

MISE AU POINT / IN-DEPTH REVIEW

THE EXTRASKELETAL ACTIONS OF VITAMIN D • MYTHS AND FACTS

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ABSTRACT • Vitamin D is well known for its effects on bone metabolism, calcium and phosphorus homeostasis. Recently, a worldwide focus has been made on the extraskeletal effects of this liposoluble vitamin. The *New York Times* even called it the “wonder drug”. Vitamin D is a liposoluble vitamin and acts via an intracellular receptor. This article reviews the extraskeletal effects of vitamin D focusing on cardiovascular effects, regulation of glucose, antitumoral properties, and effect on the immune system and the skeletal muscle and attempts to offer possible molecular explanations to these effects.

Keywords: vitamin D, extraskeletal effects

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RÉSUMÉ • La vitamine D est une vitamine bien connue pour son effet sur le métabolisme osseux ainsi que sur l'homéostasie du calcium et du phosphore. Récemment, le monde scientifique se concentre sur les effets extrasquelettiques de cette vitamine liposoluble. Le *New York Times* est allé jusqu'à la nommer « le médicament merveilleux ». La vitamine D agit via son récepteur intracellulaire. Cet article passe en revue les effets extrasquelettiques de la vitamine D en détaillant ses effets sur les systèmes cardiovasculaire, immunitaire, endocrinien (diabète) ainsi que l'effet antitumoral et sur le muscle lisse et tente de les expliquer au niveau moléculaire.

INTRODUCTION

Called the “sunshine vitamin” [1], vitamin D (VitD) is one of the essential liposoluble vitamins of the human body. Between the 4th quarter of 2008 and the 4th quarter of 2009, the orders of tests of VitD levels have surged by more than 50% and consumers have bought 235 million dollars' worth of VitD supplement compared to 40 million dollars in 2001.

In the recent years, there has been an enormous change in the understanding of the health benefits of VitD [2]. VitD is well-known for its skeletal effects (bone mineralization, calcium and phosphorous homeostasis, as well as parathyroid hormone regulation). In 2009, the *New York Times* mentioned the less known extraskeletal effects of this vitamin. Since, more than 200 studies including more than 20 meta-analysis have been published about this topic.

Optimal VitD levels are still debated [3]. There is a consensus that a value less than 20 ng/ml is equivalent to deficiency and a value of more than 150 ng/ml to intoxication. A recent expert opinion group defined a value between 20-30 as insufficiency and the optimal goal for supplementation to be around 50 [3-6].

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VITAMIN D METABOLISM (Figure 1)

As mentioned before, VitD is a one of the four liposoluble vitamins. Hence, there is two possible sources : endogenous (synthesis from the precursor 7-dehydrocholesterol under the influence of sunlight and UV light) and exogenous (fish, green vegetables, etc.) [7-8]. The first step of metabolism starts in the liver with the first hydroxylation in the liver with the resultant 25-OH-VitD. This product undergoes another hydroxylation, which is the most regulated step in VitD metabolism (Ca, PTH, 1-25 VitD, etc.) by the 1 α -hydroxylase to yield the active form vitamin D: 1-25 OH-VitD. This form acts on target organs as explained later and undergoes side chain cleavage, oxidation and 24-hydroxylation to give the inactive metabolite: calcitric acid [7-8].

As a result of its liposolubility, VitD acts through an intracellular receptor. The VitD receptor (VDR) is part of the retinoic acid receptor family. It acts as binding to the DNA sequence and initiates transcription of genes and production of target proteins [7].

Recent data suggest that VDR is ubiquitous and is found in almost every tissue in human body (parathyroid gland, thyroid, pituitary, adrenal, brain neurons, alveolar cells, cardiac muscle, esophagus, stomach, hepatocytes, small and large intestine, kidney, urethra, prostate, testis, ovary, uterus, placenta, osteoblasts, osteocytes, chondrocytes, fibroblasts, striated muscle, skin, hair follicles, breast, thymus, T-cells, B-cells, macrophages) [9]. Moreover, studies have also showed an extra renal location of the 1 α -hydroxylase enzyme in the hair follicles, the colon, skin, lymph nodes, pancreas, placenta, etc. [9].

Thus effects of VitD are not limited to skeletal effects and this article will focus on cardiac, endocrinologic, immunologic and antitumoral effects.

CARDIOVASCULAR EFFECTS

Data suggest that VDR-deficient mice are more prone to hypertension, myocardial hypertrophy as well as increased thrombogenicity. Even more, selective VDR deletion in cardiomyocytes leads to cardiac hypertrophy [4]. These findings underline the capital effect of VitD in cardiovascular homeostasis.

1. Cardiovascular disease

A recent study published in the *American Journal of Cardiology* showed that VitD deficiency was found to cause more mortality from cardiovascular disease. Moreover, VitD supplementation to VitD deficient people was showed to decrease this risk. VitD was found to be a risk factor for death (OR = 2.95), coronary artery disease (OR = 1.16), cardiomyopathy (OR = 1.29) and hypertension (OR = 1.4) [10]. These findings were also stressed by the Intermountain Heart Collaborative Study with more than 40,000 participants. This study showed that VitD < 15 ng/ml compared to > 30 ng/ml was associated with significant increases in the hypertension, hyperlipidemia, and peripheral vascular disease, myocardial

infarction, heart failure, coronary artery disease, and stroke ($p < 0.0001$), stressing the increased incidence of death ($p < 0.0001$) [11]. A recent meta-analysis in *Heart* (journal of the British Heart Association) confirmed these results with the increase of incidence of cardiovascular events (HR = 1.54 [95% CI 1.22 to 1.95]) and of mortality (HR = 1.83 [95% CI 1.19 to 2.80]) [12]. In fact, a possible explanation to the protective effects of normal VitD is shown in Figure 2 and includes decreased proliferation of cardiac muscle and increased contractility which increases cardiac function, decrease thrombogenicity and increase fibrinolysis [12]. Another effect of VitD is elevation of phosphorous. Phosphorous elevation promotes vascular calcification as was shown in an in vitro study [13]. Thus, VitD excess could promote vascular calcification and increase cardiovascular morbidity and mortality. Thus, VitD should be in a safe zone promoting vascular protection while not causing deleterious effects.

2. Vitamin D and obesity

Another contributing factor for this morbidity is obesity. Obese people were found to have a lower serum VitD [14-16]. Possible explanations to this fact are the reduced sun exposure in obese people (less exercising time) as well as more VitD sequestration in adipose tissue (Figure 3). Moreover, seasonal VitD variations are

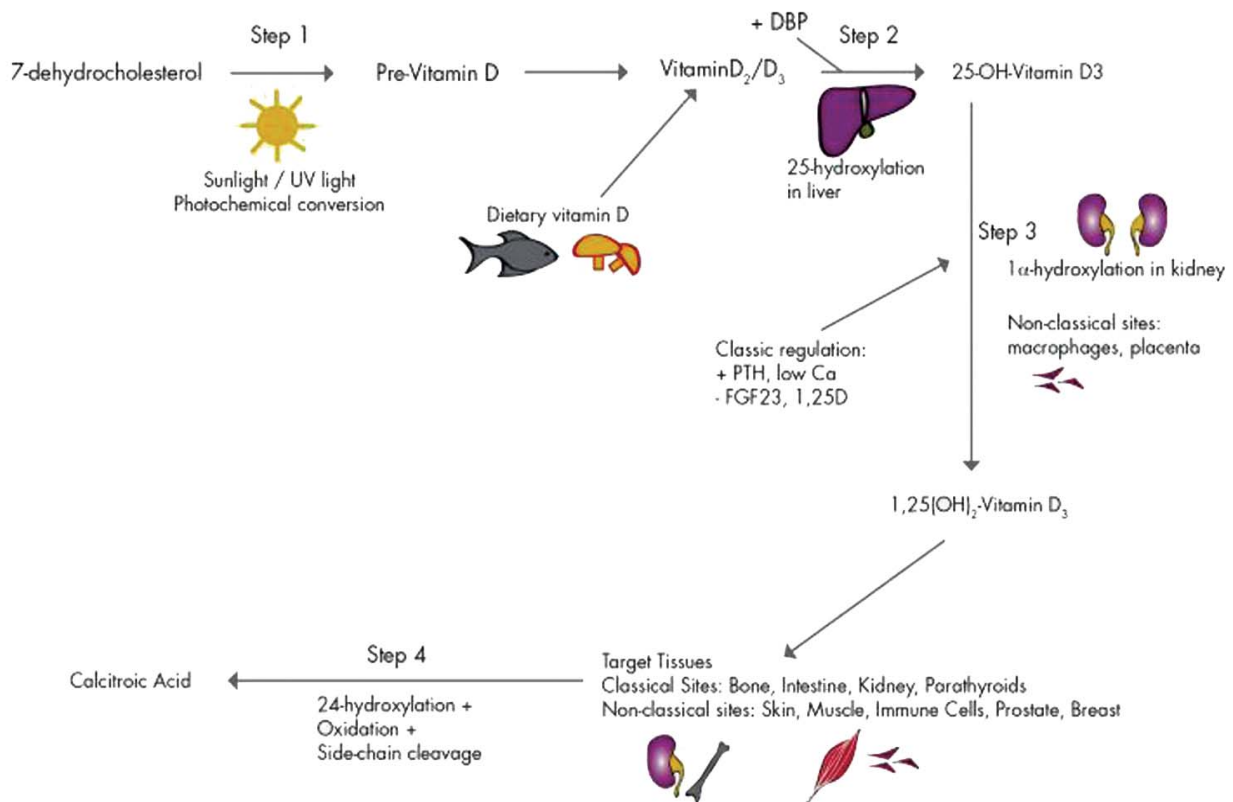


FIGURE 1. Metabolism of Vitamin D (Adapted from Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE (2013). *The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocrine Reviews* 34: 33-83.)

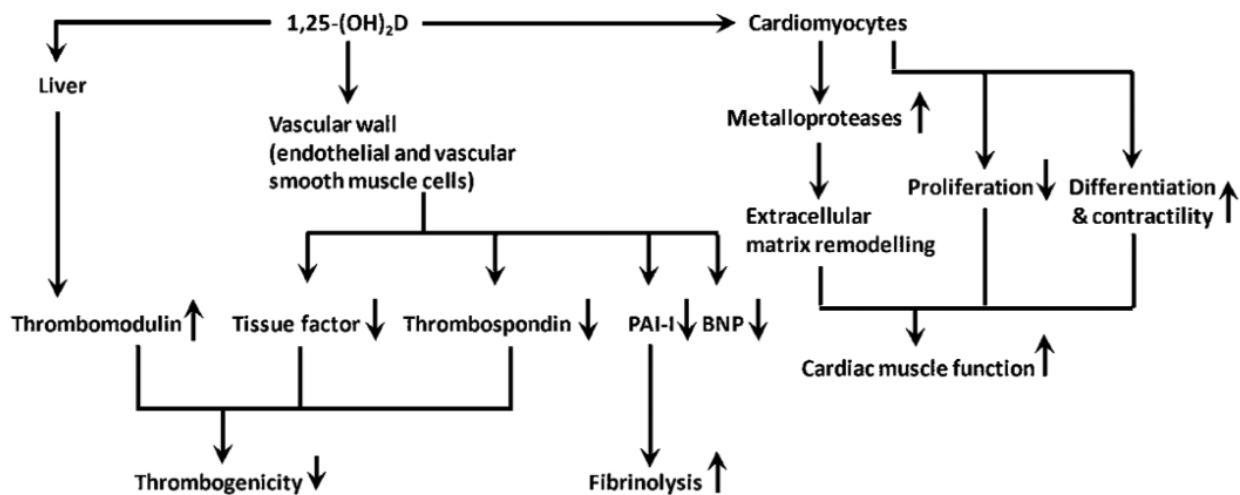


FIGURE 2. Action of vitamin D on the cardiovascular system. (Adapted from Reid IR, Bolland MJ (2012). Role of vitamin D deficiency in cardiovascular disease. *Heart (British Cardiac Society)* 98: 609-14.) PAI-1: plasminogen activator inhibitor 1 BNP: brain natriuretic peptide

not seen in the obese and this deficiency is associated with increased markers of diabetes and hypertension [17]. Metabolic syndrome was found to be more prevalent in VitD deficient morbidly obese patients [18].

3. Vitamin D and hypertension

The effect of VitD on hypertension was mentioned above. VitD was shown to protect against the incidence and morbidity of hypertension [4, 6, 10-11, 13-14, 19]. A recent meta-analysis showed an odds ratio of hypertension of 0.73 [0.63-0.84] for the highest versus the lowest category of blood VitD concentration and concluded that VitD concentration is inversely associated with hypertension [20]. Moreover, reduction of blood pressure seems to affect significantly the systolic but not the diastolic pressure [21].

As mentioned above, VitD was shown to be protective against stroke incidence [4, 10-11, 19]. A recent study in *Stroke* showed, in more than 1200 stroke cases, a higher incidence of stroke in patients with VitD deficiency compared to normal patients with a pooled relative risk of 52% [22].

Even though recent data suggest a beneficial effect of VitD on cardiovascular morbidity, a meta-analysis by El Amin in 2011 found no significant protective effect of VitD on mortality, myocardial infarction and stroke [23]. Critics to this meta-analysis were the inclusion of old moderate quality papers causing great heterogeneity among these studies. Thus, conclusions drawn from this study should be analyzed carefully and compared to other results in literature [19, 24]. This justifies the amount of future research to explain these effects [25].

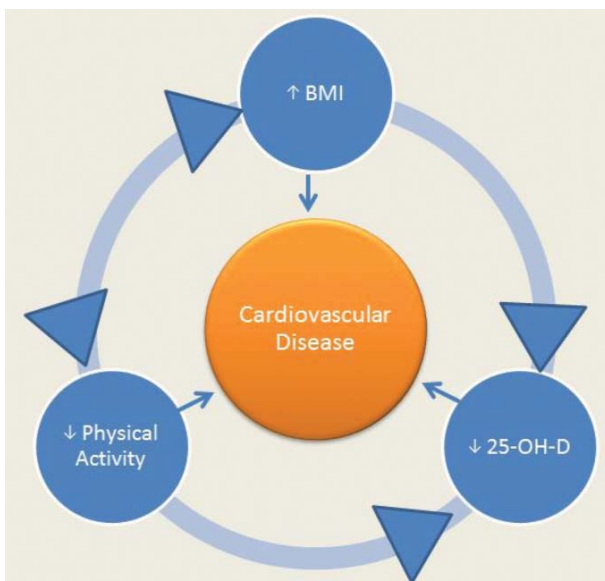


FIGURE 3. Possible relation of Vitamin D, obesity and cardiovascular disease.

VITAMIN D AND DIABETES

Multiple studies have shown the presence of VDR as well as the 1α -hydroxylase enzyme in the pancreatic islet cells. This presence would suggest an effect of this vitamin on glucose metabolism and regulation. A recent study showed that early VitD supplementation to young children is capable of reducing the incidence of diabetes type I in infants [26]. A more recent meta-analysis showed that VitD intake in infancy decreases the rate of developing type I diabetes later in life with an odds ratio of 0.71 [27].

Type II diabetes was shown also to be related to VitD concentration and metabolism. Several preclinical studies have shown the presence of VDR in the insulin gene promoter [28], an up-regulation of calcium influx in the islet cells (increasing insulin synthesis), as well as an increase of insulin receptor expression (and decrease of insulin resistance) [29]. Since then, several clinical studies have associated VitD deficiency with increased type II diabetes incidence while supplementation with

VitD was shown to be beneficial [6, 13, 30-31]. A recent meta-analysis showed that VitD deficiency was associated with a 43% increase of diabetes incidence and a 62% increase of progression from prediabetes to diabetes [32-33]. Thus many authors propose that VitD supplementation should be evaluated for the prevention of type 2 diabetes in prediabetic individuals [33]. Nonetheless, a recent study published in *JAMA* stated that there is still insufficient evidence from current data [14, 34]. Possible explanation to this fact is the presence of many confounding factors like obesity and behavioral factors and the lack of large scale randomized controlled trials.

VITAMIN D AND CANCER

Ultraviolet radiation exposure is a well-known risk factor for developing melanoma, but has also an inverse correlation with 15 types of cancer: bladder, breast, cervical, colon, endometrial, esophageal, gastric, lung, ovarian, pancreatic, rectal, renal, vulvar cancer, Hodgkin's and non-Hodgkin's lymphoma [6, 13].

In a study in 2004, colorectal cancer incidence was found to be inversely correlated to VitD concentration with a 60% decrease in the NHANES III study that included more than 16 000 patients [35]. The same study showed a trend for reduction of breast cancer incidence of more than 50% with a VitD level > 50 ng/ml. In a

recent interventional double-blind randomized placebo controlled study, and over a four-year duration, VitD supplementation was shown to reduce the relative risk of cancer to more than 60% with both serum VitD and supplementation independent predictors of cancer development [36].

Possible explanation for this protective effects of VitD is the presence of VDR in the tumor repressor promoter genes and VitD-VDR complex could recruit co-repressor complexes with DNA methyltransferase activity to gene promoters which will suppress gene transcription [37]. VitD inhibits cell proliferation and growth, angiogenesis and proinflammatory cytokines (and oxidative stress) on one hand while stimulating the apoptosis cascade, autophagy and the innate immune response thus exerting an antitumoral effect (Figure 4) [37].

Despite this biologic evidence and the worldwide enthusiasm, a recent study by Manson in the *New England Journal of Medicine* concluded that "the evidence that VitD reduces cancer incidence and mortality is inconsistent and inconclusive as to causality" [38].

More evidence is thus needed explaining the plethora of trials to determine the relation between VitD and cancer. To mention is the VITAL-D study which is a randomized prospective double-blind incidence study evaluating the incidence of cancer, heart disease and stroke. Planned completion is in June 2016 [39].

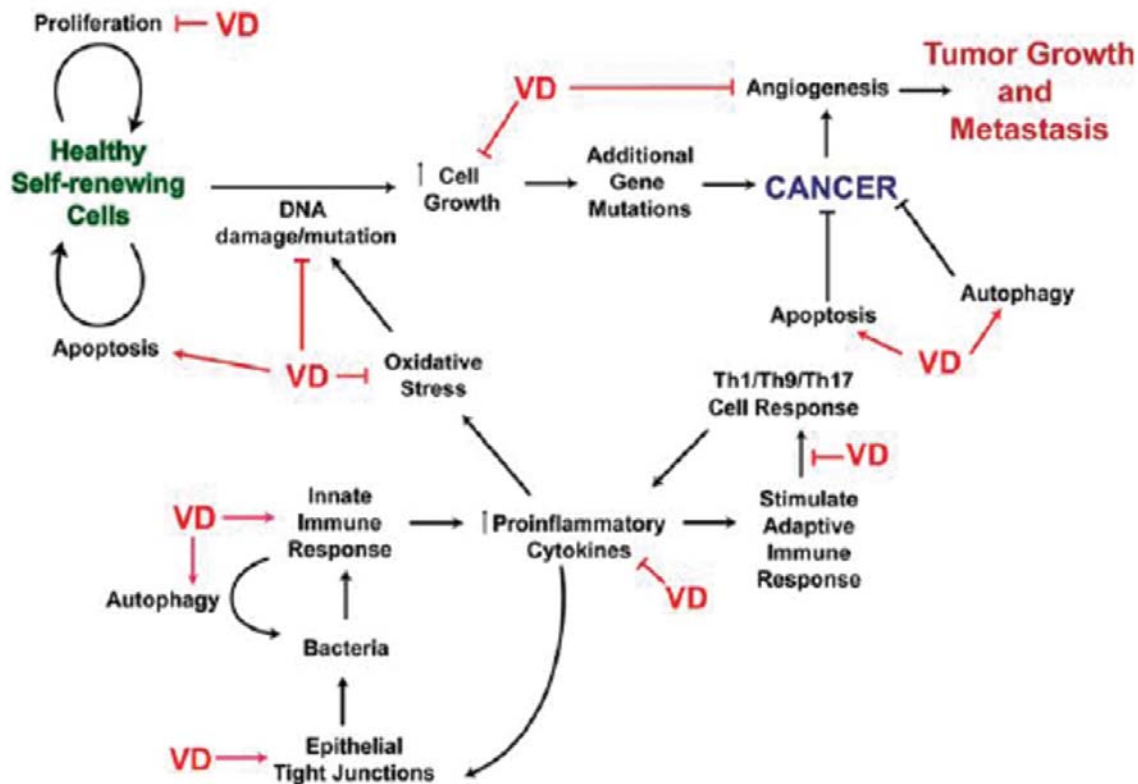


FIGURE 4. Possible effects of vitamin D on tumoral growth. (Adapted from Fleet JC, DeSmet M, Johnson R, Li Y (2012). Vitamin D and cancer: a review of molecular mechanisms. *The Biochemical Journal* 441: 61-76)

VITAMIN D AND IMMUNOLOGY

The effect of sunlight exposure is known to be protective against autoimmune disease. Even more, the incidence of multiple sclerosis, diabetes type I and Crohn's disease is increased with higher incidence according to higher location latitude in the northern hemisphere [4, 40]. Indeed the use of sunlight exposure gained significant reputation following the award of the Nobel prize for medicine in 1903 to Niels Ryberg Finsen "in recognition of his contribution to the treatment of diseases, especially *lupus vulgaris* (tuberculosis of the skin), with concentrated light radiation, whereby he has opened a new avenue for medical science" [41]. Since the beginning of the 19th century, sunlight exposure was recognized as protective against infectious diseases and especially tuberculosis, and children were given cod-liver oil to enhance resistance against tuberculosis [9, 41-42].

The main effect of VitD seems to enhance the innate immune response by activating macrophages, thus increasing the chemotaxis, phagocytosis and reactive oxygen species production. The presence of the 1 α -hydroxylase enzyme within the monocyte and macrophage cell could be another pathway for innate immunity activation [2, 41]. On the other hand, VitD inhibits the production of pro-inflammatory chemokines like IL 1 and IL 6 and increasing the production of anti-inflammatory chemokines like IL 4, IL 10 and IL 13 producing an anti-inflammatory effect. This is a possible mechanism for antibacterial and immunomodulatory effects of VitD.

VitD was shown to be protective against rheumatoid arthritis (RA). In an observational study, incidence of RA decreased by 35% in patients with VitD supplementation compared to VitD deficient [6, 43]. The risk of multiple sclerosis was shown to decrease with increasing levels of VitD with an OR of 0.59 [44]. Even more, the risk of relapse of multiple sclerosis was shown to be less with increasing levels of this vitamin [45]. In some centers, VitD has become an integral part of the therapeutic protocol of multiple sclerosis.

A recent meta-analysis concluded that genetic and epidemiological studies show a potential role of VitD in the prevention of autoimmune diseases. Conclusions should be drawn cautiously since randomized and controlled trials are needed to establish the clinical efficacy of VitD supplementation in ill or at-risk subjects [27].

VITAMIN D AND SKELETAL MUSCLE

The presence of VDR in the skeletal muscle is still debated. Many authors showed the presence of this receptors and its mRNA [6-7], a recent well-structure trial using the VDR antibody showed that the VDR concentration in skeletal muscle is low and concluded that VitD effect on the muscle is of an indirect nature [46]. Nonetheless, the effect of VitD on skeletal muscle could not be under stressed. VDR knockout mice develop a specific myopathy characterized by 20% smaller fiber

diameters leading to proximal muscle weakness, decreased swimming ability and muscle coordination [7, 47]. Bischoff-Ferrari showed an increased 8-foot walking time as well as an increase in sitting and standing time with Vit D deficiency [48]. A recent meta-analysis showed that VitD is protective against falls, increases muscle mass and power and increases gait speed. Even more, VitD deficiency is associated with increased falls and fractures following these falls [7, 49]. Another meta-analysis showed that the risk of falls following VitD supplementation was significantly reduced with an OR of 0.86 [50].

PERSONAL RECOMMENDATION

As a result of this review, we feel that we don't have enough evidence regarding the VitD and the above mentioned diseases. We strongly recommend not to give the patient false hopes to think that VitD treatment is a cure. The answer to this question will be available hopefully in 2016 with the end of the VITAL-D study [39] and more ongoing randomized controlled trials (Heike Bischoff-Ferrari; RCT; VitD + ω 3 + 20 minutes' walk evaluating effect on life expectancy).

CONCLUSION

VitD deficiency was shown to be strongly associated with multiple conditions including cardiovascular disease, diabetes, cancer, infections, etc. Despite the biological plausibility for the role of VitD in the prevention of these conditions, available evidence is still inconsistent and inconclusive and no guidelines could be drawn for such effects. Confounding factors such as obesity and behavioral factors could not be excluded. No large scale randomized prospective trials have been completed but the VITAL-D study should provide important answers on many of the unanswered questions.

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