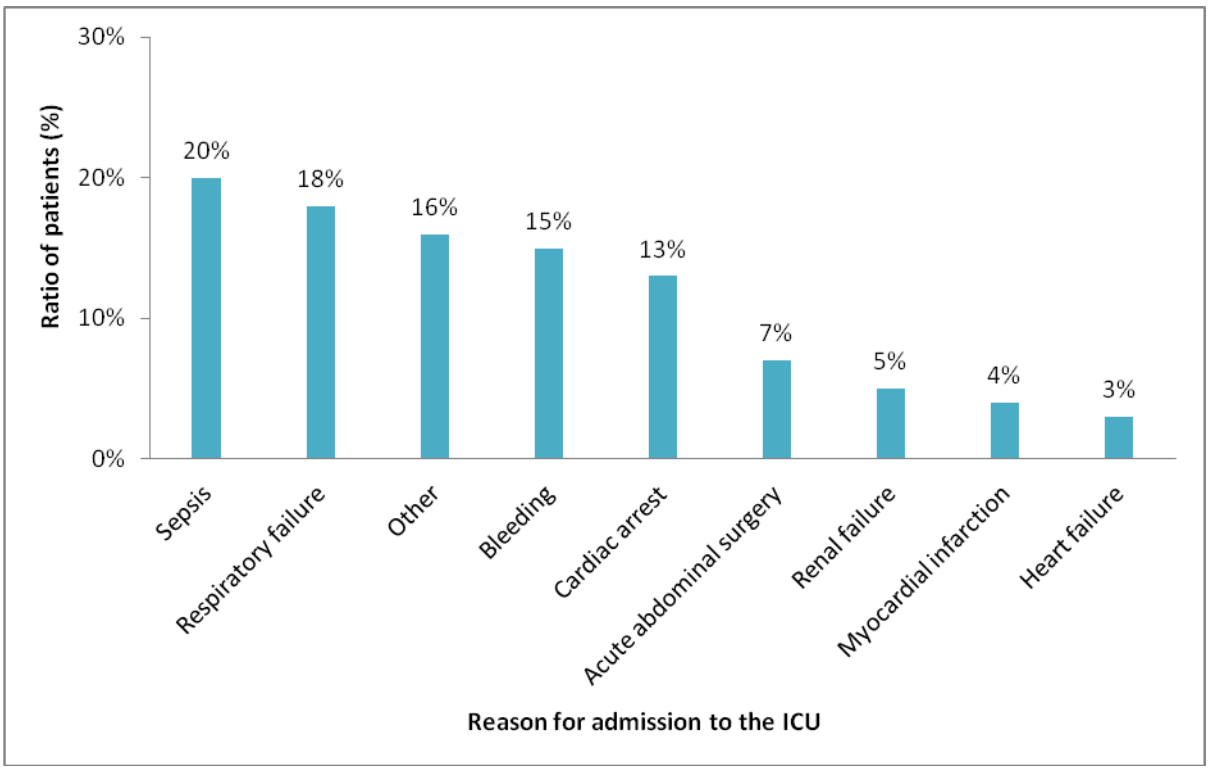

Figure 12. Reason for admission to the ICU.

Most patients had multiple comorbidities with hypertension, smoking and coronary heart disease being the most common (Table 1).

The secondary outcome variables are shown in Table 2, comparing severely vitamin D deficient patients to patients with higher serum vitamin D values, but these groups did not significantly differ in baseline characteristics except that coronary heart disease was less common in severely vitamin D deficient patients than other patients (23% vs. 44%, $p = 0.026$). Both 90-day and in hospital mortality for the study population were 25%. There was no difference in in-hospital, 30-day, 60-day or 90-day mortality between severely vitamin D deficient patients and others (13 vs. 17, $p = 0.502$, 12 vs. 16, $p = 1.000$, 14 vs. 17, $p = 0.470$ and 14 vs. 17 $p = 0.639$ respectively). The severely vitamin D deficient patients (25(OH)D > 25 nmol/L) stayed longer in the hospital, in the ICU and in mechanical ventilation compared with other patients but these results were not statistically significant (Table 2).

Table 2. Secondary outcomes. Chi-square comparison of severely vitamin D deficient and others. *P*-values < 0.05 considered statistically significant.

Outcomes	25(OH)D < 25 nmol/l ($n = 52$)	25(OH)D ≥ 25 nmol/l ($n = 70$)	Study group ($n = 122$)	*P*-value
Mortality, count (%)				
In hospital	14 (27)	17 (24)	31 (25)	0.741
30-day	12 (23)	16 (23)	28 (23)	0.977
60-day	14 (27)	17 (24)	31 (25)	0.741
90-day	14 (27)	17 (24)	31 (25)	0.741
Hospital stay				
ICU stay, count (%), days				
<2	11 (22)	25 (35)	36 (30)	0.196
2–4	18 (36)	22 (31)	40 (33)	
>4	23 (46)	23 (19)	46 (38)	
Mechanical ventilation, count (%), days				
0	15 (29)	32 (46)	47 (39)	0.166
1–3	17 (33)	17 (24)	34 (28)	
>3	20 (38)	21 (30)	41 (34)	
Hospital stay, count (%), days				
<10	18 (35)	24 (34)	42 (34)	0.435
10–19	15 (30)	27 (39)	42 (34)	
>19	19 (37)	19 (27)	38 (31)	

Table 3 shows comparison of vitamin D levels between different subgroups of patients. Patients with coronary heart disease had significantly higher vitamin D than patients without and patients with metastatic cancer had significantly lower vitamin D than patients without the disease.

Table 3. Vitamin D values in different patient categories. Mean serum values of 25(OH)D from two measurements according to binomial variables and t-test comparison. *P*-values < 0.05 considered statistically significant and are marked with *.

Binomial baseline variables	Yes (nmol/l)	No (nmol/l)	*P*-value
Male	39.3	39.8	0.931
Coronary heart disease	49.5	34.1	0.008*
Hypertension	43.5	33.6	0.060
Insulin dependant diabetes mellitus	36.6	39.7	0.717
Non-insulin dependant diabetes mellitus	49.1	38.4	0.408
Heart failure	48.9	36.3	0.091
Liver disease	26.2	40.4	0.053
Metastatic cancer	26.0	42.2	0.005*
Lung disease	44.0	37.8	0.374
Renal disease	38.7	39.6	0.924
Peripheral vascular disease	63.4	37.8	0.130
Cerebrovascular disease	45.1	38.9	0.536
Smoking	41.0	38.0	0.590
Alcohol abuse	44.9	38.6	0.453
Binomial secondary outcome variables			
Mortality			
In hospital	37.2	40.3	0.615
30-day	40.2	39.3	0.905
60-day	38.3	39.9	0.803
90-day	38.3	39.9	0.803

The first vitamin D measurements were slightly higher than the second (mean difference 2.8 nmol/L). A subgroup of 23 patients was measured three times. The third measurement was on average done 18 days (range 3–86) after the first measurement. Figure 13 shows comparison of classification of vitamin D status of these patients according to different measurements.

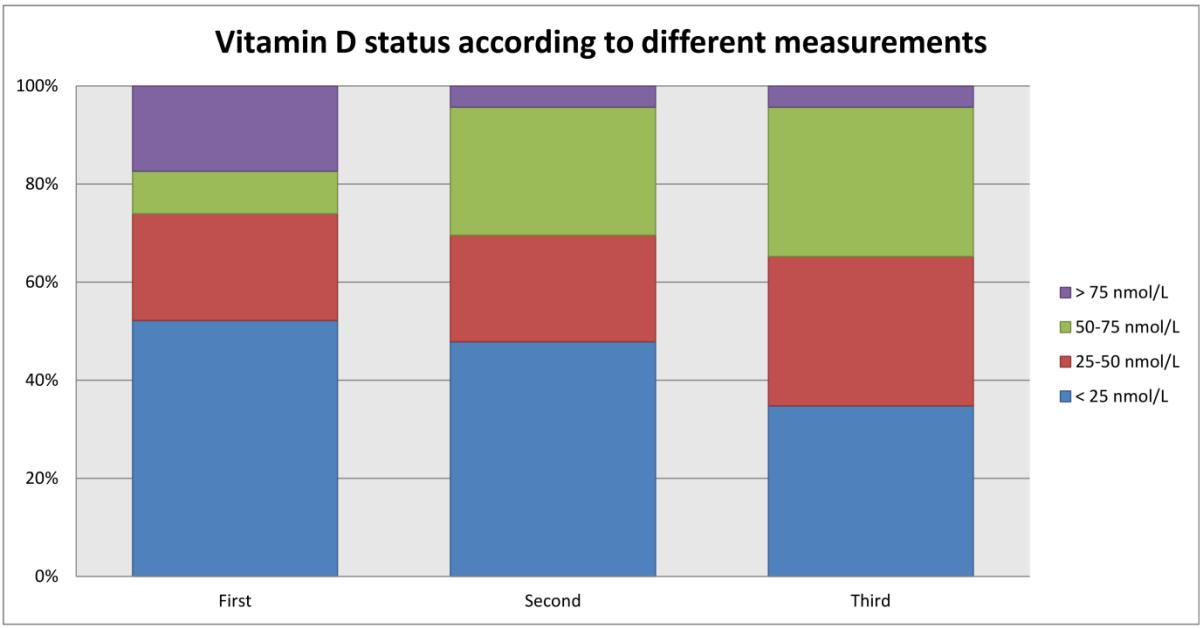

Figure 13. Vitamin D (25(OH)D) values, for the subgroup of 23 patients who were measured three times, according to classification of vitamin D status. There was not a statistically significant difference in classification of vitamin D status between the measurements.

5 Discussion

5.1 Main findings

The results of this study reveal that critically ill patients at high northern latitude (Iceland) are at high risk for being vitamin D deficient. In fact only one in every 10 patients had vitamin D levels that are currently recommended for maintaining good health (Holick et al., 2011). Furthermore, 43% of the patients had vitamin D levels below 25 nmol/L which is associated with osteomalacia; this despite the fact the level of education in the population studied is regarded high. Onto that the tradition to take vitamin D supplements in Iceland is general and popular media have in recent years repeatedly reported stories suggesting the importance of taking supplementary vitamin D, which is readily available and inexpensive. The present study also shows that vitamin D deficiency was as common and even more common than previously reported among critically ill patients at more southern latitudes (Braun, Gibbons, Litonjua, Giovannucci, & Christopher, 2012; P. Lee, 2011; Matthews et al., 2012; Moromizato et al., 2014). Furthermore, the severely vitamin D deficient patients had slightly longer absolute hospital stay compared to patients with higher serum vitamin D levels, but these results were not statistically significant.

According to Venkatesh et al., serum vitamin D levels vary over 24 hours in ICU patients (Venkatesh et al., 2012). The results of the present study show some intra-patient variability in vitamin D serum concentrations in repeated measurements over days, but the absolute difference in serum vitamin D levels was rather small and probably not clinically relevant. Therefore, a single measurement appears to be sufficient to get a reasonable picture of the vitamin D status in the critically ill.

A recent report showed low levels of vitamin D in anesthesia and ICU staff at LUH despite high ratio of staff taking vitamin D supplements (Skarphedinsdottir et al., 2014). These results raised questions about the vitamin D status of the patients warded at the ICU. The results of the present study confirm that the ICU patients' vitamin D status is even worse.

The association of vitamin D deficiency with worse outcomes in multiple chronic diseases has been demonstrated in several studies (Schöttker et al., 2013; Zittermann et al., 2012). According to a recent Cochrane meta-analysis vitamin D supplementation in the elderly might reduce overall mortality (Bjelakovic et al., 2014). The present study showed that patients with metastatic cancers had lower serum vitamin D values compared with others and patients with coronary heart disease had higher serum vitamin D values compared to other patients (Table 3). When interpreting these results it has to be taken into account that the prevalence of comorbidities was high in the current study population (Table 1) and ICU patients in general usually often have multiple comorbidities.

The discovery of vitamin D receptors in immune cells has led to researchers revealing vitamins D role in the innate and adaptive immune system. 1,25(OH)2D stimulates the production of cathelicidin, an antimicrobial peptide, in innate immune cells and a study indicated impairment of bacteriocidality in vitamin D deficiency especially in septic critically ill patients (Jeng et al., 2009). On the other hand, its role in the adaptive immune system is inhibitory. 1,25(OH)2D has a suppressive effect on cytokines and is considered to reduce the severity of systemic inflammatory response in the critically ill.

Therefore, it is rational to further study the effects of vitamin D in the critically ill. This is supported by recent review that demonstrated 38-100% prevalence of vitamin D insufficiency and deficiency in the critically ill (P. Lee, 2011).

Several studies have shown that vitamin D deficiency is a predictor of acute kidney injury (AKI), sepsis and all-cause mortality in critically ill patients (Braun, Gibbons, et al., 2012; Braun, Litonjua, et al., 2012; Moromizato et al., 2014). Recently, Flynn et al. studied the effects of vitamin D deficiency in critically ill surgical patients and showed that low vitamin D levels were associated with longer hospital stay, organ dysfunction and increased infection rates (Flynn et al., 2012). Venkatram et al. demonstrated an association between vitamin D insufficiency and hospital mortality in medical ICU patients (Venkatram et al., 2011). Results of the present study did not show higher mortality in the severely vitamin D deficient patients compared with patients who had higher serum vitamin D levels.

5.2 Limitations and strengths

It must be taken into consideration, when interpreting 25(OH)D in critical illness, that while vitamin D has been shown to reduce the systemic inflammatory response, the systemic inflammatory response has also been shown to reduce serum levels of 25(OH)D (Duncan, Talwar, McMillan, Stefanowicz, & O'Reilly, 2012; Reid et al., 2011). It is not clear how critical illness affects serum 25(OH)D levels but several other nutritional biomarkers have also been shown to be reduced during inflammatory state. 25(OH)D levels may be lowered in acute critical illness compared to normal state because of increased conversion to 1,25(OH)2D for stimulation of innate immunity. The illness leading to ICU admission may also have lowering effects on 25(OH)D by increasing immobility and decreasing sun exposure (Braun, Gibbons, et al., 2012). In addition, fluid resuscitation and hemodilution may dilute the 25(OH)D concentration. Patients admitted to the ICU often undergo massive fluid resuscitation. All these factors could lead to an overestimation of vitamin D insufficiency and deficiency when using measurements during the initiation of intensive care, and this is a potential limitation to the current study. However, a study on patients undergoing cardiac surgery under cardiopulmonary bypass showed that 25(OH)D levels almost returned to baseline 24-hours following bypass and had returned to baseline at five days following bypass (Krishnan et al., 2010). Results of another study on Vitamin D kinetics in acute critical illness, in which repeated measurements of 25(OH)D were done in 20 ICU patients, showed that the levels are changeable in this phase. Drops in 25(OH)D levels were witnessed after 12 hours of ICU care but higher levels resulted in smaller decrease. Furthermore, 25(OH)D levels stabilized after 2-3 days and then begun to increase (Czarnik et al., 2017). Taking into consideration these alterations in the serum concentration of 25(OH)D in critical illness, repeated measurements might be helpful in evaluating vitamin D status in the ICU (Krishnan et al., 2010). Vitamin D is highly bound to DBP. The critically ill have been shown to have lower levels of vitamin DBP than healthy individuals and some assays for measuring 25(OH)D underestimate the circulating amount under these circumstances. However, the laboratory assay used in the current study has not shown such underestimation (Heijboer, Blankenstein, Kema, & Buijs, 2012).

A strength of the current study is that serum 25(OH)D was measured twice for all patients reducing the effect of 24-hour variability of serum 25(OH)D value and a subgroup of patients was measured three times for comparison (Venkatesh et al., 2012). According to the results of the study, a single measurement of 25(OH)D in acute illness gives a reasonably good clue about the patients' vitamin D status and may be a useful guide in supplementation. On the other hand, a weakness of the present study is that serum 25(OH)D measured in critical illness might not accurately reflect patients' baseline value although measured more than once as stated earlier. Although serum 25(OH)D measured during acute critical illness not necessarily reflects exact vitamin D status, Quraishi et al. have demonstrated that low serum 25(OH)D level at the initiation of intensive care might predict prolonged hospital stay, readmission to the ICU and mortality (Quraishi et al., 2014).

5.3 Future perspectives

This study adds to the growing literature on vitamin D status of the critically ill by providing new information on patients at high risk for vitamin D deficiency because of their high northern latitude residency. Vitamin D supplementation is widely available, relatively inexpensive and well tolerated (Christopher, 2015; Holick et al., 2011). It is necessary to consider if vitamin D supplementation in acute critical illness might improve outcome. Some intervention studies have recently been published, however to this date the results of these have been inconclusive (Christopher, 2015). The results of the VITdAL-ICU trial by Amrein et al. were mainly negative, but the authors did a predefined subgroup analysis on the severely vitamin D deficient, defined as serum 25(OH)D levels < 30 nmol/L. In this subgroup analysis, a significant difference in overall hospital mortality was observed, with mortality being 29% in the intervention group compared to 46% in the placebo group, a hazard ratio of 0.56 and a number needed to treat of 5, showing a large treatment effect in this subgroup of patients. Of all included patients, 42% were severely vitamin D deficient. The research might have had more impact if it had focused on supplementation in the severely vitamin D deficient (Amrein et al., 2014).

Considering the results of observational studies, such as the one presented here, there is a need for further studies on this subject where a dose finding study would be important before going to a large multicenter randomized controlled interventional trial, studying the effects of vitamin D supplementation on outcomes in acute critical illness.

More intervention studies have followed the VITdAL-ICU trial (Putzu, Belletti, Cassina, Clivio, et al., 2017). The results of a meta-analysis evaluating the use of vitamin D supplements in 2017 have been confounding and the cause of debate. To some extent it may depend on the inclusion criteria (Putzu, Belletti, Cassina, & Landoni, 2017). A meta-analysis by Langlois et al. included six randomized controlled trials from the years 2011-2016 comparing supplementation with placebo (Langlois, Szwec, D'Aragon, Heyland, & Manzanares, 2017). The authors concluded that vitamin D does not improve outcome but that this might be explained by the lack of number of trials. Outcomes analyzed were mortality, length of ICU stay, length of hospital stay and length of mechanical ventilation (Langlois et al., 2017). In another meta-analysis by Putzu et al. including seven randomized controlled trials from the same period, vitamin D supplementation was associated with reduction in mortality. There was no

difference in length of ICU stay, length of total hospital stay or length of mechanical ventilation. Quality of data was considered low to moderate (Putzu, Belletti, Cassina, Clivio, et al., 2017). A third meta-analysis by Weng et al. included four studies from 2014-2016 did not show reduction in mortality with vitamin D supplementation and was inconclusive for length of hospital stay and ICU stay (Weng, Li, Mao, & Zeng, 2017). The inclusion criteria for this last meta-analysis has been criticized (McNally, Ginde, & Amrein, 2017). Considering all these results, it is at least hard to exclude that vitamin D supplementation in the ICU might be associated with a decrease in mortality and hospital stay without causing adverse effects. The multicenter randomized controlled trial, VITDALIZE, is expected to be completed in February 2021 and it has the aim to clarify once and for all if vitamin D supplementation improves survival in ICU patients or not (Langlois et al., 2017; Putzu, Belletti, Cassina, Clivio, et al., 2017; Putzu, Belletti, Cassina, & Landoni, 2017; Weng et al., 2017).

It is also likely that future studies will include frequent serial measurements of 25(OH)D and related factors to make clearer the observed variability in serum 25(OH)D in ICU patients already discussed (Czarnik et al., 2017; Krishnan et al., 2010; Venkatesh et al., 2012). A protocol for a coming study on ICU patients with AKI published in 2017, the VID-AKI study, will include such serial measurements (Cameron et al., 2017). Moreover, has it been suggested that future studies should include measurements of vitamin D binding protein (DBP) and free vitamin D metabolites to give more detailed information on the vitamin D status of each patient (Quraishi & Camargo, 2012).

Vitamin D deficiency in the ICU in Iceland can also be looked at from a public health point of view and the possibilities for prevention must be considered. The ICU patients in the current study come from a population with previously known high prevalence of vitamin D deficiency (Gunnarsson et al., 2009; Gunnarsson et al., 2004; Ramel et al., 2009; Steingrimsdottir et al., 2005). One of the possibilities for prevention is reducing vitamin D deficiency in the general population of Iceland. This might be done by launching a food fortification program, increasing the intake of vitamin D supplements and by encouraging outdoor activities during sunlight hours. A recent meta-analysis summarizing results of 25 randomized controlled trials studying vitamin D supplementation to prevent acute respiratory tract infections showed promising results (Martineau et al., 2017). The results showed that vitamin D supplementation protected against acute respiratory tract infection and the ones who benefited the most were the persons with vitamin D deficiency. Four vitamin D deficient persons were needed to treat with daily or weekly supplementation to prevent one person from having acute respiratory tract infection. Vitamin D supplementation was considered safe and patients receiving high bolus doses did not have better outcome. These results underline the possible positive effects of vitamin D supplementation in the general populations which can eventually lead to better outcome also for the ICU population (Martineau et al., 2017). When discussing prevention, it is difficult to exclude screening from the discussion. Currently, screening for vitamin D deficiency is only warranted in the individuals at risk for deficiency but not in the general population (Holick et al., 2011). It could be argued that many of ICU patients are at risk for vitamin D deficiency and should be screened if vitamin D status is earlier unknown. Another point of view might be to give supplementation to all ICU patients and save the funds that would otherwise be spent on screening

since vitamin D supplements are relatively inexpensive. This discussion must remain as reflections until further intervention and even economic studies have been carried out.

The medical problems dealt with within the ICU are often severe and complex. In the present study, the focus has been on a narrow subject within the field of intensive care medicine. This project might be looked at as some sort of a search for the magic bullet but that was not its objective. Although vitamin D status is only one of the numerous details that can affect ICU patient outcome, it is in end the sum of all these small details that decide ICU patient outcome. Optimization of every detail will lead to better outcome.

6 Conclusion

The main findings of this study are that critically ill patients staying at an ICU located at high northern latitude in Iceland seem to be at high risk of vitamin D deficiency. There was no difference in the average measurement of the first serum vitamin D concentration and the second one; suggesting that a single spot test of vitamin D is sufficient to get a broad idea about the vitamin D status in critically ill patients. The severely vitamin D deficient patients had more extended total hospital stay compared to patients with better vitamin D status, however, these differences were not statistically significant. No difference in mortality was observed when comparing the severely vitamin D deficient patients to patients with a better vitamin D status. These results warrant further studies on the role of vitamin D in ICU patients and how vitamin D supplementation in acute critical illness could be benefit these patients.

References

Amrein, K., Schnedl, C., Holl, A., Riedl, R., Christopher, K. B., Pachler, C., et al. (2014). Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. *JAMA, 312*(15), 1520-1530.

Arnson, Y., Gringauz, I., Itzhaky, D., & Amital, H. (2012). Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients. *QJM, 105*(7), 633-639.

Berry, D. J., Hesketh, K., Power, C., & Hyppönen, E. (2011). Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br J Nutr, 106*(9), 1433-1440.

Binkley, N., Novotny, R., Krueger, D., Kawahara, T., Daida, Y. G., Lensmeyer, G., et al. (2007). Low vitamin D status despite abundant sun exposure. *J Clin Endocrinol Metab, 92*(6), 2130-2135.

Bjelakovic, G., Gluud, L. L., Nikolova, D., Whitfield, K., Wetterslev, J., Simonetti, R. G., et al. (2014). Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev, 1*, CD007470.

Brannon, P. M., Yetley, E. A., Bailey, R. L., & Picciano, M. F. (2008). Overview of the conference "Vitamin D and Health in the 21st Century: an Update". *Am J Clin Nutr, 88*(2), 483S-490S.

Braun, A. B., Gibbons, F. K., Litonjua, A. A., Giovannucci, E., & Christopher, K. B. (2012). Low serum 25-hydroxyvitamin D at critical care initiation is associated with increased mortality. *Crit Care Med, 40*(1), 63-72.

Braun, A. B., Litonjua, A. A., Moromizato, T., Gibbons, F. K., Giovannucci, E., & Christopher, K. B. (2012). Association of low serum 25-hydroxyvitamin D levels and acute kidney injury in the critically ill. *Crit Care Med, 40*(12), 3170-3179.

Cameron, L. K., Lei, K., Smith, S., Doyle, N. L., Doyle, J. F., Flynn, K., et al. (2017). Vitamin D levels in critically ill patients with acute kidney injury: a protocol for a prospective cohort study (VID-AKI). *BMJ Open, 7*(7), e016486.

Cashman, K. D., Dowling, K. G., Škrabáková, Z., Gonzalez-Gross, M., Valtueña, J., De Henauw, S., et al. (2016). Vitamin D deficiency in Europe: pandemic? *Am J Clin Nutr, 103*(4), 1033-1044.

Christopher, K. B. (2015). Vitamin D supplementation in the ICU patient. *Curr Opin Clin Nutr Metab Care, 18*(2), 187-192.

Czarnik, T., Czarnik, A., Gawda, R., Gawor, M., Piwoda, M., Marszalski, M., et al. (2017). Vitamin D kinetics in the acute phase of critical illness: A prospective observational study. *J Crit Care, 43*, 294-299.

DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr, 80*(6 Suppl), 1689S-1696S.

Deluca, H. F. (2014). History of the discovery of vitamin D and its active metabolites. *Bonekey Rep, 3*, 479.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., et al. (2008). Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med, 168*(12), 1340-1349.

Duncan, A., Talwar, D., McMillan, D. C., Stefanowicz, F., & O'Reilly, D. S. (2012). Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am J Clin Nutr, 95*(1), 64-71.

Emmen, J. M., Wielders, J. P., Boer, A. K., van den Ouweland, J. M., & Vader, H. L. (2012). The new Roche Vitamin D Total assay: fit for its purpose? *Clin Chem Lab Med, 50*(11), 1969-1972.

Flynn, L., Zimmerman, L. H., McNorton, K., Dolman, M., Tyburski, J., Baylor, A., et al. (2012). Effects of vitamin D deficiency in critically ill surgical patients. *Am J Surg, 203*(3), 379-382; discussion 382.

Foods rich in vitamin D. (n.d.). Retrieved from https://www.shutterstock.com/image-photo/foods-rich-vitamin-d-top-view-465164366?src=KkojU1B7h7A4b5IoL0OeWA-1-0.

Freedman, D. M., Looker, A. C., Abnet, C. C., Linet, M. S., & Graubard, B. I. (2010). Serum 25-hydroxyvitamin D and cancer mortality in the NHANES III study (1988-2006). *Cancer Res, 70*(21), 8587-8597.

Gaksch, M., Jorde, R., Grimnes, G., Joakimsen, R., Schirmer, H., Wilsgaard, T., et al. (2017). Vitamin D and mortality: Individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium. *PLoS One, 12*(2), e0170791.

Garland, C. F., Garland, F. C., Gorham, E. D., Lipkin, M., Newmark, H., Mohr, S. B., et al. (2006). The role of vitamin D in cancer prevention. *Am J Public Health, 96*(2), 252-261.

Garland, C. F., Mohr, S. B., Gorham, E. D., Grant, W. B., & Garland, F. C. (2006). Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer. *Am J Prev Med, 31*(6), 512-514.

Ginde, A. A., Liu, M. C., & Camargo, C. A. (2009). Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Arch Intern Med, 169*(6), 626-632.
Giovannucci, E., Liu, Y., Rimm, E. B., Hollis, B. W., Fuchs, C. S., Stampfer, M. J., et al. (2006). Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst, 98*(7), 451-459.
Gorham, E. D., Garland, C. F., Garland, F. C., Grant, W. B., Mohr, S. B., Lipkin, M., et al. (2005). Vitamin D and prevention of colorectal cancer. *J Steroid Biochem Mol Biol, 97*(1-2), 179-194.
Grant, W. B. (2002). An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer, 94*(6), 1867-1875.
Gunnarsson, O., Indridason, O. S., Franzson, L., & Sigurdsson, G. (2009). Factors associated with elevated or blunted PTH response in vitamin D insufficient adults. *J Intern Med, 265*(4), 488-495.
Gunnarsson, O., Indriðason, O. S., Franzson, L., Halldórsdóttir, E., & Sigurðsson, G. (2004). [Vitamin-D homeostasis amongst adult Icelandic population.]. *Laeknabladid, 90*(1), 29-36.
Hanchette, C. L., & Schwartz, G. G. (1992). Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. *Cancer, 70*(12), 2861-2869.
Heijboer, A. C., Blankenstein, M. A., Kema, I. P., & Buijs, M. M. (2012). Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. *Clin Chem, 58*(3), 543-548.
Holick, M. F. (2007). Vitamin D deficiency. *N Engl J Med, 357*(3), 266-281.
Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., et al. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab, 96*(7), 1911-1930.
Hossein-nezhad, A., & Holick, M. F. (2013). Vitamin D for health: a global perspective. *Mayo Clin Proc, 88*(7), 720-755.
Jeng, L., Yamshchikov, A. V., Judd, S. E., Blumberg, H. M., Martin, G. S., Ziegler, T. R., et al. (2009). Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. *J Transl Med, 7*, 28.
Kantor, E. D., Rehm, C. D., Du, M., White, E., & Giovannucci, E. L. (2016). Trends in Dietary Supplement Use Among US Adults From 1999-2012. *JAMA, 316*(14), 1464-1474.
Kennel, K. A., Drake, M. T., & Hurley, D. L. (2010). Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc, 85*(8), 752-757; quiz 757-758.
Knaus, W. A., Draper, E. A., Wagner, D. P., & Zimmerman, J. E. (1985). APACHE II: a severity of disease classification system. *Crit Care Med, 13*(10), 818-829.
Krause, R., Bühring, M., Hopfenmüller, W., Holick, M. F., & Sharma, A. M. (1998). Ultraviolet B and blood pressure. *Lancet, 352*(9129), 709-710.
Kricker, A., & Armstrong, B. (2006). Does sunlight have a beneficial influence on certain cancers? *Prog Biophys Mol Biol, 92*(1), 132-139.
Krishnan, A., Ochola, J., Mundy, J., Jones, M., Kruger, P., Duncan, E., et al. (2010). Acute fluid shifts influence the assessment of serum vitamin D status in critically ill patients. *Crit Care, 14*(6), R216.
Kulie, T., Groff, A., Redmer, J., Hounshell, J., & Schrager, S. (2009). Vitamin D: an evidence-based review. *J Am Board Fam Med, 22*(6), 698-706.
Lamberg-Allardt, C., Brustad, M., Meyer, H. E., & Steingrimsdottir, L. (2013). Vitamin D - a systematic literature review for the 5th edition of the Nordic Nutrition Recommendations. *Food Nutr Res, 57*.
Langlois, P. L., Szwec, C., D'Aragon, F., Heyland, D. K., & Manzanares, W. (2017). Vitamin D supplementation in the critically ill: A systematic review and meta-analysis. *Clin Nutr*.
Lee, J. H., Gadi, R., Spertus, J. A., Tang, F., & O'Keefe, J. H. (2011). Prevalence of vitamin D deficiency in patients with acute myocardial infarction. *Am J Cardiol, 107*(11), 1636-1638.
Lee, P. (2011). Vitamin D metabolism and deficiency in critical illness. *Best Pract Res Clin Endocrinol Metab, 25*(5), 769-781.
Lee, P., Eisman, J. A., & Center, J. R. (2009). Vitamin D deficiency in critically ill patients. *N Engl J Med, 360*(18), 1912-1914.
Levis, S., Gomez, A., Jimenez, C., Veras, L., Ma, F., Lai, S., et al. (2005). Vitamin d deficiency and seasonal variation in an adult South Florida population. *J Clin Endocrinol Metab, 90*(3), 1557-1562.
Luscombe, C. J., Fryer, A. A., French, M. E., Liu, S., Saxby, M. F., Jones, P. W., et al. (2001). Exposure to ultraviolet radiation: association with susceptibility and age at presentation with prostate cancer. *Lancet, 358*(9282), 641-642.

Marshall, J. C., Bosco, L., Adhikari, N. K., Connolly, B., Diaz, J. V., Dorman, T., et al. (2017). What is an intensive care unit? A report of the task force of the World Federation of Societies of Intensive and Critical Care Medicine. *J Crit Care, 37*, 270-276.

Martineau, A. R., Jolliffe, D. A., Hooper, R. L., Greenberg, L., Aloia, J. F., Bergman, P., et al. (2017). Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ, 356*, i6583.

Matthews, L. R., Ahmed, Y., Wilson, K. L., Griggs, D. D., & Danner, O. K. (2012). Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. *Am J Surg, 204*(1), 37-43.

McCollum, E. V. (1967). The paths to the discovery of vitamins A and D. *J Nutr, 91*(2), Suppl 1:11-16.

McNally, J. D., Ginde, A. A., & Amrein, K. (2017). Clarification needed for the systematic review of vitamin D trials in the ICU. *Intensive Care Med, 43*(4), 595-596.

Melamed, M. L., Michos, E. D., Post, W., & Astor, B. (2008). 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med, 168*(15), 1629-1637.

Miller, J. W., Harvey, D. J., Beckett, L. A., Green, R., Farias, S. T., Reed, B. R., et al. (2015). Vitamin D Status and Rates of Cognitive Decline in a Multiethnic Cohort of Older Adults. *JAMA Neurol, 72*(11), 1295-1303.

Moromizato, T., Litonjua, A. A., Braun, A. B., Gibbons, F. K., Giovannucci, E., & Christopher, K. B. (2014). Association of low serum 25-hydroxyvitamin D levels and sepsis in the critically ill. *Crit Care Med, 42*(1), 97-107.

Norman, A. W. (2008). From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr, 88*(2), 491S-499S.

Norman, A. W., & Bouillon, R. (2010). Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med (Maywood), 235*(9), 1034-1045.

Putzu, A., Belletti, A., Cassina, T., Clivio, S., Monti, G., Zangrillo, A., et al. (2017). Vitamin D and outcomes in adult critically ill patients. A systematic review and meta-analysis of randomized trials. *J Crit Care, 38*, 109-114.

Putzu, A., Belletti, A., Cassina, T., & Landoni, G. (2017). Meta-analyses on Vitamin D in critically ill patients: What data can tell us. *J Crit Care*.

Quraishi, S. A., Bittner, E. A., Blum, L., McCarthy, C. M., Bhan, I., & Camargo, C. A. (2014). Prospective study of vitamin D status at initiation of care in critically ill surgical patients and risk of 90-day mortality. *Crit Care Med, 42*(6), 1365-1371.

Quraishi, S. A., & Camargo, C. A. (2012). Vitamin D in acute stress and critical illness. *Curr Opin Clin Nutr Metab Care, 15*(6), 625-634.

Quraishi, S. A., Camargo, C. A., & Manson, J. E. (2016). Low vitamin D status in Europe: moving from evidence to sound public health policies. *Am J Clin Nutr, 103*(4), 957-958.

Ramel, A., Jonsson, P. V., Bjornsson, S., & Thorsdottir, I. (2009). Vitamin D deficiency and nutritional status in elderly hospitalized subjects in Iceland. *Public Health Nutr, 12*(7), 1001-1005.

Reid, D., Toole, B. J., Knox, S., Talwar, D., Harten, J., O'Reilly, D. S., et al. (2011). The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. *Am J Clin Nutr, 93*(5), 1006-1011.

Rejnmark, L., Bislev, L. S., Cashman, K. D., Eiríksdottir, G., Gaksch, M., Grübler, M., et al. (2017). Non-skeletal health effects of vitamin D supplementation: A systematic review on findings from meta-analyses summarizing trial data. *PLoS One, 12*(7), e0180512.

Rosen, C. J. (2011). Clinical practice. Vitamin D insufficiency. *N Engl J Med, 364*(3), 248-254.

Schneider, H. A. (1973). Harry Steenbock (1886-1967)--a biographical sketch. *J Nutr, 103*(9), 1233-1247.

Schöttker, B., Haug, U., Schomburg, L., Köhrle, J., Perna, L., Müller, H., et al. (2013). Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study. *Am J Clin Nutr, 97*(4), 782-793.

Scragg, R., Stewart, A. W., Waayer, D., Lawes, C. M. M., Toop, L., Sluyter, J., et al. (2017). Effect of Monthly High-Dose Vitamin D Supplementation on Cardiovascular Disease in the Vitamin D Assessment Study : A Randomized Clinical Trial. *JAMA Cardiol, 2*(6), 608-616.

Skarphedinsdottir, S. J., Sigurdsson, M. I., Coursin, D. B., Head, D. E., Springman, S. R., Wang, S., et al. (2014). Vitamin D deficiency in anesthesia department caregivers at the end of winter. *Acta Anaesthesiol Scand, 58*(7), 802-806.

Steingrimsdottir, L., Gunnarsson, O., Indridason, O. S., Franzson, L., & Sigurdsson, G. (2005). Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. *JAMA, 294*(18), 2336-2341.

Steingrimsdottir, L., Valgeirsdottir, H., Halldorsson, T. I., Gunnarsdottir, I., Gisladottir, E., Thorgeirsdottir, H., et al. (2014). [National nutrition surveys and dietary changes in Iceland. Economic differences in healthy eating]. *Laeknabladid, 100*(12), 659-664.

Venkatesh, B., Davidson, B., Robinson, K., Pascoe, R., Appleton, C., & Jones, M. (2012). Do random estimations of vitamin D3 and parathyroid hormone reflect the 24-h profile in the critically ill? *Intensive Care Med, 38*(1), 177-179.

Venkatram, S., Chilimuri, S., Adrish, M., Salako, A., Patel, M., & Diaz-Fuentes, G. (2011). Vitamin D deficiency is associated with mortality in the medical intensive care unit. *Crit Care, 15*(6), R292.

Vitamin D deficiency. Symptoms and diseases caused by insufficient vitamin D. (n.d.). Retrieved from https://www.shutterstock.com/image-vector/vitamin-d-deficiency-symptoms-diseases-caused-296039900?src=4mz-S_vZiug1XxgtOEduLQ-1-2.

Wacker, M., & Holick, M. F. (2013). Sunlight and Vitamin D: A global perspective for health. *Dermatoendocrinol, 5*(1), 51-108.

Weng, H., Li, J. G., Mao, Z., & Zeng, X. T. (2017). Randomised trials of vitamin D3 for critically ill patients in adults: systematic review and meta-analysis with trial sequential analysis. *Intensive Care Med, 43*(2), 277-278.

Zittermann, A., Gummert, J. F., & Börgermann, J. (2009). Vitamin D deficiency and mortality. *Curr Opin Clin Nutr Metab Care, 12*(6), 634-639.

Zittermann, A., Iodice, S., Pilz, S., Grant, W. B., Bagnardi, V., & Gandini, S. (2012). Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am J Clin Nutr, 95*(1), 91-100.