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REVIEW ARTICLE

New Insights on Low Vitamin D Plasma Concentration as a Potential Cardiovascular Risk Factor.

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Abstract: The role of Vitamin D hormone in human health and disease is still debated. Recently, growing attention has been paid to its putative role in cardiovascular system homeostasis with several studies that suggested a correlation between low vitamin D levels and increased cardiovascular risk. Several mechanisms are involved in the development of cardiovascular diseases: systemic inflammation, endothelial dysfunction, arterial hypertension and insulin resistance. In the present paper, we have revised the current literature supporting a role for vitamin D in the development of these pathogenetic processes. Finally, we have evaluated the current evidence linking vitamin D to atherosclerosis and its natural consequence, cardiovascular diseases.

Keywords: Vitamin D, Cardiovascular Risk, Cardiovascular Diseases, Insulin Resistance, Inflammation, Atherosclerosis.

1. INTRODUCTION

Vitamin D is a fat-soluble hormone, the main activity of which is the regulation of calcium/phosphate metabolism. This bone-related activity is accomplished by acting with calcium-sparing effects on the gut [1], the parathyroid glands [2 - 4] and the kidney [5]. Vitamin D plays a crucial role for bone metabolism not only because calcium and phosphate are essential components of bone turnover mechanisms, but also because vitamin D can directly control the physiological turnover of bone at the level of osteoblasts and osteoclasts [6 - 9]. There is overwhelming evidence, however, that Vitamin D Receptor (VDR) is expressed not only by cognate vitamin D targets but also by other cell types and tissues, implying that vitamin D has far a wider role in human physiology than previously thought. New putative functions of vitamin D have thus been explored and confirmed by both preclinical in-vitro and in-vivo studies. For instance, vitamin D is able to induce epidermal cells differentiation [10], has a crucial role in proliferation and differentiation of the nervous system, affecting neuroprotection, neurotransmission, and neuroplasticity [11]. Furthermore, vitamin D has antiproliferative actions [12] and regulates both the innate and adaptive immune system activity [13 - 21].

Potential new functions of vitamin D have been recently suggested for the regulation of cardiovascular health, leading to the hypothesis that low vitamin D levels can be considered as a new potential marker of cardiovascular risk [22]. This inference takes origin from several associative observations, the most convincing of which are the wide expression of the VDR in the cardiovascular system [23] and the inverse correlation existing between low vitamin D levels and important cardiovascular risk factors, such as systemic inflammation [24], arterial hypertension [25], insulin resistance [26], and endothelial dysfunction [27].

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In this paper, we aim to provide an overview of current evidence linking vitamin D to the cardiovascular risk, focusing our attention on the potential role of this hormone in the pathogenesis of atherosclerosis and Cardiovascular Diseases (CVDs).

1.1. Vitamin D Metabolism and Vitamin D Deficiency

Although several foods are dietary sources of vitamin D, endogenous synthesis accounts for the largest amount of active vitamin D in humans. In the skin, 7-dehydrocholesterol is photolysed in cholecalciferol (vitamin D_3) after the exposure to UV rays [28]. Cholecalciferol is then hydroxylated to 25-hydroxyvitamin D [$25(OH)D_3$] in the liver [29]; $25(OH)D_3$, also known as calcifediol, circulates in the bloodstream bound to the vitamin D Binding Protein (DBP) and, minimally, as a free hormone. DBP, more generally, act as a carrier for all the isoforms of vitamin D [30, 31]. Vitamin D is finally activated into 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] (also termed calcitriol) by the 1α-hydroxylase (CYP27B1), in the kidney [32]. The activity of the CYP27B1 is strictly controlled by parathyroid hormone (PTH), in a positive fashion [33], and calcium and $1,25(OH)_2D_3$, in a negative fashion [34]. The inactivation of $1,25(OH)_2D_3$ is mediated by the CYP24A1 enzyme, which catalyzes the conversion to a 100-fold less active metabolite [35].

$1,25(OH)_2D_3$ acts by binding the VDR, a nuclear receptor which heterodimerizes with the Retinoid X Receptor (RXR); the VDR/RXR complex moves from the cytoplasm to the nucleus and acts to upregulate or downregulate the expression of many target genes [36].

Vitamin D deficiency causes a decrease in intestinal dietary calcium and phosphorus absorption, leading to secondary hyperparathyroidism [37, 38], which maintains serum calcium in the normal range at the expense of mobilizing calcium from the skeleton and increasing phosphorus wasting in the kidneys. This results in an inadequate calcium-phosphorus product and in a mineralization defect in the skeleton, leading to rickets in children [39] and osteomalacia in adults [40 - 42].

Although $25(OH)D_3$ is not the most active metabolite, its stable concentrations and long half-life make it a reliable plasmatic marker of vitamin D status [43]. The definition of vitamin D adequacy is historically based upon the identification of the plasma $25(OH)D_3$ threshold able to suppress PTH synthesis [44]. Although no definitive consensus currently exists, the majority of authors would agree with the definition of vitamin D status as deficient for $25(OH)D_3$ concentration lower than 50 nmol/l (20 ng/ml), insufficient for 50-75 nmol/l (20-30 ng/ml) and adequate for 75-250 nmol/l (30-100 ng/ml) [45]. Hypovitaminosis D is a diffuse health issue worldwide, with a very high prevalence either in otherwise healthy or in hospitalized adults [46 - 49]. Its prevention and correction are therefore essential for bone health, but also necessary for its putative, collateral advantages on different aspects of human physiology. Oral cholecalciferol represents the gold standard for vitamin D supplementation, in healthy subjects with neither malabsorption nor chronic kidney disease; either daily supplementation or loading-dose based regimens have been proposed, although the best supplementation strategy is still to be defined [45, 50 - 55].

2. VITAMIN D AND SYSTEMIC INFLAMMATION

One of the best characterized extra-skeletal activities of vitamin D is the regulation of the immune system and inflammatory response.

In vitro, $1,25(OH)_2D_3$ affects functional activities of monocytes and macrophages. Tumor cell cytotoxicity, phagocytosis, and mycobactericidal activity of monocytes/macrophages are enhanced by exposure to active vitamin D [56, 57], while monocyte function as an Antigen Presenting Cell (APC) is decreased [58], as well as the production of proinflammatory cytokines such as Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNFα) [59]. Furthermore, $1,25(OH)_2D_3$ promotes terminal differentiation of monocytes towards a macrophage phenotype [60] and inhibits the differentiation of murine and human Monocytes into Dendritic Cells (DCs) *in vitro* [61]. $1,25(OH)_2D_3$ also impairs DCs function as APCs, by downregulating MHC II and costimulatory molecules expression [62], as well as chemotaxis [63], thus affecting adaptive immune system, which is strictly regulated by DCs activity. However, vitamin D also directly regulates adaptive immunity. In fact, it is able to inhibit the proliferation and to induce the apoptosis of activated B cells; furthermore, $1,25(OH)_2D_3$ inhibits plasma cells and post-switch memory B cells differentiation and significantly reduces immunoglobulin secretion [64]. Finally, $1,25(OH)_2D_3$ acts on T cells: vitamin D inhibits T cells cytotoxic activity by suppressing Fas-ligand expression in activated T cells [65] and drives CD4+ differentiation, leading to a suppression of Th_1 and Th_{17} function towards a more favourable and less inflammatory Th_2 or T_{reg} phenotype. As such, $1,25(OH)_2D_3$ reduces the expression of the Th_1 associated cytokines IL-2, TNF-α, and IFNγ [66]. On the other hand, key

Th_2 cytokines like IL-4 and IL-5 are upregulated [67, 68]. Th_{17} is a specific subset of CD4+ cells able to produce IL-17A, IL-17F, IL-21, IL-22 [69], thus playing a pivotal role in inflammation. On the contrary, regulatory/suppressive T cells (T_{reg}) contribute to the maintenance of self-tolerance. T_{reg} cells account for 5-10% of total number of T CD4+ cells in healthy humans and play an important role in supporting immune homeostasis by producing anti-inflammatory cytokines, including IL-10 and TGF-β1 [70 - 72]. $1,25(OH)_2D_3$ has been shown able to induce the differentiation of T_{reg} by enhancing the expression of CTLA-4 and Foxp3, while inhibiting IL-17, IL-21 and IFNγ expression [73]. On the contrary, $1,25(OH)_2D_3$ inhibits Th_{17} proliferation [74]. As a result, the secretion of TGF-β1 is enhanced [75], paralleling a decrease in Th_{17} cytokines signature production [76].

In vitro data led many groups to investigate whether plasma $25(OH)D_3$ concentration could independently predict the risk for autoimmune diseases and whether vitamin D supplementation could benefit the treatment of inflammatory/immune conditions [77 - 85]. However, there is still a relevant gap between the strength of the *in vitro* data and the faintness of the *in vivo* findings, which do not allow, at the moment, to clarify the real relevance of vitamin D in the development of autoimmunity.

2.1. Vitamin D and Endothelial Dysfunction

Vitamin D activity has also been related to the vascular system. In fact, endothelial cells express both the VDR and the CYP27B1 enzyme, thus allowing the autocrine activation of $25(OH)D_3$ [86, 87]. This is particularly relevant, since vitamin D has a potential protective effect on the vascular endothelium. *In vitro*, vitamin D is able to induce the synthesis of Nitric Oxide (NO) through regulation of the endothelial isoform of NO synthase (eNOS) [88]. Experimentally, administration of $1,25(OH)_2D_3$ reduces inflammatory and atherosclerotic parameters [89] and blunts the deleterious effect of advanced glycation end products on the endothelium, thereby improving the activity of the NO system [90]. Furthermore, $1,25(OH)_2D_3$ stimulates the migration and proliferation of endothelial cells [91] and *in vitro* vitamin D treatment improves the capacity of endothelial progenitor cells, isolated from diabetic subjects, to form colonies [92]; taken together, these findings suggest a potential role for this hormone in vessel damage healing. In addition to its ability to modulate the effects of proinflammatory cytokines on the vascular endothelium [93] and to decrease the expression of endothelial adhesion molecules [94, 95], vitamin D can also exert antioxidant properties [96].

In vivo, $25(OH)D_3$ concentrations are related to endothelial dysfunction. In fact, vitamin D status has been inversely associated with the concentration of circulating markers of endothelial dysfunction in obese patients [97]. Consistently, hypovitaminosis D has been associated to endothelial dysfunction in patients affected by metabolic syndrome, chronic kidney disease and rheumatoid arthritis [98 - 100]. In healthy subjects, a $25(OH)D_3$ <25 mmol/l (10 ng/ml) is associated with a significantly lower brachial artery flow-mediated dilatation (FMD), an important marker of endothelial dysfunction, with respect to subjects with a normal vitamin D status. Interestingly, a high-dose supplementation regimen has been reported to lead to a significant improvement of FMD [101]. Following this finding, the effect of a vitamin D supplementation regimen on endothelial dysfunction has been investigated in different trials, leading to conflicting results. While cholecalciferol supplementation significantly increased the FMD in a trial conducted on patients affected by essential arterial hypertension and hypovitaminosis D [102], other studies presented different results. However, the discrepancies observed could depend on differences in the studied cohorts, supplementation regimens and endpoints used to define the outcome. In a trial recently published by Borgi *et al*. [103], the outcomes and the supplementation regimen were similar to those of the study by Carrara *et al*. [102]. Borgi did not find any effect of vitamin D supplementation on endothelial function. However, the populations studied were very different, being in this last trial included overweight and obese subjects who are known to have an impaired bioavailability of vitamin D [104], which could have biased the negative results. On the contrary, in a trial by Dalan *et al*. conducted on diabetic subjects, different endpoints were used and cholecalciferol supplementation was based on a lower dose regimen, which could justify the difference observed [105]. Therefore, these trials cannot be compared and further studies are required to better clarify the impact of vitamin D supplementation on endothelial function.

2.2. Vitamin D and Arterial Hypertension

High blood pressure is a recognized risk factor for disease and premature death [106]. Blood pressure is regulated by different mechanisms that include sodium and fluid balance as well as vasomotor tone. Both mechanisms are affected by genetic and environmental factors, and are controlled by hormonal, nervous, paracrine, neuroendocrine and intracellular feedback loops [107]. *In vitro* and *in vivo* data have suggested that vitamin D could be implicated in the control of blood pressure through inter-related factors, such as the Renin-Angiotensin-Aldosterone System (RAAS),

sympathetic activation and genetics. Vitamin D has been shown to exert inhibitory effects on the RAAS through modulation of the renin gene via VDR-dependent mechanism [108]. Mice lacking the VDR were prone to develop excess plasma renin activity and hypertension [109], as well as increased susceptibility to obstructive renal injury [110]. All these effects could be prevented by treatment with ACE inhibitor or AT1 receptor antagonism. Similar negative consequences were observed in mice silenced for the CYP27B1 gene, while $1,25(OH)_2D_3$ administration favored the regression of hypertension due to excess plasma renin activity, independent of calcium levels [111, 112]. However, others observed that $1,25(OH)_2D_3$ administration induced an increase in plasma renin activity [113]. Interestingly, 4-week cholecalciferol administration to normal rats, at doses ranging from deficiency to toxic levels, generated a U-shaped dose-response curve on indices of arterial stiffness and systolic hypertension, implying that the vasoactive effects related to vitamin D likely reflect a balanced vitamin D status [114]. Studies in non-hypertensive individuals maintained on dietary sodium balance showed that $25(OH)D_3$ deficiency was associated with increased renal vascular RAAS activity as well as increased angiotensin II levels [115]. Nevertheless, the effect of vitamin D on renin–angiotensin system activation and blood pressure has been analyzed in a randomized control trial, and results showed no benefit from correcting vitamin D deficiency on RAAS activity or blood pressure after 8 weeks [116].

Potential RAAS-independent mechanisms have also been claimed to explain the vitamin D-related effects on hypertension. Studies in rat showed that vitamin D deficiency results in increased cardiac contractility, hypertrophy and fibrosis and has profound effects on heart proteomics, structure and function [117]. The mechanism involves an increased expression of L-type calcium channels and sarcoplasmic reticulum calcium uptake [118]. Ablation of the VDR in mice caused profound cardiac hypertrophy in the absence of significant modifications of the RAAS [119]. Moreover, it was shown that vitamin D deficiency in growing rats promoted vascular oxidative stress and induced changes in cardiac expression of 51 genes, including genes involved in the regulation of oxidative stress and myocardial hypertrophy [120]. Based on these results, anti-inflammatory activity of vitamin D could involve vascular endothelium and smooth muscle as a potential target of action. There is also evidence that vitamin D plays a role in sympathetic activation. Vitamin D deficiency in otherwise healthy subjects is associated with increased levels of plasma metanephrine, a marker of adrenal medulla activity [121], while a study discriminating on the sympathetic effects of $25(OH)D_3$ and $1,25(OH)_2D_3$ suggested that $1,25(OH)_2D_3$ but not $25(OH)D_3$ deficiency are associated with dynamic autonomic dysfunction [122]. Furthermore, many studies pinpointed the vasoactive properties of vitamin D through modification of calcium homeostasis in vascular smooth muscle cells [123].

As VDR is present in aortic endothelial, cardiomyocytes and vascular smooth muscle cells [124] VDR polymorphisms and mutations affecting vitamin D synthesis/metabolism could play a role on the susceptibility to develop hypertension. BsmI polymorphism of the VDR gene was found to influence blood pressure in healthy men, while a positive relationship was noted between $25(OH)D_3$ levels and blood pressure only in men and not women with the BB genotype [125]. A study on an Indian cohort also found important associations between hypertension and Fok I VDR polymorphism [rs2228570], which generates long and short variants of the VDR, independent of sex, family history and smoking [126, 127]. However, allelic frequencies and genotype distribution of FokI and BsmI VDR polymorphisms were not found associated with hypertensive status or renin activity in a small study on Italian individuals with essential hypertension [128]. A recent meta-analysis reported that polymorphism in the 24-hydroxylase (CYP24A1) gene, which controls vitamin D metabolism, were the most significantly associated with systolic and diastolic blood pressure [129]. Nevertheless, results from the Women's Genome Health Study on 23,294 women and the International Consortium of Blood Pressure on 69,395 men and women of European ancestry only found associations with genes related with vitamin D metabolism and signaling which, however, disappeared after multiple testing corrections [130]. In a Mendelian randomization study on 146,581 individuals, a link was suggested between low vitamin D and increased risk of hypertension using gene variants relating to $25(OH)D_3$ synthesis and metabolism, *i.e.* DHCR7 rs12785878, CYP2R1 rs12794714, GC rs2282679, and CYP24A1 rs6013897 [131]. According to results, each 10% increase in genetically determined $25(OH)D_3$ levels was associated with a significant 0.29 mmHg decrease in diastolic blood pressure and 0.37 mmHg decrease in systolic blood pressure, conferring overall a 8.1% reduced risk of hypertension. This finding suggests that genetically affected risk factors related to the VDR are causally related to clinical outcomes [132]. On the other hand, there is no evidence of a convincing relation between DBP and hypertension except for a Mendelian randomization study based on results from the Canadian Multicentre Osteoporosis Study, which however failed to document any association between DBP polymorphism rs2282679 and arterial blood pressure [133].

Studies on oral supplementation with vitamin D is found to lower blood pressure in hypertensive rats [134, 135]. In

humans, cross-sectional data suggest an association between low vitamin D intake (<400 IU per day) and an increase in blood pressure [136], and one study in black participants reported dose-dependent reductions in systolic blood pressure after 3 months of supplementation with 1000 IU, 2000 IU, and 4000 IU of vitamin D per day, *i.e.* 0.66, 3.4, and 4.0 mm Hg, respectively [137]. However, evidence from randomized controlled trials has not provided consistent evidence of a benefit. In an interventional study on vitamin D deficient elderly women, a combination of calcium and vitamin D supplementation was found to have a greater lowering effect on blood pressure than calcium alone [138]. Another study on cholecalciferol supplementation by a dose that effectively increased vitamin D levels during winter months, showed no effects on 24-h blood pressure, yet a post-hoc subgroup analysis of 92 subjects with baseline $25(OH)D_3$ levels <32 ng/ml showed significant decreases in 24-h systolic and diastolic blood pressure [139]. Oppositely, a trial on participants with arterial hypertension and $25(OH)D_3$ levels below 30 ng/mL failed to show significant effects of cholecalciferol supplementation on 24-hour systolic ambulatory blood pressure and several cardiovascular risk factors, while it pinpointed a significant increase in triglycerides [140]. Similarly, other studies found that vitamin D supplementation did not reduce blood pressure in individuals with prehypertension or stage I hypertension and vitamin D deficiency [141].

Weaknesses inherent in observational studies, such as reverse causation, are a possible source for discrepancies between these studies, as well as differences in the therapeutic regimens and duration of vitamin D supplementation, or in baseline differences in $25(OH)D_3$ concentrations, blood pressure or obesity. Moreover, people with hypertension might move less outdoors or have poorer health than those moving actively. While current evidence remains elusive, it remains to be demonstrated that larger randomized controlled studies could really add more evidence of the benefits of vitamin D in cardiovascular health.

2.3. Vitamin D and Insulin Sensitivity

Many studies have provided *in vitro* and *in vivo* evidence that vitamin D has an effect on glucose metabolism, and hypovitaminosis D seems to detrimentally impact on insulin sensitivity both directly and indirectly via negative effects sorted by secondary hyperparathyroidism [142].

This association has been hypothesized on the basis of observational *in vivo* data. In a cross-sectional study on 3577 US adolescents, lower vitamin D concentrations were independently associated with Fasting Plasma Glucose (FPG), with the risk of Impaired Fasting Glucose (IFG) being doubled in patients at the lowest, compared to those at the highest quartile of vitamin D [143]. Similar results have been obtained on a large cohort of adults, in a recent study which proved an inverse association between $25(OH)D_3$ plasma levels and FPG [144]. Moreover, vitamin D status was inversely associated to HbA1C, as previously reported in other cross-sectional studies [145, 146]. A recent meta-analysis proved that the inverse association between $25(OH)D_3$ levels and FPG, as well as HbA1c, is confirmed both in diabetic and in nondiabetic subjects [147]. Furthermore, vitamin D predicts insulin resistance; in fact, $25(OH)D_3$ plasma concentrations have been inversely related to the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) both in children and in adults [148 - 150]. Finally, $25(OH)D_3$ is directly correlated to 2-hours plasma glucose after an oral glucose tolerance test [151]. Consistently, a higher prevalence of hypovitaminosis D was reported in diabetic patients compared to healthy controls in different populations [152, 153].

Beside these cross-sectional data, longitudinal studies strengthened the hypothesis that hypovitaminosis D could be detrimental for glucose metabolism. In 2012, Gagnon *et al*. published the results obtained in the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab), in which baseline and 5-years follow-up data on 11.247 adults demonstrated that higher $25(OH)D_3$ levels were protective against the development of metabolic syndrome. Interestingly, lower baseline 25(OH)D3 concentrations were predictive of a higher HOMA-IR and FPG after 5 years of follow-up [154]. In a pooled analysis of two nested case-control studies conducted on a Finnish population, along a 22-year follow-up period, vitamin D levels were protective against the development of T2DM [155]; consistently, Forouhi *et al*. reported an inverse association between baseline $25(OH)D_3$ levels and the 10-years risk of T2DM development [156]. However, these observations were not confirmed in another prospective cohort, when the effect of vitamin D was corrected for other relative determinants [157].

Taking into account the large number of observations published on this topic in the last few years, while many controversies still exist, there is a predominance of data suggesting a beneficial role for vitamin D in glucose metabolism. However, the mechanisms by which vitamin D regulates glucose homeostasis are only partly understood and many aspects still deserve a deeper insight.

It is now evident that vitamin D is able to both enhance insulin secretion and improve insulin sensitivity. In fact, VDR is expressed by pancreatic cells and $1,25(OH)_2D_3$ is able to directly stimulate insulin secretion [158, 159]; moreover, $1,25(OH)_2D_3$ upregulates the transcription of the insulin receptor *in vitro* [160]. As a further clue, different VDR polymorphisms have been identified and some of them have been related to glucose metabolism, with a significant effect determined by geographical belonging. In a recent meta-analysis on 28 studies and 9232 participants evaluating the effect of VDR variants on insulin sensitivity, the association between insulin resistance-related diseases and VDR polymorphisms ApaI, BsmI, FokI variant was confirmed in dark-pigmented Caucasians and Asians, but not in Caucasian with white skin [161]. The mechanisms by which VDR regulates the response to insulin are still far from being completely elucidated. However, VDR knock-out in mice muscle cells causes important effects on the insulin signalling. The skeletal muscles account for 80% of insulin-stimulated whole-body glucose disposal [162], playing a relevant role in the pathogenesis of insulin resistance. A putative mechanism of action has been recently proposed and it is related to the activity of Forkhead box O1 (FOXO1). FOXO1 is a downstream negative regulator of insulin signalling which, during fasting, promotes gluconeogenesis in the liver. After food intake, FOXO1 activity is inhibited in liver and muscle cells by insulin [163]. VDR KO mice develop insulin resistance and glucose intolerance, which are paralleled by increased expression and activity of FOXO1. Moreover, when muscle cell lines are treated with $1,25(OH)_2D_3$ FOXO1 expression and activation are downregulated. Taken together, these findings suggest that: vitamin D regulates insulin signalling in muscle cells and the KO of vitamin D activity leads to the development of insulin resistance; vitamin D action on muscle cells is VDR mediated; a deficient vitamin D signalling induces an increase in FOXO1 activity which can be suppressed by the administration of $1,25(OH)_2D_3$ which, in turn, could be supposed to be a promising therapeutic tool in insulin resistance [164]. There are further data, obtained in animal models, which support the hypothesis that the positive effect of vitamin D on muscle cells can be therapeutically exploited. For example, in mice affected by diet-induced obesity and insulin resistance, the administration of vitamin D significantly improves the response to an oral glucose load and ameliorates the HOMA index; this is related to a direct effect on muscle cells which is paralleled by a reduction of lipid storage and myosteatosis [165, 166]. Similar data have been obtained on diabetic rats [167].

Unfortunately, randomized clinical trials (RCTs) in humans led to conflicting results being therefore absolutely inadequate to support the use of vitamin D as a therapeutic tool for the improvement of glucose metabolism. In fact, recent trials failed to disclose a beneficial effect for vitamin D supplementation on insulin sensitivity in vitamin D deficient overweight/obese [168] or in diabetic subjects [169 - 171], despite the use of large cholecalciferol doses. Consistently, the weekly administration of 20,000 IU of cholecalciferol was shown to be ineffective in preventing the progression from prediabetes to T2DM in an RCT conducted on 511 patients. In obese subjects, high-dose cholecalciferol administration was recently shown able to selectively increase plasma levels of high-molecular weight adiponectin, a mediator of glucose homeostasis [172].

The inconsistency of data from RCTs is reflected in the divergent conclusions obtained by the meta-analysis published on this topic. In fact, a very recent meta-analysis on 24 controlled trials showed a beneficial effect of vitamin D supplementation, at a minimum daily dose of 4000 IU, on FPG, HbA1c and HOMA-IR [173]; on the contrary the large prevalence of meta-analysis failed to disclose an effect for vitamin D supplementation in T2DM patients [174] but also in normoglucotolerant and prediabetic subjects [175, 176].

2.4. Vitamin D and Atherosclerosis

Systemic inflammation, glucose metabolism impairment and endothelial dysfunction are well-known risk factors for atherosclerosis; the observation that vitamin D has both an anti-inflammatory activity and a positive effect of endothelial function led many authors to postulate a potential detrimental effect of vitamin D deficiency on the development and the progression of atherosclerotic plaques *in vivo*. In the last years, many data have been reported in literature, supporting this hypothesis.

Lower $25(OH)D_3$ levels have been associated with higher IMT and to an increased risk of atherosclerotic plaques, although these findings are controversial and still debated [177, 178]. A possible explanation of these different conclusions could be related to differences in patients selection, being the association more convincing amongst diabetic subjects [179, 180] than in the general population. Moreover, the correlation between IMT and $25(OH)D_3$ levels sounds relatively weak and, probably, a large number of subjects is required to disclose this association [181]. Recently, a meta-analysis published by Lupoli *et al*. included data from twenty-one studies (3,777 vitamin D-deficit patients and 4,792 controls) evaluating the association between vitamin D and IMT, and 6 studies (1,889 vitamin D-deficient patients and 2,883 controls) evaluating the different prevalence of carotid plaques. According to this analysis,

vitamin D deficiency was associated with a higher IMT and an increased prevalence of carotid plaques; the attributable risk for vitamin D deficiency was 35.9%. As previously stated, the risk of carotid plaques seems to be even higher when vitamin D deficiency develops in association with T2DM (OR: 2.29, 95%CI: 1.03-5.11, p=0.043 in general population; OR: 3.27; 95%CI: 1,62-6.62, *p*=0.001 in diabetic subjects) [180].

Importantly, there is evidence that vitamin D levels are inversely related to the risk of Coronary Artery Disease (CAD). In a large prospective cohort on 1859 patients undergoing a non-urgent coronary angiography, low vitamin D levels were related to the prevalence and severity of CAD [182]. The detrimental impact of hypovitaminosis D on the development of CAD seems to be influenced by gender. In fact, hypovitaminosis D seems to be a more relevant risk factor for CAD in females than in males [183]. However, also middle-aged male patients with vitamin D deficiency show and increased risk for coronary artery calcification assessed by computed tomography [184]. It is important to underline that, as per the association with IMT, if on one side many authors agreed in identifying hypovitaminosis D as a risk factor for a more severe CAD [185], according to others, vitamin D levels are not predictive of the extent of atherosclerotic coronary disease [186, 187]. To summarize, although vitamin D has strong pre-clinical data supporting its involvement in the development of atherosclerosis and CAD, clinical data are conflicting and, again, this is the results of the profound differences which distinguish one study from another: the cohorts are generally different for gender and age and for comorbidities, which is particularly relevant if we consider stronger evidence for vitamin D involvement in the pathogenesis of atherosclerosis in diabetic subjects. Recently, an association between $25(OH)D_3$ levels and cardiovascular risk factors has been observed in patients affected by hypopituitarism, underpinning the potential role of contextual endocrine disorders in strengthening the detrimental effect of hypovitaminosis D [188]. Moreover, CVDs are the result of a complex pathogenetic model which includes many different risk factors; even in prospective studies, the control of all these elements might be difficult. Finally, different imaging techniques have been used and this is obviously a factor affecting the sensitivity in the detection of subclinical atherosclerosis. Novel imaging techniques, such as intravascular imaging modalities might be considered in the future to better disclose the role of vitamin D in the pathogenesis of CAD. However, at the moment, data are still conflicting and not enough persuasive of the effective role of vitamin D *in vivo*.

Finally, it is still debated whether the postulated association of vitamin D with coronary and peripheral vascular diseases is the result of hypovitaminosis per se or the effect of secondary hyperparathyroidism accompanying vitamin D deficiency. In fact, serum PTH concentration, but not vitamin D, has been directly associated with IMT in a large cohort of more than 8.000 patients [189]. Similarly, hyperparathyroidism has been associated to the extent of CAD [190].

Although evidence about the role, *in vivo*, of hypovitaminosis D as a risk factor for atherosclerosis are inconclusive, there is a general consensus on the significance of vitamin D status as a general marker of good health. In a very recent meta-analysis including almost 27.000 subjects recruited in eight different prospective studies, the global mortality risk significantly increased for lower $25(OH)D_3$ concentrations [191]. Interestingly, hypovitaminosis D was specifically associated with cardiovascular mortality. This observation replicates the results of a very large meta-analysis by Zhang *et al*., in which a total of 34 publications with 180.667 participants were considered. The authors described an inverse association between plasma $25(OH)D_3$ concentration and total cardiovascular events and cardiovascular mortality [192]. However, different trials have tested the effect of cholecalciferol supplementation on cardiovascular health. Specifically, in a trial on 36.282 postmenopausal women randomized to either calcium and vitamin D or to placebo and followed-up for seven years, the treatment had no effect on cardiovascular and cerebrovascular risk [193]. Similarly, in a subgroup of the same study, calcium/cholecalciferol supplementation seems not to affect the development of coronary artery calcification [194].

On this basis, no recommendations can be made to date for the use of vitamin D supplementation in the prevention and treatment of cardiovascular diseases, as well as for the other extra-skeletal chronic diseases.

CONCLUSION

In conclusion, vitamin D is a pleiotropic hormone, the activity of which is supposed to be much wider than previously known at the cardiovascular level. There is a significant gap between the large evidence relating vitamin D activity to vascular function and healing *in vitro*, and the relatively poor strength of *in vivo* data, which are often conflicting and/or inconclusive. While the achievement of a satisfactory vitamin D status can be considered advisable as a general marker of good health, there is still no consensus on the screening and correction of hypovitaminosis D for cardiovascular health.

Large population-based studies are thus required to strengthen the currently available evidence, and to contribute to the understanding of the causal mechanisms underlying the association between vitamin D and cardiovascular health and diseases.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise

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