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Vitamin D: A magic bullet or a myth?

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Abstract

The interest in Vitamin D (Vit D) is increased after the finding of Vit D receptors in many different cells. This led to the hypothesis that Vit D may have more impact on human health than its role in bone health. Epidemiological studies found associations between low plasma levels of Vit D and the prevalence of many diseases. However, Large RCTs did not find convincing evidence for a positive effect of Vit D supplementation on cancer, cardiovascular disease, auto-immune disease and inflammatory diseases. In this review, the results are described of a literature search regarding the relationship between Vit D status and different diseases.

Pubmed was used to find systematic reviews of observational studies describing the association between Vit D status, diseases (cancer, coronary heart diseases, auto-immune diseases, sepsis) and mortality. Subsequently, a search was performed for RCTs and the results of large RCTs are described. Studies with a positive intervention effect on primary or secondary outcome variables are summarized. No exclusion criteria were used.

The metabolism of Vit D is reviewed, its endogenous production and the intake from food, its activation and transport in the body. The article addresses the effects of diseases on the metabolism of Vit D with special focus on the role of Vit D Binding Protein and its effects on assessing Vit D status. Studies addressing the association between vitamin D status and cancer, cardiovascular diseases, auto-immune diseases, inflammation and severe illness are reviewed. A search for RCTs with positive effects of Vit D supplementation on different diseases yielded only a few studies. The vast majority of RCTs showed no significant positive effects. The presumed high prevalence of Vit D deficiency is questioned based on these results and on altered concentrations of Vit D binding protein, leading to low Vit D levels in plasma but not to low active Vit D levels during disease related inflammation In these conditions, plasma levels of Vit D are therefore not a valid reflection of Vit D status. Reversed causality is described as a possible factor interfering with the correct assessment of the Vit D status. It is concluded that further widespread fortification of foods and stimulation of supplement use should be reconsidered.

Keywords:

Vitamin D, deficiency, metabolism, vitamin D binding protein, supplementation.

1. Introduction

The relationship between Vitamin D (Vit D) and health is studied extensively. A search in Pubmed leads to more than 80.000 hits for "Vitamin D" with a steady annual increase, leading to 4343 articles published in 2018. Despite this large amount of research, the confusion about the effects of Vit D in health and disease has not decreased but seems to be increasing.

Vit D is a fat-soluble vitamin that plays an essential role in calcium and phosphorus homeostasis and bone health. Unlike other vitamins, Vit D levels are not solely dependent on dietary intake. Endogenous production in the skin contributes for a large part to overall Vit D status. Modern lifestyle includes indoor living combined with sun avoidance and use of sunscreen to prevent skin burns and skin cancer and leads to decreased endogenous production of the vitamin. Preventing deficiencies has become more dependent on dietary intake. Only few foods contain high levels of Vit D. Consequently, fortification of nutrients with Vit D is common in many parts of the world. Furthermore, use of oral supplements is advised, especially for elderly and individuals with dark skin.

Results of epidemiological studies and in vitro studies are suggested to indicate that the vitamin might have much broader effects on health, including cancer, cardiovascular diseases, autoimmune diseases and infections. The renewed interest in Vit D has led to a large increase in the number of studies published over the last two decades. However, in this rapidly growing scientific area several uncertainties remain regarding effects, status and function of Vit D.

Although no consensus on the definition of Vit D deficiency is reached yet, generally used cut-off values have recently been increased. As a result, measured prevalence of Vit D deficiencies ranges from 20 to 92% of the world's population, with highest values in the Middle East and Northern Europe (1). From an evolutionary point of view, it seems unlikely that half of the population is currently unable to reach healthy levels of Vit D. On the basis of observational epidemiological studies, these presumably deficient levels are related to an increased risk for development of a number of diseases. However, recent RCT's fail to show the expected positive effects of Vit D in many diseases.

This paper describes factors affecting Vit D metabolism and the difficulty of reliably assessing Vit D deficiency. Factors that may lead to misclassification of Vit D status and the consequences of misclassification for the interpretation of randomized studies or meta-analyses are discussed. Reversed causality will be highlighted as a potential factor interfering with correct assessment of the Vit D status. Finally, the question if further widespread supplementation is needed or if supplementation should be limited to people at risk for deficiencies will be discussed.

2. Metabolism of vitamin D

Vit D is a fat-soluble steroid derivative present in only a few nutritional products. Food of animal origin contains not only more Vit D but also a more active form of the vitamin (D_3 or cholecalciferol). In some fungi and molds, Vit D is present in the form of D_2 (ergocalciferol). Although dietary intake of Vit D is important, exposure to sunlight is the main factor affecting Vit D status, contributing about 80% to the total Vit D level (2). Endogenous production of Vit D in the skin during exposure to Ultraviolet- B radiation in sunlight (UV-B) starts with the synthesis of cholecalciferol from 7-dehydrocholesterol (figure 1). After binding to the vitamin D binding protein (VDBP), cholecalciferol and ergocalciferol are transported to the liver where they are hydroxylated by the enzyme CYP27B1 (a member of the cytochrome P450 enzymes) to 25(OH)D (calcidiol) which can be stored. 85 to 90% of the 25(OH)D in the circulation is tightly bound to the VDBP, 10-15% loosely to albumin and less that 1% is in the free form (3). Only the albumin bound and free 25(OH)D are accessible for hydroxylation yielding the active form. Further hydroxylation to the active form $1,25(OH)_2D$ (calcitriol) is catalyzed by the enzyme CYP27B1 and happens mainly in the kidneys under tight control of Calcium, Phosphate, Parathyroid Hormone (PTH) and fibroblast growth factor 23 (FGI23) (4). PTH stimulates the hydroxylation while FGI23 inhibits it. Other cells, like intestinal, pancreatic, prostatic and immune cells, can also hydroxylate 25(OH)D to the active form 1,25(OH)2D with a wide range of functions. The active form can enter cells and bind to the Vit D receptor (VDR), a nuclear hormone receptor.

There is consensus that the serum or plasma total 25(OH)D concentration should be used to assess Vit D status. It reflects both contribution from diet and synthesis in the skin (5,6). These Vit D assays do not measure the active $1,25(OH)_2D$, but instead measure the total pool of its precursor, total 25(OH)D. Because only 10-16% of total serum 25(OH)D is bio-available (the free and albumin bound fraction), the total 25(OH)D level can vary without changes in the concentration of $1,25(OH)_2D$ which is regulated between strict norms. The half-life of 25(OH)D is 2 to 3 weeks while for the active form this is only one day. Consequently, this indirect measure of the Vit D status gives no information about the concentration of the actually active, hormone-like acting form of Vit D. This complicates the interpretation of study results, especially during diseases when the normal relationship between the non-active and active, the bound and unbound fractions are changed (see later).

Vit D is degraded by 24-hydroxylases (CYP24A1) mainly in the liver, followed by oxidation leading to water soluble metabolites that are excreted in bile and urine (4). CYP24A1 is also present in all cells expressing the Vit D receptor (VDR) (3).

Vitamin D binding protein (VDBP)

VDBP, discovered in 1959, is a member of the albuminoid superfamily produced in the liver (7,8,9,10). Besides the three major phenotypes (DBP1F, DBP1S, DBP2) many variants of this polymorphic protein have been described with differences in affinity for Vit D. Besides binding and transport of Vit D and its metabolites, it has many other functions like binding fatty acids, binding of endotoxins (11) and others. VDBP plays a role in the immune system and is like albumin a constitutive protein. Levels decrease at times of inflammation, including infection, chronic inflammatory disease, after severe trauma or surgery (12,13,14). It also acts as an actin scavenger. During tissue injury and inflammation damaged or lysed cells release actin which needs to be cleared to prevent polymerization that can damage endothelial cells (15). How these functions of VDBP affect its concentration and thereby the Vit D assay during disease is unknown.

A decrease in the VDBP level one day after elective surgery was reported. This was related to decreased levels of total and free 25(OH)D. Levels returned to baseline after 6 weeks without Vit D supplementation (16). The results of this study show that assessing Vit D status immediately after surgery lead to misclassification and superfluous interventions. Fluid shifts after surgery can cause alterations in the distribution volume of the VDBP and thus of Vit D. These changes might also be present during inflammatory states.

Genetic variants of VDBP differ markedly between racial groups. Black Americans have lower total serum Vit D than white Americans but, as their VDBP levels are also lower, bio-available Vit D is similar (17). Many other factors affect VDBP levels. VDBP is lower in elderly women and related to estrogen concentrations. Levels can increase 50% in a high estrogen state and decrease in certain disease states such as hepatic disease (7,8,9,10). Oral contraceptive use and hormone replacement therapy increase hepatic VDBP synthesis and raise serum concentrations (18,19). During pregnancy, levels are also increased due to an estrogen mediated increase in its synthesis (10). VDBP is 16% lower in obese subjects compared with normal weight individuals. Free and bio-available Vit D were also decreased (20). The high prevalence of Vit D deficiency in obese subjects is not related to low bone mineral density and might be caused

by volumetric dilution (fat, serum, muscle) (21). Supplementation of Vit D in obesity has not been shown to be consistently beneficial (22).

The complex relationship between VDBP and total and free 25(OH)D is described by Jassil NK et al (23). Conditions with a decreased VDBP level include nephritic syndrome, end stage liver disease, critical illness, smoking, menopause and cystic fibrosis. In these conditions free 25(OH)D was found to be low or normal. Increased levels of VDBP were reported in acromegaly, pregnancy, use of oral contraceptive pills, psychosis and multiple sclerosis. Free 25(OH)D levels were however low or normal (23). Aspirin therapy in cerebral thrombosis prevention was shown to increase the VDBP concentration by 100% (24).

Knowledge about the effects of different kinds of medication on VDBP, especially in elderly, is not available. Because assays rely on total 25(OH)D for assessing Vit D status while VDBP levels can change in many situations, the proportion of bio-available Vit D is not known. Consequently, widespread misclassification of the Vit D status seems plausible. This is especially the case during the inflammatory response to stressful events (trauma, infection, disease) where constitutive proteins like plasma-albumin levels decrease without exception. The concentration of micronutrients bound to carrier proteins (e.g. Albumin, retinol binding protein and VDBP) will decrease in this situation due to a redistribution of these proteins to the extravascular space (25,26,27). This also happens during life events like pregnancy, lactation, puberty which are also driven by inflammatory stimuli (14). The increase in VDBP during pregnancy, despite belonging to the albuminoid family, is enigmatic and suggests that in pregnancy VDBP acts like an acute phase protein. Alternatively its increased level may be caused by specific oestrogen/progesterone stimulation. Including measures of the presence of inflammatory activity in chronic conditions or of the acute phase response in assessing the Vit D status appears to be necessary to improve our understanding of the actual active Vit D status.

3. Endogenous production and intake

The major contribution to the Vit D status is endogenous production in the skin during exposure to UV-B. In the Netherlands daily sun expose for 15 to 30 minutes with hands and head uncovered between 11 am and 3 pm is advised. People with a dark skin require six-time longer exposure than fair-skin individuals to achieve the same Vit D serum levels (28). In people living near the equator where sun-exposure is high, the level of Vit D is often lower than in places at higher latitude. Not only skin pigmentation, but also low skin exposure for cultural or religious reasons plays a role. Severe Vit D deficiency is most common in the Middle East and South Asia. In these areas the prevalence of rickets is high (1).

Dietary intake contributes only 10 % to the overall Vit D level. According to some authors an indoor lifestyle and sun avoidance greatly contribute to the development of global Vit D deficiency (29). Natural dietary sources of Vit D are salmon, fatty fish, organ (e.g. liver), meat and eggs. In most countries several products such as margarine, milk and cereals, are fortified with Vit D in order to prevent deficiencies. The recommended intake of Vit D is 10 µg/d (400 IU/d). For elderly 20 µg/d (800 IU/d) is advised. In cases of a low sun exposure, dietary intake becomes crucial. In elderly a higher intake is advised because the efficiency of producing cholecalciferol when exposed to UV-B is decreased. Also, their time spent outdoors is limited especially in institutionalized elderly persons. Data reporting dietary intake of Vit D are scarce. A median intake below 5 µg/d (200 IU) in the greater part of the world has been reported (4). Several studies have shown that vegetarians and vegans are particularly at risk to develop Vit D insufficiency and deficiency (30,31). In patients with malabsorption (e.g.cystic fribosis, small bowel syndrome, pancreatic insufficiency) a higher intake of Vit D is needed to prevent a deficiency (32).

In the 1940s fortification of food with Vit D has been widely introduced to prevent rickets in children. Different foods are fortified depending on the local policies. In the US, milk, dairy products, beer and hot dogs are fortified. In Europe fortification of margarines, cereals and bread is common.

4. Norms for Vit D

In Europe the optimal serum level of total 25(OH)D is set at 75 nmol/L. Values below 30 nmol/L are considered as Vit D deficient (33). However, for women over 50 years and men older than 70 years the lower limit is set at 50 nmol/L. Levels above 250 nmol/L are toxic and lead to hypercalcemia with symptoms of vomiting, dehydration, pain and loss of appetite (4). Different cut-offs are used in studies. Based on a lower limit of 50 nmol/L 25(OH)D, global prevalence of Vit D deficiency varies from 20 to 90% (40% in Europe, 24% in the US, 37% in Canada, 90% middle East) (34,4). However, a review on worldwide Vit D status showed a high degree of variability across studies, countries and regions (35).

Currently most methods used for analyzing total 25(OH)D are radio immunoassays, manual immuno assays, automated immune-assays and LC-MS/MS (liquid chromatography-tandem mass spectrometry). Large variations in the assays used complicate standardization of defining Vit D deficiency (33). Bias of the different methods, assessed by measuring standard reference material, is mostly > 15% and in some cases > 30% (5). The low accuracy of most methods complicates accurate measurements of changes in time. Consequently, the number of participants in intervention studies must be large in order to measure differences between groups. Also, in meta-analyses the different methods used in individual studies

lead to results that are not comparable. There is a great need for standardization and optimization of methods. The LC-MS is considered the most accurate method and is currently regarded as the gold standard for measurement (23).

5. Deficiency symptoms

The classical actions of Vit D are promoting calcium homeostasis and bone health. Absorption of calcium in the small intestine, osteoclast differentiation and calcium re-absorption in bone are enhanced. The relationship between vit D status and bone health is usually studied by measuring bone mineral density.

The best known symptoms of Vit D deficiency are rickets and osteomalacia (36). Rickets develops in children when serum calcium and phosphorus are low. A low mineralization of growth plates in bones leads to softening and deformations of bones, development delay or widening of the joints. Also stunted growth, bone pain and muscle spasms are described although stunting may also be caused by chronic or recurrent infectious diseases. Osteomalacia develops in existing bones with closed growth plates due to defective mineralization (36). The global prevalence of reported Vit D deficiency is very high. This is not in agreement with the prevalence of skeletal defects like rickets (in children) and osteomalacia (in adults). Also, from an evolutionary point of view it is hard to accept that most people at a time that food is abundantly available in a greater part of the world, would suffer from vitamin D deficiency. Lack of exposure to UV-B due to modern life style and use of sunscreen are often considered to be the cause of low circulating Vit D levels. Several studies reported a high prevalence of Vit D deficiency in women wearing total skin covering clothing, ranging from 37 to 90 % (37,38,39,40,41,42). Surprisingly, no abnormalities in bone status were found (40). This again raises the question about the accuracy of the currently used norms for Vit D and about the validity of plasma 25(OH)D levels as adequate status parameter.

Vit D deficient rickets is associated with serum total 25(OH)D levels < 12,5 nmol/L (43). In Europe, the prevalence of rickets in Caucasian children is very low but in children with a dark skin the prevalence is high. In Denmark the annual incidence is 2 per 100.000 in the ethnic Danish population and 100 per 100.000 in children from immigrant families (44). In Africa, Middle East and Asia prevalence rates of rickets of 10 to 70% have been reported (44). Causes are lack of sun exposure (clothing of the baby, living indoors) leading to low Vit D levels and chronic calcium deficiency. An additional cause may be that some of these children suffer from severe malnutrition and have very low cholesterol levels which may lead to

a decrease in Vit D synthesis (45). In Bangladesh a rickets prevalence of 1,2% was measured. Of these children 70% had normal total 25(OH)D levels. The main cause was insufficient calcium intake (46). Also in Africa the main cause of rickets was calcium deficiency while low Vit D levels were uncommon (43).

Interest in Vit D increased after the finding that many cells express the Vitamin D receptor (VDR) (bone marrow, brain, colon, breast, pancreas, thyroid, prostate, uterus, immune cells, malignant cells). After binding to the VDR, $1,25(OH)_2D$ exerts an effect on gene transcription. In vitro studies showed that the synthesis of many proteins in different tissues is regulated by the active form of Vit D (47). This finding has led to many hypotheses about the role of Vit D in several diseases, especially cancer, cardiovascular disease (CVD) , diabetes and respiratory diseases (48). In addition, other tissues than the kidneys are able to transform 25OHD to $1,25(OH)_2D$ making Vit D act in an autocrine manner (49). If the correlations between Vit D status and the above-mentioned diseases is based on causality, than the prevalence of these diseases would be much higher in Asia, the Middle East and Africa where the majority of people is Vit D deficient according to the currently used norms. The fact that this is not the case means that either the norms for Vit D deficiency are set too high, or that low Vit D Levels have less impact on health than claimed. Also, disease related changes in binding proteins may cause the correlations between Vit D status and diseases.

6. Public health and vitamin D

After the discovery of the VDR in many tissues, the possible role of Vit D in different diseases has been studied extensively. The role of Vit D in cellular proliferation, differentiation, apoptosis and the innate and adaptive immune system was recognized.

Cell culture studies have revealed that $1,25(OH)_2D$ appears to prevent cancer development or retard its propagation and development of metastases. The many mechanisms involved are cell specific (blocking cell cycle, interfering signaling processes, apoptosis, stimulating DNA damage repair) (3,50). Also, anti-inflammatory and immune regulating properties were found in vitro (51). In view of studies reporting the expression of VDR in several brain structures, Vit D has also been associated with neurological disorders such as multiple sclerosis, stroke, Alzheimer and Parkinson disease (52).

Observational studies have shown an association between low total serum 25(OH)D levels and a variety of non-skeletal disorders such as infectious diseases, diabetes, cardiovascular disease and cancer. In a recent systemic review of 84 observational studies the association between all-cause mortality and Vit D status, it was concluded that most epidemiological observational studies show an inverse relation-

ship between 25(OH)D and all-cause mortality (53). The lowest mortality was found at Vit D levels between 50-75 nmol/L. However, these results may be caused by reverse causality e.g. that a poor health is related to a low sun exposure leading to low Vit D levels (54,55). Also, a decrease in VDBP during inflammatory states, leading to incorrect assessment of the Vit D status, must have affected the results.

To assess whether correlations found in observational studies are based on causality, randomized controlled trials (RCTs) should confirm these findings. However, in a large recent RCT (N=25.871, VITAL study) with supplementation of 2000 IU per day and a follow-up of 5,3 years, no effect of Vit D supplementation on overall mortality was found (56). It can be argued that the power of intervention studies is low due to the fact that participants are not selected based on their Vit D status at the start of the study (57). Targeted interventions in individuals with low Vit D status can increase the power of RCTs. Although the VITAL study did not select participants based on their baseline Vit D Levels, 12,7% of the participants had Vit D levels below 50 nmol/L and 32,2% between 50 and 75 nmol/L. Although the power of this study was high enough, it failed to show an effect on mortality (56).

Cardio Vascular diseases (CVD)

Epidemiological observational studies revealed an association between low serum 25OHD and an increased risk for cancer and CVD. (58,59,60). However, Zhang et al recently published a case cohort study in a large population (N=80000) of postmenopausal women free of CVD at baseline with a follow up of 11 years and found no correlation between levels of Vit D (total, free and bio-available 25(OH)D and CVD risk (61).

Based on results of observational studies, RCTs were started. Some found beneficial effects of Vit D supplementation, but the effects were much smaller than expected (62,63). In a RCT published in 2017 (N=5110, age >50 years) with high dose Vit D supplementation (bolus of 200 000 IU Vit D followed by monthly 100 000 IU) for 3,3 years, no effect was found on cardiovascular events and on cancer outcome (64). A recent large RCT (N=25.5871) on the effect of Vit D supplementation of 2000 IU/d, with a follow up of 5 years, again did not show effects on cardiovascular events (56).

Cancer

In observational studies, a relationship was found between low serum levels of total 25(OH)D and increased risk for cancer (59,60). In a large observational study, total 25(OH)D did correlate with colorectal cancer risk. However, this relationship was not found for VDBP, free 25(OH)D or bio-available 25(OH)D (65). Meta-analyses of observational studies revealed a correlation between low levels of Vit D and

breast, prostate, colon, lung and other cancers (66). Furthermore, high levels of Vit D were related to reduced mortality in cancer patients and tumor prognostic indicators (67). A meta-analysis including colorectal cancer patients, revealed a decreased risk with increasing total 25(OH)D levels up to 100 nmol/L, with optimal levels between 75-100 nmol/L (68). A meta-analysis of observational studies showed a significant reverse correlation between total 25(OH)D and cancer mortality (69). Higher total 25(OH)D concentration was associated with better cancer outcome and overall survival (hazard ratio (HR=0.74, 95% CI: 0.66-0.82). However, these studies might also suffer from reverse causality. The type and phase of cancer will affect VDBP levels. A decrease in VDBP results in a decrease of measured total 25(OH)D, but gives no information about the concentration of active Vit D. Furthermore, the overall condition of patients might affect their time spend outside, limiting their UVB-B exposure.

In RCTs some beneficial effects of Vit D supplementation were reported, but the effects were much smaller than expected (70,71). In postmenopausal women supplementation of Vit D and Ca for 4 years did not result in altered cancer risks of all types. Post hoc analysis however revealed an inverse relationship between serum levels of Vit D and cancer incidence (72). The relationship between Vit D and breast cancer was studied by pooling data of two RCTs and in a prospective cohort study (73). A dose response decrease in breast cancer risk was found. Concentrations > 150 nmol/L were reported to be most protective for breast cancer risk. However, because only few women have such high Vit D concentrations the confidence limits for the calculated hazard ratio are very wide, limiting the extrapolation of this finding. A review of the effects of Vit D supplementation on cancer mortality reported a reduced risk (RR=0,88) for D3 supplementation (74). However, this analysis was based on data from only four studies with the largest contribution (60,1%) of data from a study in postmenopausal women, limiting extrapolation of these results (75). In a recent large RCT (N=255871), Vit D supplementation of 2000 IU/d was combined with n-3 fatty acid 1,0 g/d with a follow up of 5 years (56). The study did not show any effect on the incidence of invasive cancers. Because of the lack of effects in intervention studies, a causal relationship between Vit D status and cancer or CVD seems unlikely.

7. Vitamin D and immunology

Almost all immune cells express the VDR, indicating that Vit D can exert effects on their metabolism. Furthermore, various immune cells (monocytes, dendritic cells, macrophages, B cells and T cells) have the capacity to convert 25(OH)D into the active $1,25(OH)_2D$. This allows local regulation of the active form at the site of inflammation (76,77).

The active 1,25(OH)$_2$D binds to the VDR and this complex is translocated to the cell nucleus where it can influence the expression of hundreds of genes, including genes involved in cytokine production (78). Also, the complex induces the expression of antimicrobial proteins (β-defensin or cathelicidin), enhancing innate immunity (79,80,81,82). Human cathelicidin (LL-37) and beta defensins are antimicrobial peptides (AMPs) of the innate immune system. AMPs protect against bacterial invasion, LL-37 promotes wound healing and reduces inflammation (83). In vitro studies showed a role of Vit D in enhancing mechanisms of pathogen elimination (84,85). Several studies suggest that Vit D also plays a role in the defense against viral infections (85,86,87).

In observational studies, the relationship between Vit D deficiency and influenza infections is described. The risk for RTI (respiratory tract infection) was found to be associated with Vit D levels in both a large cross-sectional study in British adults (88) and in the large American NHANES study (89). These findings do not prove causality and may again suffer from reversed causality. Intervention studies with Vit D supplementation did show contradictory results regarding the prevention of RTIs. A meta-analysis revealed that the number of people needed to treat with Vit D supplementation to reduce the risk of experiencing at least one RTI was 34. The protective effect was greatest in subjects with low Vit D levels (90). One RCT in children showed a decrease in the incidence of influenza A, but not in influenza B, in the group that received Vit D supplements for 15 days (table 1) (91). Unfortunately, plasma Vit D levels were not measured in this study.

A positive correlation between lung function and Vit D levels in asthma patients has been described in observational studies (92). However, results of intervention studies are controversial. Several meta-analyses have been published, some show no effects of Vit D supplementation (93) while others show modest (94,95) or low quality evidence (96,97) of a positive effect. The most recent meta-analysis showed only a positive effects in a subgroups. A reduction in the rate of asthma exacerbation was only found in patients with Vit D levels below 75 nmol/L (98).

Autoimmune diseases

Vit D deficiency has been linked to autoimmune diseases (Multiple Sclerosis (MS), Rheumatoid Arthritis (RA), Diabetes Mellitus type I (DMI), Inflammatory Bowel Disease) (99,100,101). Because VDR is expressed in immune cells (B cells, T cells, macrophages) Vit D may modulate the innate and adaptive immune response (102). Vit D was shown to decrease in vitro the production of inflammatory cytokines

(IL-17, IL-21) and to increase the production of anti-inflammatory cytokines such as IL-10. Also, it inhibits monocyte production of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12 and TNFα (103).

Epidemiological observational studies showed a correlation between many immune-related diseases such as asthma, atherosclerosis, diabetes and autoimmune diseases and low Vit D levels (104,105). Based on these findings the use of Vit D to decrease symptoms was studied. Clinical trials are reviewed by Dankers W et al (106): In MS, results are contradictory, for RA no definite conclusion could be drawn, for Crohn's disease (CD) a beneficial effect was found (table 1) but not for DM1. Also, a meta-analysis of the effects of Vit D supplementation in DM2 showed no effect of Vit D on fasting blood glucose, HbA1c and fasting insulin levels (107). In a recent RCT (N=2423, follow-up of 2,5 years) daily supplementation of 4000 IU D_3 in adults at high risk for DM2 did not result in a significantly lower risk of diabetes than placebo (108).

8. Inflammation and severe illness

Low levels of Vit D are common in critical illness. Some observational studies showed an association between low Vit D levels and unfavorable outcome during critical care (109,110), while others did not find this association (111,112,113). Meta-analyses also yielded conflicting results. Upala et al (114) published a systemic review and meta-analysis of observational studies (N=10) of the relationship between Vit D deficiency (defined as 25(OH)D< 50 nmol/L) assessed before or during admission and sepsis. Patients with Vit D deficiency had higher odds for sepsis compared to patients without deficiency (OR 1,78). However, no significant difference in 25(OH)D levels was found between patients who had and did not have sepsis. This finding does not support a strong causal relationship between Vit D status and sepsis. A meta-analysis of 24 studies of the association between Vit D status and sepsis showed that sepsis cases had significant lower Vit D levels than non-septic patients. The Vit D status was not correlated with Albumin, PLT, IL-6, CRP levels or mortality. Sepsis death was not associated with Vit D deficiency. It was concluded that Vit D had no impact on biochemical indexes and prognosis of sepsis (110). In a study with 461 patients with suspected sepsis, Vit D and sepsis biomarkers were monitored. No relation could be found between 25(OH)D or 1,25(OH)2D with sepsis and mortality. The authors stated that their data support the hypothesis that reduced levels of Vit D are rather a marker of systemic inflammation than a marker of severe infection (111). Vitamin D levels had no predictive value for assessment of the likelihood to progress to sepsis and mortality.

Recently a prospective observational study in septic shock patients (N=75) was published. Levels of VDBP, 25OHD and $1,25(OH)_2D$ were measured on admission. Only VDBP was associated with in-hospital mor-

tality. No relationship of 25OHD and 1,25$(OH)_2$D or levels of cathelicidin and beta- defensin with mortality were found (115). The authors state that VDBP could be used as a prognostic marker in these patients. Others described a reduction in VDBP of 35% during first 24 hours of critical illness (116,117). In critical illness, increased vascular permeability is universally occurring in these conditions, leading to leakage of proteins to the extra vascular space and its expansion. Like Albumin, VDBP levels in blood are likely to decrease in this situation. How this affects the availability of 25(OH)D for activation and the level of 1,25$(OH)_2$D is unclear. Most studies measure total serum 25(OH)D of which usually 85% is strongly bound to VDBP and not immediately bio-available. The relationship between changes in VDBP and the level of active 1,25$(OH)_2$D is usually not assessed.

In view of the role of Vit D in the innate and adaptive immune response, restoring Vit D levels has been proposed in order to improve the patient's condition and reduce mortality. It is hypothesized that Vit D has a role in the regulation of the inflammatory responses against infection (118,82). The mechanism involves upregulation of the antimicrobial peptide cathelicidin (LL-37). Vit D supplementation in critically ill patients was shown to prevent excessive production of pro-inflammatory cytokines (IL-6) and CRP. It also increased the production of anti-inflammatory mediators (IL10) and increases the antibacterial capacity of macrophages, leukocytes migration, local inflammation and innate responses against bacteria (119, 120). However, although increasing total Vit D level can be achieved, no convincing significant improvement in hospital stay or mortality has been described.

RCTs evaluating effect of Vit D supplementation in patients with sepsis did not demonstrate a difference in clinical outcome (121,122). Amrein et al studied the effects of Vit D supplementation in 475 critically ill patients and did not find beneficial effects on mortality and LOS (123). They noticed a low response to D3 supplementation on increasing 25(OH)D levels and discussed that a compromised hepatic cytochrome 450 system that is implicated in the 25-hydroxylation of Vit D, can be responsible. In their study, large dosages of Vit D were administered by nasogastric tube (see table 1), while 1,25$(OH)_2$D was also monitored. Only on day 7, levels were different between groups (22,3 pg/ml in intervention and 9,42 pg/ml in control) and after 6 months levels were increased to 36 pg/ml in both groups. These results indicate that during severe illness metabolic changes are responsible for the decrease in Vit D status and that supplementation has at best a marginal effect on the concentration of the active form of the vitamin. Acute host response leads to a decrease of proteins of the extracellular actin scavenger system. Circulating levels of Gc-Globulin decrease shortly after severe trauma. In patients, who develop organ dysfunction and sepsis, this decrease is more pronounced (124). The same holds true for VDBP leading to misclassification of Vit D status during inflammatory states. The same mechanisms seem to be responsible for the assessment of vitamin A status. Data obtained in the NHANES study showed that an acute phase

response (CRP>10mg/L) was associated with lower serum retinol levels, leading to misclassification of Vitamin A status (125). Studies in infants showed that increases in CRP were correlated with decreases of Vitamin A levels leading to overestimation of vitamin A deficiency (25). This decrease in serum retinol as a consequence of the acute phase response should be differentiated from nutritional deficiency. In fact, Retinol Binding Protein (RBP) is decreased in critically ill patients and this is a general response in critical illness independent of the origin of the disease (126). The authors hypothesize that the acute decrease in its concentration may be explained by reduced synthesis (liver) or increased removal by extravasation due to capillary leakage or increased metabolic clearance. If the metabolism of RBP in critically illness follows the same order of events as albumin, increased capillary escape and increased extracellular fluid volume may be responsible for the decrease in serum RBP concentration. In addition accelerated break-down (shortened half life) may be an additional factor decreasing the total RBP pool and contributing to its decreased serum concentrations. The same mechanism may be responsible for the decrease in serum VDBP. Duncan et al. studied the plasma concentrations of trace elements and vitamins during different degrees of inflammation and concluded that a reliable clinical interpretation of Vit D and Vit A can be made only if the CRP is below 10 mg/L (127).

9. Positive interventions (table 1)

In an attempt to assess the impact of Vit D supplementation on health, a search for published RCTs with positive outcomes of different diseases was performed. Studies referred to in reviews and meta- analyses were studied and Pubmed was used to search for RCTs on Vit D supplementation and a variety of diseases (cancer, diabetes, CVD, auto-immune diseases, infectious diseases). Studies describing positive effect in primary, or secondary outcome variables or in post hoc analyses were included. This search is possibly not complete, but in face of the large amount of studies on Vit D, it can be argued that if a positive effect is evident, the list would and should be large. However, most studies did not yield statistically significant effects. Studies with a positive outcome are summarized in table 1 (83,128,91,72,123,129). Results were predominantly negative. In most studies, primary outcomes were not significantly different between the control group and the intervention group. Only two studies showed a positive effect, one addressing the protective effect of Vit D on the incidence of influenza A in children (91) and one study addressing the development of MS in patients with optic neuritis (129). Post hoc analysis of the results of some studies showed positive effects. However, it can be argued that these analyses mainly show that people with low levels of Vit D suffer from a mild to severe inflammatory state, leading to decreased total serum 25(OH)D levels due to a decrease of their binding protein VDBP. Overall,

the therapeutic effects of Vit D are non-existent or very modest, because in the vast majority of RCT's and meta-analyses no significant effects have been shown.

10. Discussion

The metabolism of Vit D is complex. Besides sun exposure and dietary intake, many other factors affect plasma levels. The liver and kidney play an essential role in the activation of the vitamin. During disease these functions often are impaired. The fact that the body can synthesize Vit D and the presence of VDR in many different cells, with the in vitro evidence that the active form of Vit D is involved in many metabolic pathways, the vitamin appears to act more like a hormone than as a vitamin.

Defining Vit D deficiency is currently based on the level of total 25(OH)D in blood of which 85% is not available for hydroxylation. Because in many clinical disease conditions the levels of VDBP decrease, assessing also the free and bound levels of 25(OH)D should give more insight into the actual availability of the vitamin. With the currently used method, a decrease in VDBP will inevitably classify a patient as Vit D deficient while the levels of available 25(OH)D and of the active 1,25(OH)2D may be within normal ranges. Misclassification of the Vit D status might explain the overall lack of positive intervention effects. Furthermore, in order to compare results of studies, laboratories should use the same validated assays. Besides the technical aspects of defining deficiency, seasonal changes are responsible for fluctuations in blood levels of Vit D due to variations in endogenous production. In many studies this is not taken into account.

According to current norms for Vit D plasma levels, there would be a global deficiency. However, serious deficiency symptoms like rickets are only found in children from immigrants with a dark skin living at high latitudes. Rickets in children living in places with an abundance of sunshine are often caused by calcium deficiency (43,46). Complete avoidance of sun exposure may lead to true vit D deficiency. Global deficiency implies that supplementation of Vit D, as advised by health authorities, would improve overall health. However, the correlations between a low Vit D status and many diseases appear not to be based on causality as intervention studies fail to show beneficial effects. Rather, reversed causality largely explains the relationship between low levels of Vit D and overall health.

Because the costs of global Vit D supplementation and the risks for adverse effects are low, it may be argued that even small positive effects would warrant treatment and might be beneficial to overall health. However, in large RCTs these positive effects are not found. Even when there would be a modest benefit, further investigation may be preferred to identify the small group that benefits and why, and to

restrict vitamin D supplementation to the group that is truly at risk to develop Vit D deficiency. In addition, more basic information regarding the effects of disease and use of medication on the synthesis and activation of Vit D is needed for a better understanding. Also, the interaction between Vit D and cholesterol, its precursor, and steroid hormones has not been studied intensively.

As Vit D does not seem to be a magic bullet, further widespread fortification of foods and stimulation of supplements use should be reconsidered. If the reported prevalence of vit D deficiency is indeed significantly overestimated, the question arises whether the prophylactic administration of vit D (and A) should be limited to populations that are truly at risk to develop clear physical symptoms of Vit D deficiency. These populations are severely malnourished children with hypocholesterolemia in areas with endemic malnutrition, people with dark skin living at high latitudes with little exposure to UV-B, religious groups, covering their skin continuously, elderly living mainly indoors and individuals suffering from malnutrition or renal and hepatic failure. Clear lack of calcium intake or increased calcium losses should be taken into account as causes of osteomalacia or rickets.

In clinical practice the assessment of Vit D status is especially indicated in patients with symptoms of malabsorption, renal failure, hepatic failure, patients with low bone mineral density , patients prone to malnutrition and patients who lack sun exposure. Measurements should include longitudinal data during different seasons and should include not only total 25(OH)D but also free and bound levels of the vitamin.

11. Conclusion

The high incidence of low vit D levels in disease related inflammatory states is predominantly based on reversed causality. Inflammation causes a decrease in total plasma Vit D due to a decrease of its binding protein, but active Vit D does not change.

In practice the assessment of Vit D status is especially indicated in patients with symptoms of malabsorption, renal failure, hepatic failure, patients with low bone mineral density, patients prone to malnutrition and patients who lack sun exposure. Measurements should include longitudinal data during different seasons and should include not only total 25(OH)D but also free and bound levels of the vitamin. A deficient Calcium intake should be considered in individuals with osteomalacia or rickets. Widespread fortification of food with Vit D and use of supplements should be reconsidered.

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915 **Legends**

916

917 Figure 1. Chemical structures of the Vit D metabolites.

918

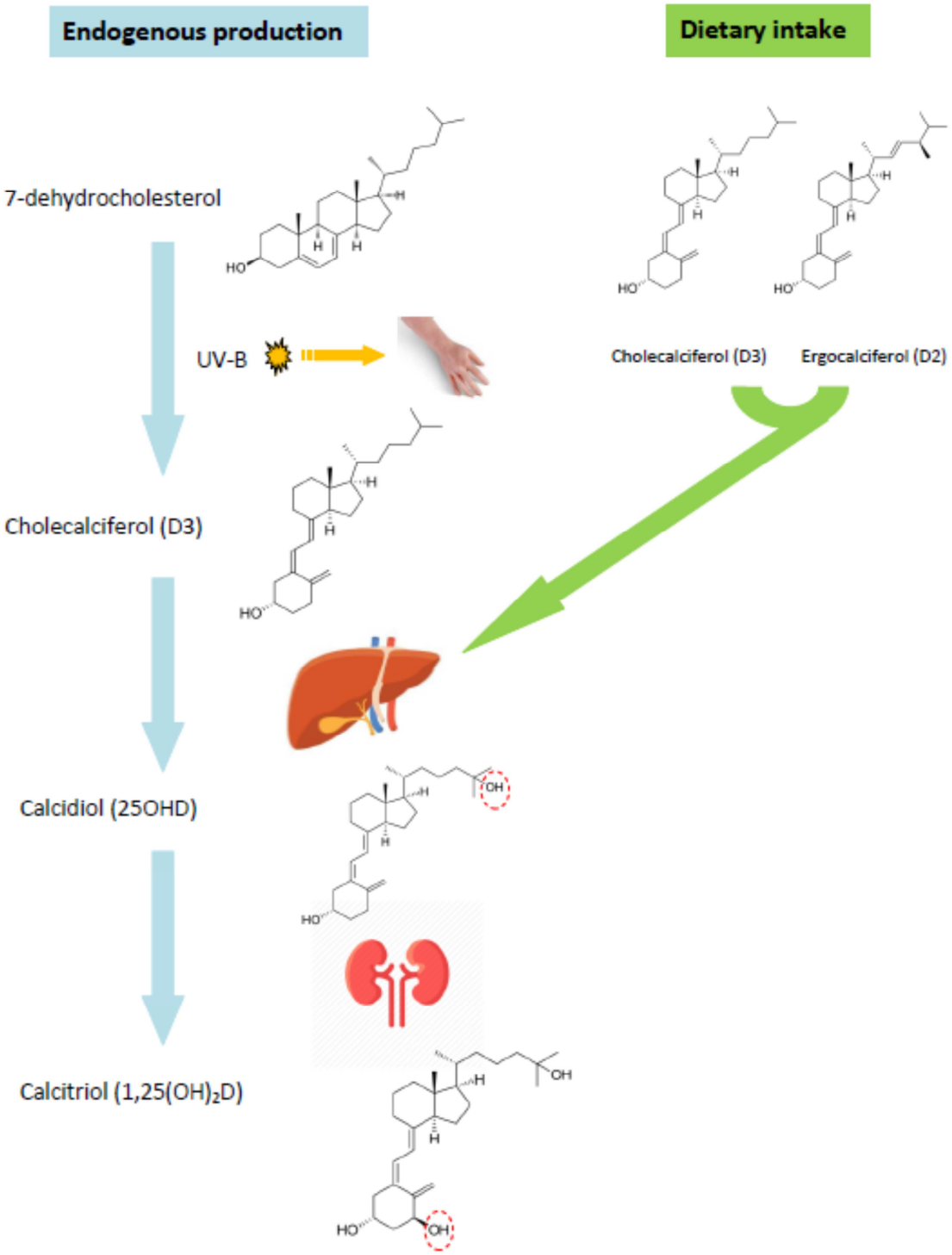
919 Table 1. Randomized controlled trials with positive intervention effects of Vit D supplementation.

920

Study	Population	Number	Mean age	Baseline 25(OH)D (nmol/L)	Intervention	Follow-up duration	25(OH)D (nmol/L) After intervention	Assay used	Primary outcome	Secondary Outcome	Post Hoc analysis
Raftery 2015 (83)	Crohn's disease in remission	27	37	VD group: 51,8 Control :69,2	2000 IU/d 3 months	3 months	VD group: 91,6* Control :40,4	Liquid chromatography-tandem mass spectroscopy	Intestinal permeability: No effects	Disease markers: No effects	LL-37 levels increased only in VD group (no diff between groups). In Pt with 25(OH)D>75 nmol/L higher LL-37, higher QoL, lower CRP compared to Pt with 25(OH)D<75 nmol/L
Soilu-Hänninen 2012 (128) (funded by Bayer)	MS patients	66	37	VD group: 54 Control :56	20,000 IU weekly 12 months	12 months	VD group: 110* Control :50	RIA kit	MRI T2 Burden of disease No effects % of patients with 25(OH)D>85 nmol/L: VD group 85%, C:ontrol:3 %	EDSS changes No effects Relapse time Time to first relapse: No effects T1 Gd enhancing lesions: decreased more in VD groep*	
Urashima 2010 (91)	Healthy children	430	10	Not assessed	1200 IU/d 15 days	4 months	Not assessed	-	Incidence of influenza A VD group 10,8%, control 18,6% RR=0,58*	Incidence of influenza B No effects	Incidence of asthma in children diagnosed with asthma (N=241) VD group: 2 Control : 12 RR=0,17*
Lappe J 2017 (72)	Healthy postmenopausal women	2303	65	81,9	2000 IU VD/d+ 1500 mg Ca/d	4 years	VD group: 109,6 Control :78,9	Liaison Analyzer (Diasorin)	Cancer incidence (all types). No effects	Hypertension, CVD, osteoarthritis, colonic adenomas, diabetes, resp	Exclusion of participants who withdrew, died, developed cancer before start of study (N=162).

										tract infections No effects	Difference in cancer incidence: HR 0,65*
Amrein 2014 (123)	Critically ill patients (ICU)	475	65	VD group:32,4 Control :32,7	Bolus of 540,000 IU and monthly 90,000 IU for 5 months	6 months	Day 7 VD group: 88,6 Control :36,2 Day 28 VD group: 81,6 Control :43,2	Chemiluminescence technology (IDS-iSYS)	Hospital LoS No effects	Length of ICU stay, hospital mortality, No effects	Subgroup analysis in pt with 25(OH)D <30 nmol/L(N=200): LOS no effect . Lower mortality in VD group (28,6% vs 46%) 6 months mortality no effects
Derakhshaudi H 2013 (129)	Patients with optic neuritis with serum 25(OH)D< 75nmol/L	24	25	VD group:34,2 Control :41,0	50,000 IU/week until serum levels are 250 nmol/l	12 months	Not available	Radio-immunoassay kit	Optic neuritis conversion rate to MS: VD group:0 Control: 5 partients RR=0,316*	Brain MRI lesions no effects	Relation between serum 25(OH)D and number of MRI plaques: no effects.

* Statistically significant (P<0,05).


Endogenous production
Dietary intake
7-dehydrocholesterol
UV-B
Cholecalciferol (D3)
Ergocalciferol (D2)
Cholecalciferol (D3)
Calcidiol (25OHD)
Calcitriol (1,25(OH)2D)

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.