Role of Vitamin D in Atherosclerosis

Eva Kassi, MD, PhD; Christos Adamopoulos, BSc, MSc; Efthimia K. Basdra, DDS, PhD; Athanasios G. Papavassiliou, MD, PhD

Atherosclerosis, the principal cause of cardiovascular diseases (CVDs), is a process that involves a complex interplay among different factors and cell types, including cells of the immune system (T cells, B cells, natural killer cells, monocytes/macrophages, dendritic cells) and cells of the vessel wall (endothelial cells [ECs], vascular smooth muscle cells [VSMCs]). The atherogenic process evolves in different stages, starting from inflammatory endothelial activation/dysfunction and resulting in plaque vulnerability and rupture.[1]

Several cardiovascular risk factors have been recognized. Among them, vitamin D deficiency [25(OH)D <20 ng/mL] is emerging as a new one. In addition to its well-defined role in bone and calcium metabolism, vitamin D has been identified as an important factor in cardiovascular health.[2–8]

Vitamin D deficiency affects almost 50% of the population worldwide. It has been suggested that this pandemic might contribute to the worldwide increased prevalence of CVD.[9–11]

Several mechanisms have been proposed to account for this inverse relationship. In addition to its effects exerted on numerous tissues and organs that indirectly participate in the atherosclerosis, vitamin D is directly involved in this systemic inflammatory process.[12,13] Vitamin D receptors (VDRs) are present in all cells implicated in atherosclerosis, including ECs, VSMCs, and immune cells. Vitamin D appears to regulate a wide range of physiological and pathological processes like vascular cell growth, migration, and differentiation; immune response modulation; cytokine expression; and inflammatory and fibrotic pathways, all of which play a crucial role, starting from the early stage of endothelial activation/dysfunction to the later stages of the plaque vulnerability and rupture.

In this review, we provide current data on the effects of vitamin D on cells directly implicated in atherosclerosis such as ECs, VSMCs, and immune cells (lymphocytes, monocytes, macrophages, etc) with a focus on the underlying molecular mechanisms, which are still largely unknown. We also summarize reports related to the favorable (antiatherogenic) actions of vitamin D in tissues and organs that indirectly participate in the atherogenic process. Finally, we critically discuss clinical studies to assess the protective role of vitamin D and the efficacy of vitamin D and VDR agonists in CVD. Because a comprehensive background is a prerequisite for further discussions of vitamin D–induced effects, we provide a brief description of vitamin D metabolism and mechanism of action.

Vitamin D Metabolism and Mechanism of Action

Vitamin D is a steroid hormone that comes in 2 forms that differ chemically in their side chain, D_2 and D_3 (Figure 1). Either produced in the skin (D_3) from 7-dehydrocholesterol by exposure to ultraviolet-B light or ingested with foods of plant or animal origin (D_2 and D_3, respectively), vitamin D is biologically inert and requires 2 hydroxylations to form its active metabolite.[10] The first hydroxylation is constitutive and takes place in the liver by vitamin D-25-hydroxylase to form 25(OH)D. The second hydroxylation is catalyzed by 25(OH)D-1aOHase (CYP27B1) to form the biologically active form of vitamin D, 1,25$(OH)_2$D (calcitriol; see Figure 1). This latter 1a-hydroxylation of 25(OH)D takes place in most tissues and cells of the body; however, serum levels of 1,25(OH)D are determined mainly by renal 1a-hydroxylase activity. This activity is regulated by serum calcium, phosphate, parathormone, and fibroblast growth factor 23.

It is important to know that circulating 25(OH)D levels are a main determinant of extrarenal tissue levels of 1a,25$(OH)_2$D and thus are the best indicator of whole-body vitamin D status. Actually, 25(OH)D is used for the classification of the vitamin D status as deficient or sufficient.[11]

The biological responses to the 1a,25$(OH)_2D_3$ hormone and its analogs are mediated by the VDR, which is a DNA-binding transcription factor. However, VDR is also localized to the plasma membrane caveolae and may result in activation of signal transduction pathways that generate rapid nongenomic responses.[14]

Interestingly, genome-wide associations studies revealed a genetic influence on 25(OH)D levels by 25-hydroxylase, 24-hydroxylase, and 7-dehydrocholesterol reductase, the enzyme that catalyzes the last step in cholesterol biosynthesis.[15] Moreover, the genes *CYP2R1*, *CYP24A1*, and *DCHR7* encoding the aforementioned enzymes, respectively, were also significant at the methylation level according to a genome-wide methylation study.[16]

Details on vitamin D metabolism and mechanism of action are given in the online-only Data Supplement.

From the Department of Biological Chemistry, University of Athens Medical School, Athens, Greece.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.113.002654/-/DC1.

Correspondence to Athanasios G. Papavassiliou, MD, PhD, Department of Biological Chemistry, Medical School, University of Athens, 75 Mikras Asias St, 11527 Athens, Greece. E-mail papavas@med.uoa.gr

(*Circulation*. 2013;128:2517-2531.)

***Circulation* is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.113.002654**

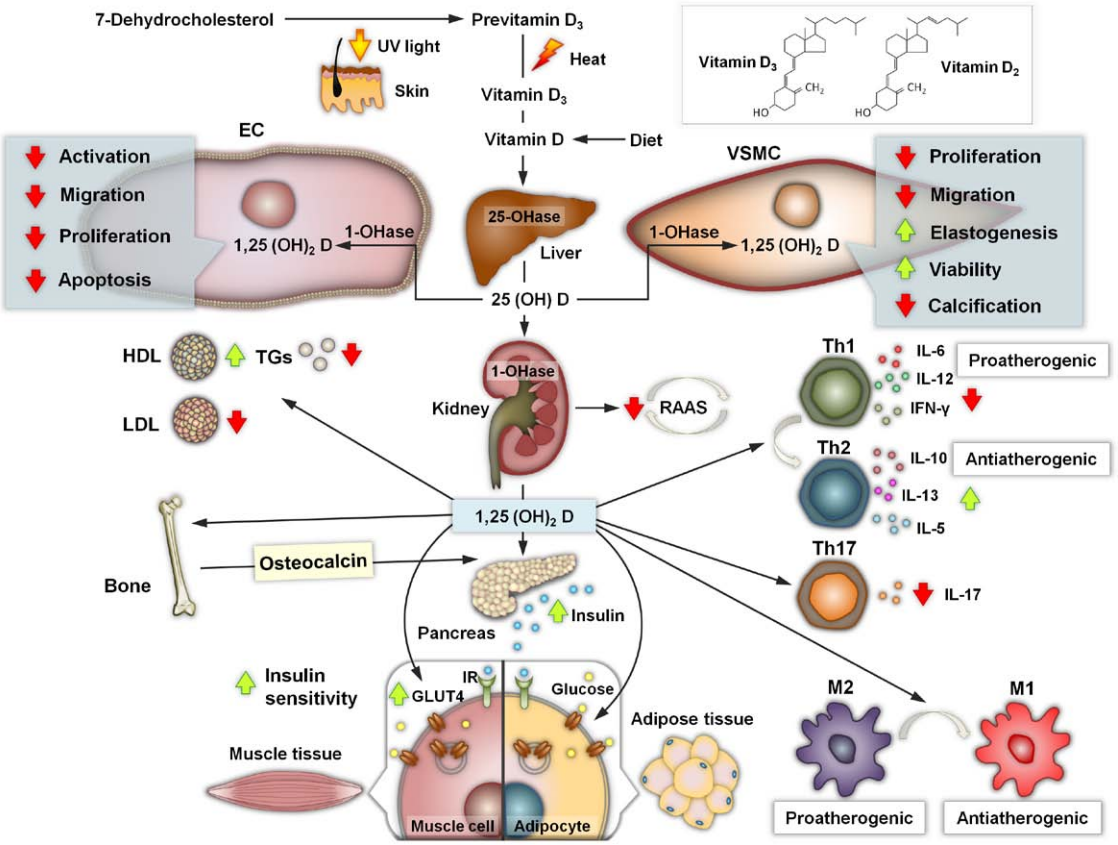

Figure 1. Schematic representation illustrating synoptically the metabolism and actions of vitamin D in cells and tissues that are implicated directly and indirectly in the atherogenic process. EC indicates endothelial cell; Glut-4, glucose transporter 4; HDL, high-density lipoprotein; IL, interleukin; IR, insulin receptor; LDL, low-density lipoprotein; M1, macrophage/monocyte 1; M2, macrophage/monocyte 2; RAAS, renin-angiotensin-aldosterone system; TGs, triglycerides; Th, T helper; and VSMC, vascular smooth muscle cell.

Direct Effects of Vitamin D on the Atherogenic Process

Effects of Vitamin D on ECs

It is well known that endothelium is the key vessel wall component in the initiation of the atherogenic process. Its possible role in the later stages has been strongly suggested.[17,18]

Studies revealed that ECs express VDRs and have the ability to synthesize calcitriol [1a,25(OH)$_2$D$_3$] because they express 1a-hydroxylase.[19] The coexistence of these 2 crucial elements of vitamin D metabolism strengthened the hypothesis of an autocrine/intracrine mechanism of vitamin D action as a modulator of endothelial functions.[20]

Vitamin D exerts protective effects on endothelial activation/dysfunction, an inflammatory process that precedes atherosclerosis, through several mechanisms both genomic and nongenomic (Figure 2). Among the main alterations ascribable to endothelial dysfunction are the reduced availability of nitric oxide (NO) and increased production of reactive oxygen species.[18]

Vitamin D found to stimulate NO production in human umbilical vein ECs cultures through endothelial NO synthase activation. This effect was dose dependent, VDR mediated, and accompanied by a significant increase in the level of phosphorylation of intracellular kinases such as p38, protein kinase B (AKT), and extracellular signal-regulated kinases.[21,22]

The multimeric enzyme complex NADPH oxidase is the major enzyme system that generates superoxide (the main reactive oxygen species) in the vasculature. In vivo and in vitro experiments have recently demonstrated that a vitamin D analog (22-oxacalcitriol) significantly suppressed the elevated expression of p22(phox) and NADPH oxidase subunit and improved endothelial NO synthase coupling, thus reducing oxidative stress in endothelium.[23]

Moreover, vitamin D protected ECs against H_2O_2 oxidative stress, counteracting superoxide anion generation and apoptosis and blocking the extrinsic caspase cascade by positively controlling the level of phospho-active extracellular signal-regulated kinases. Exploring in vitro the 1a,25(OH)$_2$ D downstream effector, Polidoro et al[24] found that 1a,25(OH)$_2$ D upregulated SirT-1 expression in human umbilical vein ECs and reverted the SirT-1 downregulation induced by H_2O_2.

The above currently identified antioxidative stress mechanisms of vitamin D are in line with the observed reduction of the basal reactive oxygen species level in the ECs of spontaneously hypertensive rat treated long term with 1a,25(OH)$_2$D$_3$.[25]

Vitamin D appears also to be implicated in the modulation of the vascular tone via regulation of the release of vasoconstrictor metabolites of arachidonic acid called endothelium-derived contracting factors.[26] The release of endothelium-derived contracting factors occurs via a calcium-dependent process in which the calcium influx

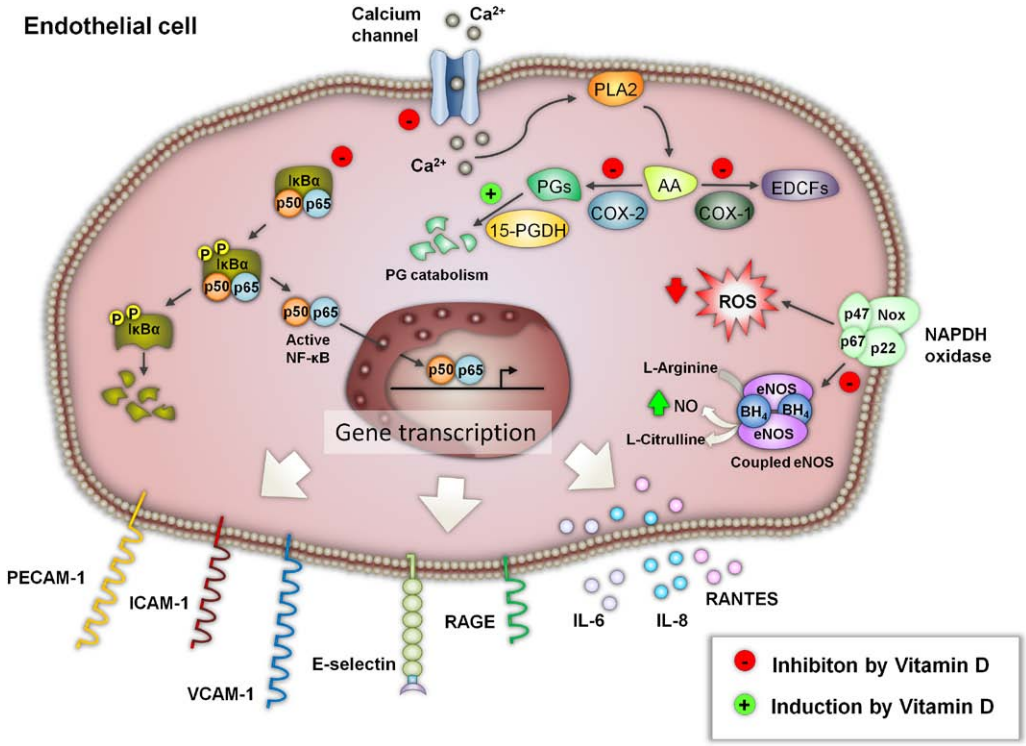

Figure 2. Vitamin D exerts protective effects on endothelial activation/dysfunction. Vitamin D suppresses the NADPH oxidase subunit p22(phox) and improves the endothelial nitric oxide (NO) synthase (eNOS) coupling, thus increasing NO production while decreasing reactive oxygen species (ROS) production. 1a,25$(OH)_2$D inhibits the expression of interleukin (IL)-6, IL-8, RANTES (regulated on activation, normal T cell expressed, and secreted), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), receptor of advanced glycation end products (RAGE), and E-selectin through a nuclear factor-κB (NF-κB)–mediated mechanism. Vitamin D represses the expression of cyclooxygenase (COX)-2 and upregulates the expression of 15-hydroxyprostaglandin dehydrogenase (15PGDH), the enzyme initiating prostaglandin (PG) catabolism, thereby reducing the levels of prostaglandins and suppressing the production of several proinflammatory cytokines. It can also acutely modulate vascular tone by reducing calcium influx into the endothelial cells (ECs) and hence decreasing the production of endothelium-derived contracting factors (EDCFs). However, a recent study also recognized a calcium-independent mechanism through a direct effect of vitamin D downregulating the expression of COX-1, which is the major source of endothelium-derived contracting factors, in ECs. AA indicates arachidonic acid.

activates phospholipase A2, which in turn converts the membrane phospholipids to arachidonic acid. Of note, vitamin D acutely modulated vascular tone by reducing calcium influx into the ECs and hence decreasing the production of endothelium-derived contracting factors.[27] However, a recent study also recognized calcium-independent mechanism through a direct effect of vitamin D downregulating the expression of cyclooxygenase-1, the major source of endothelium-derived contracting factors, in ECs.[28]

In addition to their effects on vascular tone, prostaglandin metabolism and signaling appear to play important role in the inflammatory component of atherosclerosis. It is well known that cyclooxygenase catalyzes the conversion of arachidonic acid to prostaglandin endoperoxides, which in turn are converted enzymatically into prostaglandins and thromboxane A2, both of which play pathological roles in vascular function as proinflammatory molecules. Cyclooxygenase-2, although absent from most normal tissues, is expressed in atherosclerotic lesions by ECs and VSMCs, probably in response to proliferative and inflammatory stimuli.[29]

It is noteworthy that calcitriol significantly repressed the mRNA and protein expression of cyclooxygenase-2 but upregulated the expression of 15-hydroxyprostaglandin dehydrogenase, the enzyme initiating prostaglandin catabolism, thereby reducing the level of prostaglandins and suppressing the production of several proinflammatory cytokines. This could successfully prevent the immune-mediated changes in the ECs.[30]

Equils et al[31] also demonstrated an effect of 1a,25$(OH)_2D_3$ on microbial antigen-induced EC activation. According to their data, lipopolysaccharide stimulation led to the release of proinflammatory cytokines, which induced 1a-OHase activity and consequently calcitriol expression. Calcitriol in turn inhibited interleukin (IL)-6, IL-8, and RANTES (regulated on activation, normal T cell expressed, and secreted) expression through inhibition of nuclear factor-κB (NF-κB) in an autocrine/paracrine fashion to "switch off" the immune activation.

The subendothelial migration of the monocytes and subsequent transformation into foam cells depend on the interaction between adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin expressed by ECs, and integrins (eg, LFA-1, VLA-4) expressed by leucocytes.[32] Recent studies showed that the tumor necrosis factor-α (TNF-α)–induced increase in protein levels of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 was significantly abolished after incubation of human ECs with 1a,25$(OH)_2D_3$, an effect independent of NF-κB activation.[33,34]

Talmor et al[35] demonstrated inhibitory effects of calcitriol on the expression of intercellular adhesion molecule-1, platelet EC adhesion molecule-1, and IL-6 in ECs, effects mediated through NF-κB and p38 mitogen-activated protein kinase. Moreover, recent studies have revealed that pretreatment with $1a,25(OH)_2D_3$ significantly inhibited the TNF-α–induced expression of E-selectin, vascular cell adhesion molecule-1, and IL-8 on human coronary arterial ECs via inhibition of NF-κB.[36,37]

Finally, calcitriol may also act as a vascular protective agent counteracting the deleterious effects of advanced glycation end products on endothelial function.[38] Increased concentrations of glucose and advanced glycation end products acting through its receptor of advanced glycation end products can induce the expression of proinflammatory molecules by ECs. Calcitriol acting via VDR has been found to decrease the expression of genes involved in advanced glycation end products–activated inflammatory pathway such as *RAGE*, *IL-6*, and *IL-8* through an NF-κB–mediated mechanism.[39]

Interestingly, by acting on ECs, vitamin D may also affect the later stages of atherosclerosis. It is believed that angiogenesis is mainly responsible for the rupture of an unstable atherosclerotic plaque. Vitamin D inhibited angiogenesis, which is greatly influenced by the proliferation and migration of ECs.[40] It is of interest that Mantell et al[40] showed that vitamin D inhibits vascular endothelial growth factor–induced EC sprouting and the formation of EC networks within 3-dimensional collagen gels. They further showed that $1a,25(OH)_2D_3$ promoted cellular regression as a result of apoptosis, specifically within the sprouting cell population.

Moreover, in vitro experiments in human ECs showed that $1a,25(OH)_2D_3$ attenuates the expression of membrane type 1 matrix metalloproteinase and platelet activation through a reduction of the CD62p platelet adhesion molecule, further highlighting its possible therapeutic effects against destabilization of atherosclerotic plaques and thrombosis.[34]

Confirming the in vitro data, several clinical studies showed both an inverse relationship between vitamin D levels and endothelial dysfunction and favorable effects of the vitamin D replacement on endothelial function and indicated the important role of NF-κB in mediating most of the beneficial action of vitamin D on endothelial dysfunction.[41–43] Indeed, Jablonski et al[42] found that vascular EC expression of NF-κB was greater in 25(OH)D-deficient than -sufficient subjects, whereas inhibition of NF-κB improved flow-mediated dilation to a greater extent in subjects with lower versus higher 25(OH)D.

Effects of Vitamin D on VSMCs

Studies have shown that the antiatherogenic effects of vitamin D extend beyond the endothelium to the VSMCs. VSMCs, which constitute the most common cell type in the medial layer of the arterial wall, play a pivotal role in the pathogenesis of atherosclerosis through proliferation and migration from the media to the intima layer, through morphological changes, or via secreting inflammatory molecules.[44]

The presence of VDRs in the VSMCs, which also express an enzymatically active 1a-hydroxylase, highlights the biological importance of $1a,25(OH)_2D$ in the regulation of VSMC homeostasis and function[45,46] (Figure 1).

Antiproliferative effects of $1a,25(OH)_2D_3$ on VSMCs have been demonstrated by several studies, but little is known about the molecular mechanisms involved.[47–49] It has been shown that $1a,25(OH)_2D_3$ inhibits the proliferative effects of both epidermal growth factor and endothelin on VSMCs, the latter via decreasing cyclin-dependent kinase 2 activity, that actually regulate the cell cycle machinery.[47,50] Additionally, calcitriol inhibited proliferation by an acute influx of Ca^{2+} into the VSMCs[51] (Figure 1).

The effects of $1a,25(OH)_2D_3$ on VSMC migration appear to be divergent. At high doses, calcitriol can induce VSMC migration, an effect that involved both nongenomic (through activation of PI3K pathway) and genomic (through reducing the expression of β_1-integrin receptors on VSMC surface) actions.[52,53] However, in physiological doses, 25(OH)D and calcitriol inhibited VSMC migration and proliferation by reducing the activity of vitamin D–binding protein, an effect mediated through the attenuation of extracellular signal-regulated kinase 1/2 phosphorylation.[54]

Other aspects of VSMC biology besides proliferation and migration are affected by vitamin D. Vitamin D appears to exert morphological effects, including increased elastogenesis and stabilization of musculoelastic multilayer of VSMCs, via regulation of the production of proteins that are related to the vascular wall, including myosin, collagen type 1, matrix metalloproteinase-9, and elastin.[55] Furthermore, $1a,25(OH)_2D$ has been shown to enhance prostacyclin production, a prostanoid that inhibits the aggravation of atherosclerosis, in VSMCs via the cyclooxygenase pathway.[56]

$1a,25(OH)_2D_3$ increased the superoxide dismutase activity in VSMCs via stimulation of the expression of IκB-α, thus reducing vascular cell–mediated oxidation of low-density lipoprotein.[57] Moreover, it improved the recovery of stressed VSMCs and their viability via regulating heat-shock protein 70 and possibly via increasing telomerase activity. It has recently been shown that vitamin D_3 supplementation improved telomerase maintenance and prevented peripheral blood mononuclear cells senescence.[58]

In accordance with the aforementioned in vitro data, a 16-week clinical trial showed that 2000 IU daily vitamin D_3 supplement counterbalanced the progression of arterial stiffness, as estimated by the carotid-femoral pulsed-wave velocity.[59]

Vitamin D also regulates the expression of profibrotic and antifibrotic factors.[60] Plasminogen activator inhibitor-1 is produced by a broad range of cells, among them vascular ECs and VSMCs. It is considered an inflammatory response gene that is associated with increased risk for thrombosis and atherosclerosis.[61,62] Activated vitamin D analogs (calcitriol and paricalcitol) have been reported to suppress plasminogen activator inhibitor-1 in human coronary artery smooth muscle cells.[48] Of interest, Chen et al[63] found that $1a,25(OH)_2D_3$ suppressed the upregulation of plasminogen activator inhibitor-1 induced by TNF-α and lipopolysaccharide by blocking NF-κB activation.

Additionally, vitamin D has been found to regulate the expression of antifibrotic factors. It was demonstrated that exposing mesenchymal multipotent cells to $1a,25(OH)_2D$

increased through VDR-mediated genomic effects the expression of bone morphogenetic protein-2 and BMP7 (transforming growth factor-β1 antagonists), matrix metalloproteinase-8 (a collagen breakdown inducer), and follistatin (an inhibitor of the profibrotic factor myostatin) and decreased the expression of collagen I and III, which define a fibrotic process.[60]

Vascular calcification is nearly a universal feature of atherosclerosis; hence, it can be considered synonymous in this perspective. In particular, intima calcification is gaining interest in the context of its association with atherosclerosis and its prognostic significance of coronary artery disease.[64] Studies have shown that osteogenic transformation of VMSCs enables them to secrete an osteoid-like extracellular matrix that calcifies over a defined time course.[65] Various proteins involved in osteogenesis have been detected in VSMCs and atherosclerotic lesions such as osteopontin, bone morphogenetic protein-2 and matrix Gla protein, fetuin-A, osteoprotegerin, and receptor activator of NF-κB ligand, playing either a stimulatory or an inhibitory role in the calcification process.[66] Data on the role of vitamin D in the vascular calcification are conflicting. In vitro experiments support that VSMCs undergo calcification when treated with 1a,25$(OH)_2$D through several mechanisms. Among them, a VDR-mediated stimulation of calcium influx in VSMCs, a VDR-dependent suppression of parathormone-related peptide and parathormone receptor signaling, and downregulation of fetuin-A expression have been proposed.[67–69] . Of note, a recent study by Shalhoub et al[70] identified an altered gene expression pattern that was representative of multiple pathways implicated in the calcitriol-induced vascular calcification such as osteochondrogenesis, mineralization, and apoptosis. However, it should be noted that the inductive effects of 1a,25$(OH)_2$D on vascular calcification are exerted under specific culture conditions (ie, in the presence of high phosphate medium).

On the other hand, protective effects of calcitriol and other VDR agonist on vascular calcification have also been recognized in vitro. Among the proposed mechanisms is upregulation of the expression of matrix Gla protein and osteopontin by VSMCs, which are both potent inhibitors of vascular calcification.[9] Interestingly, Zitman-Gal et al,[71] using microarray technology to detect gene expression profiles in VSMCs exposed to diabetic-like conditions, found that the addition of physiological concentration of calcitriol caused a decrease in the receptor activator of NF-κB ligand/osteoprotegerin mRNA ratio and in receptor activator of NF-κB expression, as well as in NF-κB p50/p65 protein expression, an outcome that might attenuate the calcification process.

Moreover, a recent study showed that VDR agonists (both calcitriol and maxacalcitol) suppressed the TNF-α–induced expression of the *runx2, osteocalcin*, and *MMP-2* genes in VSMCs.[72]

Clinical studies have shown either a positive or an inverse relationship between vitamin D levels and vascular calcification. Studies conducted in subjects with chronic kidney disease (CKD) reported an inverse relationship between serum 1a,25$(OH)_2D_3$ levels and total (intimal and medial) coronary artery calcification.[73–75] On the other hand, studies conducted in patients with CKD found a significant positive correlation between the extent of vascular calcification and 1a,25$(OH)_2D_3$ concentration.[76–78] However, in CKD, particular factors may contribute to vascular calcification, among them elevated levels of phosphorus, calcium, parathormone, and other potential uremic toxins that may influence the transformation of VSMCs into osteoblast-like cells. Of note, elevated phosphate was found to directly induce the expression of Runx2 and Osterix in VSMCs.[79]

These contradictory data probably reflect the complex, vascular-renal-endocrine-bone axis implicated in this process. Interestingly, Shroff et al[80] reported that in CKD patients both low (<20 pg/mL) and high (>60 pg/mL) levels of 1a,25$(OH)_2$D are related to increased carotid intima-media thickness and vascular calcification, possibly through a dual effect on parameters implicated in the calcification process such as calcium/phosphorus metabolism and inflammation. It is important to note that the protective or harmful effect of active vitamin D on vascular calcification remains a contentious issue, presumably because of the differences in the experimental model or the dose or type of active vitamin D used. In terms of dose, there is probably a fine balance between the protective and vascular calcifying effects of vitamin D; the latter may itself occur with either high or low doses of vitamin D analog. In terms of the type of active vitamin D used, clinical observations demonstrated that vitamin D analogs such as paricalcitol provide benefit for patients with CKD not only because of their less calcemic and phosphatemic effects but also by exerting direct protective effects on vascular calcification.[81]

Recently, observations clearly suggest the emerging roles of the fibroblast growth factor 23–Klotho axis in atherosclerosis. Current data on the role of vitamin D regulation of the fibroblast growth factor–Klotho axis in the pathogenesis of vascular calcification in CKD and in non-CKD subjects are provided in the online-only Data Supplement.

Effects of Vitamin D on Immune Cells

Both innate and adaptive immune responses are involved in the atherogenic process. Several subsets of T cells such as naive, T helper (Th) 1, Th2, T regulatory cells, Th17, and B cells, along with leucocytes and transformed monocytes, are present within atherosclerotic lesions.[11] Interestingly, the Th1 subtype primarily drives the inflammatory response in atherogenesis process by producing cytokines such as interferon-γ, TNF-α, IL-6, and IL-12, which are known to be proatherogenic.[82,83]

On the other hand, Th2, by secreting antiatherogenic cytokines such as IL-5, IL-10, and IL-13, neutralizes the Th1 effect.[84] It has been demonstrated that 1,25$(OH)_2$D shifts the immune response away from a Th1 and toward a Th2 profile, thus promoting an antiatherogenic immune profile[85] (Figure 1).

Although the exact role of IL-17 and its producing Th17 cells has not been fully addressed, recent studies have demonstrated the existence of IL-17A$^+$ cells within atherosclerotic lesions, suggesting a potential role in atherosclerosis.[86] Actually, the IL-17A–dependent response was found to occur in parallel with the Th1-dominant and unfavorable immune response during the atherogenic process. Treatment of T cells in vitro with 1a,25$(OH)_2D_3$ suppressed the development of Th17 and inhibited IL-17 production via a posttranscriptional mechanism[87] (Figure 1).

It has been shown that vitamin D is carried by low-density lipoprotein particles and is internalized by various cells, including monocytes, through low-density lipoprotein receptor either in its active form or as 25(OH)D. Once monocytes migrate transendothelially and are transformed into foam cells, 25(OH)D is accumulated in subendothelial space or in the atherosclerotic plaque and may be converted to its active form by 1a-hydroxylase, which expressed by monocyte-derived cells.[88,89] In turn, $1,25(OH)_2D$ may alter macrophage gene expression, influencing the expression of a variety of factors that are implicated in the atherosclerosis process (eg, type I collagen, vascular endothelial growth factor, matrix metalloproteinases, elastin) by ECs and VSMCs.[52]

Riek et al[90] found that $1a,25(OH)_2D_3$ affects macrophages by lowering acetylated and oxidized low-density lipoprotein uptake, thus reducing foam-cell formation. Interestingly, $1a,25(OH)_2D$ supplementation suppressed cholesteryl ester formation and enhanced cholesterol efflux in macrophages compared with vitamin D–depleted cells, suggesting facilitation of cholesterol egress in the presence of $1a,25(OH)_2D$.[90] Moreover, $1a,25(OH)_2D_3$ acting as a natural endoplasmic reticulum stress reliever was found to induce an M1 antiatherogenic macrophage/monocyte phenotype over M2 (with increased cholesterol uptake and deposition) and to decrease mRNA expression of the monocyte adhesion molecules PSGL-1, β(1)-integrin, and β(2)-integrin, resulting in an increase of monocyte adhesion to activated endothelium.[91]

Moreover, $1a,25(OH)_2D_3$ dose-dependently inhibited the production of IL-6 and TNF-α by monocytes, an effect exerted at a posttranscriptional level, and possibly contributed to the aforementioned suppression of Th1 proatherogenic immune response.[92] In addition to their immunoregulatory effects, monocytes are implicated in the development of atherothrombotic lesions by regulating coagulant and anticoagulant factors. It has been demonstrated that vitamin D, via VDR, upregulates the expression of the anticoagulant glycoprotein thrombomodulin and downregulates the expression of a critical coagulation factor, tissue factor, in human peripheral monocytes.[93] The physiological role of vitamin D–VDR in the maintenance of antithrombotic homeostasis has been confirmed with VDR knockout mice.[94]

Supporting the in vitro and animal studies, ex vivo experiments showed that $1a,25(OH)_2D_3$ modulates inflammation in monocytes isolated from diabetic patients through downregulating the expression of TNF-α, IL-6, IL-1, and IL-8.[95] Additionally, in 2 cohort studies, 25(OH)D levels were inversely associated with C-reactive protein and IL-6, whereas supplementation with vitamin D was found to suppress serum TNF-α, IL-6, and C-reactive protein levels and to increase IL-10 levels.[96–98]

In summary, the beneficial action of vitamin D on endothelial dysfunction, VSMC proliferation and migration, and calcification, as well as on the inflammatory/immune process of atherosclerosis, is well documented in the literature, even though the signal mechanisms by which these responses are elicited need to be fully characterized. NF-κB appears to play a crucial role in mediating many of the benefit effects of vitamin D. However, other signaling pathways, either genomic or nongenomic, appear to be implicated (Figure 2).

Indirect Effects of Vitamin D on the Atherogenic Process

In addition to the direct effects on cells implicated in the atherogenic process, vitamin D exerts protective effects against systemic conditions that promote atherosclerosis such as insulin resistance, β-cell dysfunction, dyslipidemia, the renin-angiotensin-aldosterone system (RAAS), and consequent hypertension (Figure 1).

Insulin Secretion/Insulin Resistance

Vitamin D deficiency has been reported to exert negative effects on β-cell function and insulin sensitivity, whereas it may be causally related to the development of diabetes mellitus type 2.[99–101]

Although the exact mechanism for such a pathogenic role has not yet been fully established, it was found that the promoter region of the *insulin* gene contains vitamin D–responsive elements, postulating a direct regulation of its transcription by $1a,25(OH)_2D_3$.[102] Additionally, insulin secretion is a calcium-dependent process, and vitamin D is known to regulate the calcium flux and intracellular cytosolic calcium $[Ca]^{2+}_i$ pool in the pancreatic β cell. Intriguingly, pancreatic β cell expresses 1a-hydroxylase and 24-hydroxylase, indicating a possible autocrine link between vitamin D status and pancreatic function.[100]

Current data demonstrated a significant downregulation of several genes related to islet growth and function such as *FOXO1, TCF7L2*, and *IRS-1* in vitamin D–deficient mouse islets.[102] Furthermore, hypovitaminosis D decreased the islet renin-angiotensin system activity, which in turn induces β-cell dysfunction in mice.[103]

Along with β-cell function, experimental data suggest effects of vitamin D against insulin resistance, which per se is associated with endothelial dysfunction, and increased carotid intima-media thickening. VDR is expressed in adipose tissue and skeletal muscle, tissues that play a central role in peripheral insulin sensitivity. Adipocytes also express the *CYP27B1* (1a-hydroxylase) gene.[104] Vitamin D directly activated the transcription factor peroxisome proliferator-activated receptor-γ, which is implicated in the regulation of fatty acid metabolism in muscle and adipose tissue.[105] Moreover, vitamin D contributed to normalization of extracellular calcium, ensuring normal calcium influx through cell membranes and adequate $[Ca]^{2+}_i$ pool to promote proper insulin-mediated intracellular processes such as phosphorylation of insulin receptor and translocation of glucose transporter-4[106] (Figure 1).

Finally, *osteocalcin*, which is another gene classically induced by $1a,25(OH)_2D_3$ in osteoblasts, has been identified as a bone-secreted hormone that both improves insulin release from β cells and increases insulin metabolic responsiveness[107] (Figure 1).

Lipid Profile

An association between vitamin D deficiency and atherogenic dyslipidemia has also been suggested. In a cross-sectional study that included 107 811 patients, vitamin D was associated with a significant increase in total cholesterol and high-density lipoprotein cholesterol.[108] Recently, using the filaggrin

genotype as an instrumental variable to estimate the causal effect of vitamin D on serum lipids, Skaaby et al[109] showed a 23.8% higher high-density lipoprotein cholesterol level and a 30.5% lower serum level of triglycerides per doubling of vitamin D.

The mechanisms by which vitamin D may affect the lipid metabolism are largely unknown. In vitro studies showed that incubation with calcitriol increases lipoprotein lipase expression and activity in cultured adipocytes.[110]

Recently, serum 25(OH)D was found to be positively associated with lipoprotein lipase concentration, a finding that could explain the inverse association between serum 25(OH)D and triglycerides.[111]

Moreover, a 25(OH)D receptor binding site modifying the *APOA5* promoter polymorphism was found to be associated with lower high-density lipoprotein in 25(OH)D-deficient individuals.[112]

The RAAS

The RAAS is the major regulator of blood pressure. It components, angiotensin II and aldosterone, appear to be directly involved in the pathogenesis of atherosclerosis (see the online-only Data Supplement).[113]

A growing body of evidence supports that vitamin D is a potent endocrine suppressor of the RAAS.[114] In vitro studies using a juxtaglomerular cell model showed that vitamin D suppresses both *renin* gene expression via a vitamin D–responsive element in the promoter of the *renin* gene and the expression of the *angiotensinogen* gene by blocking the NF-κB pathway.[115,116] Interestingly, VDR knockout mice have elevated circulating levels of renin and angiotensin II and develop hypertension.

Corroborating the in vitro data, clinical studies revealed that vitamin D is inversely associated with plasma renin activity, whereas both 25(OH)D and 1a,25$(OH)_2$D emerged as independent predictors of plasma renin and angiotensin II in a large cohort of patients referred for coronary angiography.[117,118]

In summary, it could be argued that vitamin D, acting either directly or indirectly, has a variety of favorable effects on the function and pathology of cells and tissues involved in the atherogenic process, therefore suggesting a potential role in the management of disorders associated with dysfunctional vascular wall such as atherosclerosis (Figure 1).

Vitamin D Deficiency and CVD Risk: Epidemiological and Clinical Studies

Ecological studies have indicated a higher incidence of CVD disease with increasing distance from the equator, suggesting an association with vitamin D insufficiency in regions with less sun exposure.[119]

In addition, the majority of observational studies suggested an inverse association between 25(OH)D levels and clinical CVD events (Table).[4–6,96,120–124] Notably, Martins et al,[3] analyzing data from >13 000 adults in the Third National Health and Nutrition Examination Survey (NHANES III), demonstrated a strong association between hypovitaminosis D and key CVD risk factors (diabetes mellitus, blood pressure, overweight, hypertriglyceridemia) that was independent of multiple variables. Furthermore, concentrations of 25(OH)D were inversely associated with CVD mortality among adults with hypertension in the United States.[123] The weight of the evidence in support of a strong inverse relationship between vitamin D levels and CVD events has become stronger with the Framingham Offspring Study and the Health Professionals Follow-up Study, showing an approximately doubled risk for cardiovascular events in vitamin D–deficient subjects.[4,5]

Along those lines, the Intermountain Heart Collaborative (IHC) Study Group analysis of a retrospectively collected database of 27 686 patients reported that vitamin D levels were highly associated with coronary artery disease and myocardial infarction (Table).[6]

In a prospective cohort study, cardiovascular mortality was higher for patients in the lower two 25(OH)D quartiles (median, 7.6 and 13.3 ng/mL) compared with patients in the highest 25(OH)D quartile (median, 28.4 ng/mL).[96] In accordance, Semba et al[122] reported a strong increased risk (2.64-fold) in the lowest quartile (<10.5 ng/mL). Moreover, a recent case cohort study by Karakas et al[124] showed that the hazard ratios comparing the tertile extremes of serum levels of 25(OH)D were 0.42 (95% confidence interval, 0.19–0.93) for women and 0.84 (95% confidence interval, 0.52–1.35) for men after adjustment for potential confounders (Table).

When this evidence was analyzed in recent meta-analyses of observational studies, an inverse association between 25(OH)D and cardiovascular risk emerged, although the strengths of associations in longitudinal studies were attenuated compared with cross-sectional studies.[127,128] However, it should be noted that in the Framingham Offspring Study the association appeared to be nonlinear (U shaped) with the suggestion of a slightly increased risk in CVD events at 25(OH)D levels >30 ng/mL.[4] A similar U-shaped relationship for 25(OH)D, with increased total mortality not only at low (<20 ng/mL) but also at high (>50 ng/mL) levels, has also been suggested in an NHANES III analysis.[120]

Notably, although several studies have evaluated the prognostic value of 25(OH)D for CVD incidence and mortality, population-based studies specifically looking at 25(OH)D and atherosclerosis are scarce. Lower 25(OH)D concentration was associated with an increased risk for incident coronary artery calcification, a measure of coronary atherosclerosis, in the Multi-Ethnic Study of Atherosclerosis (Table).[125] In addition, Lim et al[126] recently demonstrated that serum 25(OH)D levels were negatively associated with significant coronary artery stenosis evaluated with a multidetector-row cardiac computed tomography scanner. Interestingly, the odds ratios were 2.08 for a 25(OH)D concentration of 15 to 29.9 ng/mL versus at least 30 ng/mL and 3.12 for 25(OH)D concentration <15 ng/mL versus at least 30 ng/mL (both $P<0.05$; the Table).

Although observational studies suggest that vitamin D deficiency is related to a higher risk for CVD, numerous interventional studies that have recently been conducted showed conflicting results. However, it should be noted that randomized, controlled studies with CVD and, more specifically, atherosclerosis as the primary prespecified outcome are lacking.

The recently published Randomised Evaluation of Calcium or Vitamin D (RECORD) trial with a secondary outcome of CVD events showed no effects of vitamin D_3

Table. Summary of the Most Important Population-Based Studies Evaluating Atherosclerosis and CVD Risk in Patients With Vitamin D Insufficiency/Deficiency

		Study Population						
Author	Study Design	Total (Cases), n	Mean Age, y	Male, %	Setting	Follow-Up Length, y	25(OH)D Measurement	Type of Outcome
Giovannucci et al[5]	NCCS (prospective)	1354 (454)	63.8	100	Health care professionals, initially healthy	10	RIA	Fatal CHD and non-fatal MI
Wang et al[4]	CS (prospective)	1739 (120)	59	45	Population-based, initially healthy	7.6 (max) 5.4 (mean)	RIA (DiaSorin)	Cardiovascular events (MI, stroke, angina, transient ischemic attack, peripheral claudication)
Bolland et al[121]	CS	1471 (52)	74	0	Healthy postmenopausal women who participated in an RCT for calcium supplementation	5	RIA (DiaSorin)	MI
Dobnig et al[96]	CS (prospective)	3217 (463)	62	70	Symptomatic patients scheduled for coronary angiography at a single tertiary center	7.7 (median)	RIA* (DiaSorin)	Fatal cardiovascular events
Melamed et al[120]	CS (prospective)	13 331 (777)	44.8	45	Population-based	8.7 (median)	RIA‖ (DiaSorin)	Fatal cardiovascular events
Anderson et al[6]	CS (retrospective)#	27 686 (763)	66.6 (≥50)	25	General healthcare population**	1.3 (mean) 6.6 (max)	RIA (DiaSorin)	Coronary artery disease/MI
Zhao et al[123]	Cohort study (prospective)	2609 (68)	57.5	49	Hypertensive adults selected from a nationally representative survey sample	3.7 (mean)	RIA (DiaSorin)	Fatal cardiovascular events
Semba et al[122]	CS (prospective)	1006 (107)	74 (median) ≥65	75	Population-based	6.5	RIA (DiaSorin)	Fatal cardiovascular events
De Boer et al[125]	CS (prospective)	647 (135)	45-84 (range)	37	Population-based	3 (median)	RIA (DiaSorin)	Coronary artery calcification‡‡
Lim et al[126]	CS	921 (156)	76	44	Population-based	…	UPLC-MS/MS	Coronary artery stenosis >50%‖‖

Confounders Adjusted in Analysis	Relative Risk (95% CI) According to 25(OH)D Levels (Range or Median), ng/mL	Limitations
Adjustment for age, month, and year of blood sampling, smoking status, family history of MI before 60 y of age, history of DM, history of hypertension, alcohol intake, BMI, physical activity, region, race, multivitamin use, marine omega-3 intake, and fasting status. HDL-C-, LDL-C, and triglyceride levels	≥30 1.00 22.6–29.9 1.60 (1.1–2.32) 15.1–22.5 1.43 (0.96–2.13) ≤15 2.09 (1.24–3.54)	The number of fatal CHD cases was too small to make definitive conclusions on the association between 25(OH)D levels and fatal CHD
Adjustment for age, sex, systolic BP, antihypertensive treatment, DM, serum creatinine, total-to-HDL-C ratio, cigarette smoking, BMI, and CRP	≥15 1.00 <15 1.59 (1.03–2.45) <10 1.81 (1.03–3.18)	Lack of adjustment for season of blood sampling. Inadequate statistical power to evaluate the effect of milder degrees of vitamin D deficiency (15–30 ng/mL) and vitamin D sufficiency given the low proportion of individuals in the cohort with levels >30 ng/mL (10%)
Adjustment for treatment allocation (calcium or placebo) and baseline age, body weight, smoking status, systolic BP, and history of ischemic heart disease, stroke or transient ischemic attack, dyslipidemia, DM, and adjustment for seasonal variation in 25(OH)D concentrations	≥20 1.00 <20 1.2 (0.7–2.2)	Participants in the study were volunteers for an RCT for calcium supplementation. It is possible that volunteer bias occurred so that these women were healthier than their peers; therefore, findings are not generalizable to the general population
Adjustment for age, sex, BMI, and physical activity level, active smokers, DM, albumin level, cystatin C level, triglyceride level, N-terminal pro-BNP level, systolic and diastolic BP, LDL-C and HDL-C levels, and the use of statins, aspirin, β-blockers, bronchodilators, and angiotensin-converting enzyme inhibitors	Q4†: 28.4 1.00 Q3: 18.9 1.4 (0.9–2.1)‡ Q2: 13.3 1.82 (1.29–2.58) Q1: 7.6 2.22 (1.57–3.13)	Limitations of the study are related to 25(OH)D level determination and possibly race/ethnicity and changes of serum 25(OH) D levels over time§
Adjustment for age, sex, race, season of blood sampling, BP, history of CVD, DM, smoking, HDL-C, triglycerides, use of cholesterol-lowering medications, estimated GFR categories, serum albumin level, log albumin to creatinine ratio, log CRP, BMI, physical activity level, use of vitamin D supplementation, and low socioeconomic status	Q4: >32.1 1.00 Q3: 24.4–32.1 0.83 (0.65–1.07) Q2: 17.8–24.3 0.88 (0.69–1.14) Q1: <17.8 1.2 (0.87–1.64)	Northern states were only sampled in the summer in NHANES III; therefore, the full extent of 25(OH)D deficiency in the population is probably underestimated
Adjustment for age, sex, BP, hyperlipidemia, DM, and peripheral vascular disease	>30 1.00 16–30 1.15 (1–1.35)†† ≤ 15 1.45 (1.2–1.78)	The incident events were limited to those identified within the Intermountain Healthcare system. Both recognized (eg, female sex, risk of osteoporosis) and unrecognized selection biases could exist relating to the reasons that providers performed the vitamin D measurements. Lack of adjustment for season of blood sampling
Adjustment for age, examination period, sex, race/ethnicity, education, BMI, smoking, physical activity, heavy alcohol drinking, concentrations of serum non-HDL, HDL, calcium, elevated CRP, estimated GFR, use of dietary and vitamin supplements, and history of DM, CAD, stroke, and cancer	Q4: >29 1.00 Q3: 23–28 2.33 (0.88 –6.12) Q2: 17–22 2.42 (0.85–6.9) Q1: <17 3.2 (1.14–8.99)	The follow-up period of mortality (3.7 y) was relatively short, resulting in a relatively small number of deaths, especially deaths resulting from CVD. Inability to distinguish those adults with severe conditions at baseline who may contribute to early death
Adjustment for age, sex, education, season of blood sampling, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL-C, MMSE score, and renal insufficiency	Q4: >25.6 1.00 Q3: 16.1-25.6 2.19 (1.05–4.6 Q2: 10.5-16 1.68 (0.76–3.62) Q1: <10.5 2.64 (1.14–4.79)	The present study may possibly underestimate the relationship between low 25(OH)D and mortality because the reference quartile of 25(OH)D consisted of many participants who were still in the insufficient range of 25(OH)D. There are other unmeasured confounding factors: traditional risk factors for CVD such as DM, BP
Adjustment for age, sex, race/ethnicity, season, physical activity, BMI, DM, BP, smoking, CRP, total-C, HDL-C, triglycerides, and measurement batch§§	≥15 1.00 <15 1.38 (0.95–1.99)	It cannot be determined whether lower vitamin D concentrations are associated with the development of atherosclerotic plaque per se or with its calcification
Adjustment for age, sex, BMI, BP, alcohol intake smoking status, exercise habit, HbA1C, and triglycerides HDL-C, LDL-C, ALT, creatinine, hsCRP, HOMA-IR, phosphorus, iPTH	≥30 1.00 15–29.9 2.08 (1.16–4.68) <15 3.12 (1.89–5.59)	No data on hard end points such as the incidence or mortality of CVD. Data that can affect vitamin D levels such as seasonal variation were not captured

(*Continued*)

Table. Continued

Author	Study Design	Study Population: Total (Cases), n	Mean Age, y	Male, %	Setting	Follow-Up Length, y	25(OH)D Measurement	Type of Outcome
Karakas et al[124]	CCS (prospective)	964 (225)	35-74##	100	Population-based, initially healthy	11	EIA (IDS)	Fatal and nonfatal MI, SCD
		819 (73)	35-74***	0				

25(OH)D has a relatively long circulating half-life (≈3 weeks) and is considered the best indicator of whole-body vitamin D status, but a single measurement cannot fully capture cumulative vitamin D exposure. ALT indicates alanine aminotransferase; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CCS, case cohort study; CHD, coronary heart disease; CI, confidence interval; CLIA, chemiluminescence immunoassay; CS, cohort study; CVD, cardiovascular disease; DM, diabetes mellitus; EIA, enzyme immunoassay; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; IP-10, interferon-γ–inducible protein 10; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; MMSE, Mini-Mental State Examination; N/A, not available; NCCS, nested case–control study; Q, quartile; RCT, randomized, controlled trial; RIA, radioimmunoassay; SCD, sudden cardiac death; sICAM-1, soluble intercellular adhesion molecule 1; T, tertile; and UPLC-MS/MS, ultraperformance liquid chromatography–tandem mass spectrometry.

*In a random sample of 100 study participants, 25-hydroxyvitamin D level was also determined using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and a highly significant correlation was noted between the RIA and LC-MS/MS (R=0.87).

†Monthly quartiles.

‡Graphically determined.

§A retrospective classification of all individual 25 (OH)D levels into quartiles was performed on the basis of 202 to 358 measurements of 25 (OH)D levels each month.

‖The RIA kit was calibrated using high-performance liquid chromatography–purified 25(OH)D every 6 months.

#Prospectively designed analysis of a retrospectively collected and observational medical records database.

**25(OH)D levels were drawn at the providers' discretion for clinical indications (eg, osteoporosis risk).

††The 95% confidence intervals are graphically determined.

‡‡Coronary artery calcification was quantified using an electron-beam computed tomography (CT) scanner or a multidetector CT system. Any Agatston score >0 defining the presence of coronary artery calcification.

§§Measurements were completed using the same assay in three batches by estimated GFR. Because measurement batch was determined by estimated GFR, adjustment for batch also adjusts for chronic kidney disease.

‖‖Coronary artery stenosis was evaluated by CT angiography was performed with a 64-slice multidetector-row cardiac CT scanner.

##Weighted means 56.8 y (0.53) for cases and 51.9 y (0.42) for noncases.

***Weighted means 57.7 y (0.76) for cases and 52.5 y (0.39) for noncases.

supplementation on vascular disease mortality and all-cause mortality.[129] The Women's Health Initiative (WHI) reported a nearly statistically significant harmful effect with combined vitamin D_3 and calcium supplementation on a composite cardiac outcome that included nonfatal myocardial infarction, coronary heart disease death, or need for revascularization (relative risk, 1.08; 95% confidence interval, 0.99–1.19).[130] In both studies, participants were followed up for 2 to 7 years but were tested only for 800 and 400 IU of vitamin D_3 daily, respectively, a dose that can increase the serum 25(OH)D concentration only by 2 to 4ng/mL, which is unlikely to be associated with any change in risk of CVD outcomes. Another trial that tested 100 000 IU vitamin D_3 or placebo every 4 months for up to 5 years, with CVD as a secondary outcome, did not show statistically significant results. However, the authors noted that although the concentration of 25(OH)D in the treatment group was 40% higher than placebo, it still did not achieve physiological concentrations.[131] The recently completed PRIMO (Paricalcitol Capsules Benefits Renal Failure Induced Cardiac Morbidity in Subjects With Chronic Kidney Disease Stage 3/4) trial found no change in left ventricular mass index with 48 weeks of paricalcitol treatment (2 μg/d) in patients with CKD and mild to moderate left ventricular hypertrophy, although in post hoc analysis paricalcitol reduced left atrial volume, a measure linked to adverse cardiovascular events.[132]

Elamin et al[133] conducted a systematic review of the literature to summarize the available evidence of the possible cardiovascular benefits of vitamin D supplementation. According to their meta-analysis of 51 trials, vitamin D–raising interventions were associated with an insignificant effect on myocardial infarction (relative risk, 1.02; 95% confidence interval, 0.93–1.13; P=0.64; I^2=0%, representing low inconsistency), stroke (relative risk, 1.05; 95% confidence interval, 0.88–1.25; P=0.59; I^2=15%, representing low inconsistency), lipid fractions, glucose, or blood pressure.

Of note, the long-term effects of high-dose vitamin D supplementation on CVD events (myocardial infarction, stroke, and CVD mortality) are expected to be evaluated by the ongoing Vitamin D and Omega-3 Trial (VITAL), which is a large, randomized, double-blind, placebo-controlled, 2×2 factorial trial of vitamin D (vitamin D_3, 2000 IU/d) and omega-3 fatty acid supplements in the primary prevention of CVD in a multiethnic population of 20 000 US men ≥50 years of age and women ≥55 years of age. The mean treatment period will be 5 years.[134]

Several potential reasons could explain the lack of apparent concordance between the various cross-sectional, longitudinal observational, and randomized studies. The substantial heterogeneity of studies with respect to definition of vitamin D status, age structures, definition and determination of cardiovascular outcome, and exposure definitions and the different

Confounders Adjusted in Analysis	Relative Risk (95% CI) According to 25(OH)D Levels (Range or Median), ng/mL		Limitations
Adjustment for age, sex, season, survey, BMI, smoking, physical activity, alcohol, systolic BP, total cholesterol/HDL, or parental history of MI, CRP, IL-6, sICAM-1, and IP-10	T1: 10.81	1.00	The number of female cases was considerably lower than male cases. No data on comedication influencing bone metabolism and thereby altering vitamin D levels in serum. Lack of adjustment for DM (traditional risk factor for CVD)
	T2: 17.42	0.66 (0.43–1.02)	
	T3: 0.84	0.84 (0.52–1.35)	
	T1: 10.57	1.00	
	T2: 15.86	0.67 (0.35–1.29)	
	T3: 23.43	0.42 (0.19– 0.93)	

adjustments for confounders such as the seasonality of 25(OH) D or even the existence of residual confounding are identified as a significant shortcomings in the literature. Finally, the use of single baseline vitamin D measurements, which may not reflect long-term vitamin D status, and the duration, dosing regimen, intervention formulations, use of concomitant therapies, and methodology used for vitamin D assessment may affect the quality of the intervention studies.

Evaluation and Treatment of Vitamin D Deficiency

The Institute of Medicine and the Endocrine Society Task Force both published recently guidelines for the evaluation, treatment, and prevention of vitamin D deficiency.[11,135] On the basis of their data, the Endocrine Society Task Force defined vitamin D deficiency as a 25(OH)D level <20 ng/mL, vitamin D insufficiency as a 25(OH)D level of 21 to 29 ng/mL, and vitamin D sufficiency as a 25(OH)D level ≥30 ng/mL.[11] However, the Institute of Medicine committee found optimal bone health outcomes with 25(OH)D levels between 16 and 20 ng/mL.[135]

In any case, both committees used skeletal health as an end point, and they both concluded that the available scientific evidence supports a causal role for vitamin D only in skeletal health but is not yet compelling that either confers benefits for or is causally related to non–skeletal health outcomes like CVD. Thus, prescribing vitamin D supplementation for the purpose of preventing CVD beyond recommended daily needs cannot be recommended.

The Endocrine Society Task Force suggests using either vitamin D_2 or vitamin D_3 for the prevention and treatment of vitamin D deficiency. However, there is currently a lack of clarity in the literature as to whether there is a definitive difference in the potency between D_2 and D_3 as modulators of bone metabolism factors, and it is more unclear whether any differences translate to different effects of these 2 forms (D_2/D_3) on cardiovascular risk factors.

Of interest, total 25(OH)D levels have been shown to be correlated with various adverse cardiometabolic risk factors in adolescents, whereas recently published studies aiming to compare prospective distinct associations of 25(OH)D_2 and 25(OH)D_3 with cardiovascular risk factors in younger ages have shown that higher 25(OH)D_3 concentrations are associated with higher levels of high-density lipoprotein cholesterol in childhood and lower fasting insulin in adolescence.[136,137] Moreover, 25(OH)D_2 was inversely associated with apolipoprotein A1 and triglycerides and positively associated with C-reactive protein and IL-6.[138]

Taking into account that pharmaceutical preparations in some countries (ie, the United States) contain only vitamin D_2 whereas multivitamin preparations contain either D_2 or D_3, the identification of possible differences is of great importance in suggesting the use of one or the other form for achieving favorable effects on cardiovascular risk factors. Details on the treatment of vitamin D deficiency in special conditions and the re-evaluation of the subjects supplemented with vitamin D are provided in the online-only Data Supplement.

Conclusions and Future Perspectives

Vitamin D exerts a variety of favorable effects on endothelial dysfunction, VSMC proliferation and migration, and calcification, as well as on the inflammatory/immune process of atherosclerosis; moreover, it exerts beneficial effects against systemic conditions that promote atherosclerosis such as insulin resistance, β-cell dysfunction, dyslipidemia, RAAS, and consequent hypertension, therefore suggesting a potential therapeutic role. Elucidating the molecular foundation of these diverse effects and the possible mediating effects of genetic factors on the relationship between vitamin D and CVD is also of great importance.

Mendelian randomization studies, which are believed to overcome unmeasured confounding, should be conducted to estimate the causal effect of vitamin D status on atherosclerosis and CVD risk.

In addition, rigorous large-scale, randomized, clinical trials to test the effects of vitamin D (D_3 and D_2) on atherosclerosis and CVD as the primary outcome emerge as a particular priority to firmly establish the role of vitamin D supplementation on CVD risk and mortality. Dose-response randomized trials are important to identify threshold effects and possible adverse effects.

Disclosures

None.

References

1. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol*. 2006;6:508–519.
2. Lavie CJ, Lee JH, Milani RV. Vitamin D and cardiovascular disease: will it live up to its hype? *J Am Coll Cardiol*. 2011;58:1547–1556.
3. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States:

data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2007;167:1159–1165.
4. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. *Circulation*. 2008;117:503–511.
5. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-Hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med*. 2008;168:1174–1180.
6. Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, Lappé DL, Muhlestein JB; Intermountain Heart Collaborative (IHC) Study Group. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol*. 2010;106:963–968.
7. van de Luijtgaarden KM, Voute MT, Hoeks SE, Bakker EJ, Chonchol M, Stolker RJ, Rouwet EV, Verhagen HJ. Vitamin D deficiency may be an independent risk factor for arterial disease. *Eur J Vasc Endovasc Surg*. 2012;44:301–306.
8. Fruchart JC, Nierman MC, Stroes ES, Kastelein JJ, Duriez P. New risk factors for atherosclerosis and patient risk assessment. *Circulation*. 2004;109(suppl 1):III15–III19.
9. Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br J Nutr*. 2005;94:483–492.
10. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281.
11. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–1930.
12. Danik JS, Manson JE. Vitamin D and cardiovascular disease. *Curr Treat Options Cardiovasc Med*. 2012;14:414–424.
13. Wallis DE, Penckofer S, Sizemore GW. The "sunshine deficit" and cardiovascular disease. *Circulation*. 2008;118:1476–1485.
14. Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1α,25(OH)$_2$vitamin D$_3$: genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab*. 2011;25:543–559.
15. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. 2010;376:180–188.
16. Zhu H, Wang X, Shi H, Su S, Harshfield GA, Gutin B, Snieder H, Dong Y. A genome-wide methylation study of severe vitamin D deficiency in African American adolescents. *J Pediatr*. 2013;162:1004–1009.e1.
17. Walski M, Chlopicki S, Celary-Walska R, Frontczak-Baniewicz M. Ultrastructural alterations of endothelium covering advanced atherosclerotic plaque in human carotid artery visualised by scanning electron microscope. *J Physiol Pharmacol*. 2002;53(pt 1):713–723.
18. Vanhoutte PM. Endothelial dysfunction and atherosclerosis. *Eur Heart J*. 1997;18(suppl E):E19–E29.
19. Merke J, Milde P, Lewicka S, Hügel U, Klaus G, Mangelsdorf DJ, Haussler MR, Rauterberg EW, Ritz E. Identification and regulation of 1,25-dihydroxyvitamin D3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D3: studies in cultured bovine aortic endothelial cells and human dermal capillaries. *J Clin Invest*. 1989;83:1903–1915.
20. Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, Stewart PM, Hewison M. Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. *J Am Soc Nephrol*. 2002;13:621–629.
21. Molinari C, Uberti F, Grossini E, Vacca G, Carda S, Invernizzi M, Cisari C. 1α,25-Dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. *Cell Physiol Biochem*. 2011;27:661–668.
22. Queen LR, Ji Y, Xu B, Young L, Yao K, Wyatt AW, Rowlands DJ, Siow RC, Mann GE, Ferro A. Mechanisms underlying beta2-adrenoceptor-mediated nitric oxide generation by human umbilical vein endothelial cells. *J Physiol*. 2006;576(pt 2):585–594.
23. Hirata M, Serizawa K, Aizawa K, Yogo K, Tashiro Y, Takeda S, Moriguchi Y, Endo K, Fukagawa M. 22-Oxacalcitriol prevents progression of endothelial dysfunction through antioxidative effects in rats with type 2 diabetes and early-stage nephropathy. *Nephrol Dial Transplant*. 2013;28:1166–1174.
24. Polidoro L, Properzi G, Marampon F, Gravina GL, Festuccia C, Di Cesare E, Scarsella L, Ciccarelli C, Zani BM, Ferri C. Vitamin D protects human endothelial cells from H_2O_2 oxidant injury through the Mek/Erk-Sirt1 axis activation. *J Cardiovasc Transl Res*. 2013;6:221–231.
25. Wong MS, Delansorne R, Man RY, Svenningsen P, Vanhoutte PM. Chronic treatment with vitamin D lowers arterial blood pressure and reduces endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol*. 2010;299:H1226–H1234.
26. Vanhoutte PM, Feletou M, Taddei S. Endothelium-dependent contractions in hypertension. *Br J Pharmacol*. 2005;144:449–458.
27. Wong MS, Delansorne R, Man RY, Vanhoutte PM. Vitamin D derivatives acutely reduce endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol*. 2008;295:H289–H296.
28. Wong MS, Man RY, Vanhoutte PM. Calcium-independent phospholipase A(2) plays a key role in the endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol*. 2010;298:H1260–H1266.
29. Chenevard R, Hürlimann D, Béchir M, Enseleit F, Spieker L, Hermann M, Riesen W, Gay S, Gay RE, Neidhart M, Michel B, Lüscher TF, Noll G, Ruschitzka F. Selective COX-2 inhibition improves endothelial function in coronary artery disease. *Circulation*. 2003;107:405–409.
30. Krishnan AV, Feldman D. Molecular pathways mediating the anti-inflammatory effects of calcitriol: implications for prostate cancer chemoprevention and treatment. *Endocr Relat Cancer*. 2010;17:R19–R38.
31. Equils O, Naiki Y, Shapiro AM, Michelsen K, Lu D, Adams J, Jordan S. 1,25-Dihydroxyvitamin D inhibits lipopolysaccharide-induced immune activation in human endothelial cells. *Clin Exp Immunol*. 2006;143:58–64.
32. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. *FASEB J*. 1995;9:899–909.
33. Martinesi M, Bruni S, Stio M, Treves C. 1,25-Dihydroxyvitamin D3 inhibits tumor necrosis factor-alpha-induced adhesion molecule expression in endothelial cells. *Cell Biol Int*. 2006;30:365–375.
34. Stach K, Kälsch AI, Nguyen XD, Elmas E, Kralev S, Lang S, Weiss C, Borggrefe M, Kälsch T. 1α,25-Dihydroxyvitamin D3 attenuates platelet activation and the expression of VCAM-1 and MT1-MMP in human endothelial cells. *Cardiology*. 2011;118:107–115.
35. Talmor Y, Bernheim J, Klein O, Green J, Rashid G. Calcitriol blunts proatherosclerotic parameters through NFkappaB and p38 in vitro. *Eur J Clin Invest*. 2008;38:548–554.
36. Suzuki Y, Ichiyama T, Ohsaki A, Hasegawa S, Shiraishi M, Furukawa S. Anti-inflammatory effect of 1alpha,25-dihydroxyvitamin D(3) in human coronary arterial endothelial cells: implication for the treatment of Kawasaki disease. *J Steroid Biochem Mol Biol*. 2009;113:134–138.
37. Kudo K, Hasegawa S, Suzuki Y, Hirano R, Wakiguchi H, Kittaka S, Ichiyama T. 1α,25-Dihydroxyvitamin D(3) inhibits vascular cellular adhesion molecule-1 expression and interleukin-8 production in human coronary arterial endothelial cells. *J Steroid Biochem Mol Biol*. 2012;132:290–294.
38. Talmor Y, Golan E, Benchetrit S, Bernheim J, Klein O, Green J, Rashid G. Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. *Am J Physiol Renal Physiol*. 2008;294:F1059–F1064.
39. Zitman-Gal T, Golan E, Green J, Bernheim J, Benchetrit S. Vitamin D receptor activation in a diabetic-like environment: potential role in the activity of the endothelial pro-inflammatory and thioredoxin pathways. *J Steroid Biochem Mol Biol*. 2012;132:1–7.
40. Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 Alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. *Circ Res*. 2000;87:214–220.
41. Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O, Akalin S. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab*. 2009;94:4023–4030.
42. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked

vascular endothelial dysfunction in middle-aged and older adults. *Hypertension*. 2011;57:63–69.
43. Harris RA, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H, Dong Y. Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *Am J Hypertens*. 2011;24:557–562.
44. Doran AC, Meller N, McNamara CA. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2008;28:812–819.
45. Kawashima H. Receptor for 1,25-dihydroxyvitamin D in a vascular smooth muscle cell line derived from rat aorta. *Biochem Biophys Res Commun*. 1987;146:1–6.
46. Somjen D, Weisman Y, Kohen F, Gayer B, Limor R, Sharon O, Jaccard N, Knoll E, Stern N. 25-hydroxyvitamin D3-1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation*. 2005;111:1666–1671.
47. Carthy EP, Yamashita W, Hsu A, Ooi BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. *Hypertension*. 1989;13(pt 2):954–959.
48. Wu-Wong JR, Nakane M, Ma J. Effects of vitamin D analogs on the expression of plasminogen activator inhibitor-1 in human vascular cells. *Thromb Res*. 2006;118:709–714.
49. Mitsuhashi T, Morris RC Jr, Ives HE. 1,25-Dihydroxyvitamin D3 modulates growth of vascular smooth muscle cells. *J Clin Invest*. 1991;87:1889–1895.
50. Chen S, Law CS, Gardner DG. Vitamin D-dependent suppression of endothelin-induced vascular smooth muscle cell proliferation through inhibition of CDK2 activity. *J Steroid Biochem Mol Biol*. 2010;118:135–141.
51. Davies MR, Hruska KA. Pathophysiological mechanisms of vascular calcification in end-stage renal disease. *Kidney Int*. 2001;60:472–479.
52. Rebsamen MC, Sun J, Norman AW, Liao JK. 1Alpha,25-dihydroxyvitamin D3 induces vascular smooth muscle cell migration via activation of phosphatidylinositol 3-kinase. *Circ Res*. 2002;91:17–24.
53. Tukaj C, Trzonkowski P, Pikuła M, Hallmann A, Tukaj S. Increased migratory properties of aortal smooth muscle cells exposed to calcitriol in culture. *J Steroid Biochem Mol Biol*. 2010;121:208–211.
54. Raymond MA, Désormeaux A, Labelle A, Soulez M, Soulez G, Langelier Y, Pshezhetsky AV, Hébert MJ. Endothelial stress induces the release of vitamin D-binding protein, a novel growth factor. *Biochem Biophys Res Commun*. 2005;338:1374–1382.
55. Norman PE, Powell JT. Vitamin D, shedding light on the development of disease in peripheral arteries. *Arterioscler Thromb Vasc Biol*. 2005;25:39–46.
56. Wakasugi M, Noguchi T, Inoue M, Kazama Y, Tawata M, Kanemaru Y, Onaya T. Vitamin D3 stimulates the production of prostacyclin by vascular smooth muscle cells. *Prostaglandins*. 1991;42:127–136.
57. Tukaj S, Trzonkowski P, Tukaj C. Regulatory effects of 1,25-dihydroxyvitamin D3 on vascular smooth muscle cells. *Acta Biochim Pol*. 2012;59:395–400.
58. Zhu H, Guo D, Li K, Pedersen-White J, Stallmann-Jorgensen IS, Huang Y, Parikh S, Liu K, Dong Y. Increased telomerase activity and vitamin D supplementation in overweight African Americans. *Int J Obes (Lond)*. 2012;36:805–809.
59. Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo DH, Thomas J, Pierce GL, White J, Holick MF, Zhu H. A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. *J Clin Endocrinol Metab*. 2010;95:4584–4591.
60. Artaza JN, Norris KC. Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. *J Endocrinol*. 2009;200:207–221.
61. Ha H, Oh EY, Lee HB. The role of plasminogen activator inhibitor 1 in renal and cardiovascular diseases. *Nat Rev Nephrol*. 2009;5:203–211.
62. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135–1143.
63. Chen Y, Kong J, Sun T, Li G, Szeto FL, Liu W, Deb DK, Wang Y, Zhao Q, Thadhani R, Li YC. 1,25-Dihydroxyvitamin D_3 suppresses inflammation-induced expression of plasminogen activator inhibitor-1 by blocking nuclear factor-κB activation. *Arch Biochem Biophys*. 2011;507:241–247.
64. Simon A, Chironi G, Megnien JL. Relevance of screening symptom-free population for coronary and noncoronary calcification burden. *Hypertension*. 2010;55:840–841.
65. Speer MY, Yang HY, Brabb T, Leaf E, Look A, Lin WL, Frutkin A, Dichek D, Giachelli CM. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. *Circ Res*. 2009;104:733–741.
66. Hsu JJ, Tintut Y, Demer LL. Vitamin D and osteogenic differentiation in the artery wall. *Clin J Am Soc Nephrol*. 2008;3:1542–1547.
67. Inoue T, Kawashima H. 1,25-Dihydroxyvitamin D3 stimulates $45Ca^{2+}$-uptake by cultured vascular smooth muscle cells derived from rat aorta. *Biochem Biophys Res Commun*. 1988;152:1388–1394.
68. Jono S, Nishizawa Y, Shioi A, Morii H. 1,25-Dihydroxyvitamin D3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. *Circulation*. 1998;98:1302–1306.
69. Ix JH, Barrett-Connor E, Wassel CL, Cummins K, Bergstrom J, Daniels LB, Laughlin GA. The associations of fetuin-A with subclinical cardiovascular disease in community-dwelling persons: the Rancho Bernardo Study. *J Am Coll Cardiol*. 2011;58:2372–2379.
70. Shalhoub V, Shatzen EM, Ward SC, Young JI, Boedigheimer M, Twehues L, McNinch J, Scully S, Twomey B, Baker D, Kiaei P, Damore MA, Pan Z, Haas K, Martin D. Chondro/osteoblastic and cardiovascular gene modulation in human artery smooth muscle cells that calcify in the presence of phosphate and calcitriol or paricalcitol. *J Cell Biochem*. 2010;111:911–921.
71. Zitman-Gal T, Green J, Pasmanik-Chor M, Oron-Karni V, Bernheim J, Benchetrit S. Vitamin D and vascular smooth muscle cells: gene modulation following exposure to a diabetic-like environment. *J Diabetes Metab*. 2012;3:1–9.
72. Aoshima Y, Mizobuchi M, Ogata H, Kumata C, Nakazawa A, Kondo F, Ono N, Koiwa F, Kinugasa E, Akizawa T. Vitamin D receptor activators inhibit vascular smooth muscle cell mineralization induced by phosphate and TNF-α. *Nephrol Dial Transplant*. 2012;27:1800–1806.
73. Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R, Demer LL. Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation*. 1997;96:1755–1760.
74. Braam LA, Hoeks AP, Brouns F, Hamulyák K, Gerichhausen MJ, Vermeer C. Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. *Thromb Haemost*. 2004;91:373–380.
75. Doherty TM, Tang W, Dascalos S, Watson KE, Demer LL, Shavelle RM, Detrano RC. Ethnic origin and serum levels of 1alpha,25-dihydroxyvitamin D3 are independent predictors of coronary calcium mass measured by electron-beam computed tomography. *Circulation*. 1997;96:1477–1481.
76. McCullough PA, Sandberg KR, Dumler F, Yanez JE. Determinants of coronary vascular calcification in patients with chronic kidney disease and end-stage renal disease: a systematic review. *J Nephrol*. 2004;17:205–215.
77. Memon F, El-Abbadi M, Nakatani T, Taguchi T, Lanske B, Razzaque MS. Does Fgf23-klotho activity influence vascular and soft tissue calcification through regulating mineral ion metabolism? *Kidney Int*. 2008;74:566–570.
78. Razzaque MS, Lanske B. Hypervitaminosis D and premature aging: lessons learned from Fgf23 and Klotho mutant mice. *Trends Mol Med*. 2006;12:298–305.
79. Schoppet M, Shroff RC, Hofbauer LC, Shanahan CM. Exploring the biology of vascular calcification in chronic kidney disease: what's circulating? *Kidney Int*. 2008;73:384–390.
80. Shroff R, Egerton M, Bridel M, Shah V, Donald AE, Cole TJ, Hiorns MP, Deanfield JE, Rees L. A bimodal association of vitamin D levels and vascular disease in children on dialysis. *J Am Soc Nephrol*. 2008;19:1239–1246.
81. Zittermann A, Schleithoff SS, Koerfer R. Vitamin D and vascular calcification. *Curr Opin Lipidol*. 2007;18:41–46.
82. Robertson AK, Hansson GK. T cells in atherogenesis: for better or for worse? *Arterioscler Thromb Vasc Biol*. 2006;26:2421–2432.
83. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci U S A*. 1995;92:3893–3897.
84. Young JL, Libby P, Schönbeck U. Cytokines in the pathogenesis of atherosclerosis. *Thromb Haemost*. 2002;88:554–567.
85. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. *Rheum Dis Clin North Am*. 2012;38:125–139.
86. Smith E, Prasad KM, Butcher M, Dobrian A, Kolls JK, Ley K, Galkina E. Blockade of interleukin-17A results in reduced atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 2010;121:1746–1755.
87. Chang SH, Chung Y, Dong C. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. *J Biol Chem*. 2010;285:38751–38755.

88. Teramoto T, Endo K, Ikeda K, Kubodera N, Kinoshita M, Yamanaka M, Ogata E. Binding of vitamin D to low-density-lipoprotein (LDL) and LDL receptor-mediated pathway into cells. *Biochem Biophys Res Commun*. 1995;215:199–204.
89. Gottfried E, Rehli M, Hahn J, Holler E, Andreesen R, Kreutz M. Monocyte-derived cells express CYP27A1 and convert vitamin D3 into its active metabolite. *Biochem Biophys Res Commun*. 2006;349:209–213.
90. Riek AE, Oh J, Bernal-Mizrachi C. 1,25(OH)2 vitamin D suppresses macrophage migration and reverses atherogenic cholesterol metabolism in type 2 diabetic patients. *J Steroid Biochem Mol Biol*. 2013;136:309–312.
91. Riek AE, Oh J, Sprague JE, Timpson A, de las Fuentes L, Bernal-Mizrachi L, Schechtman KB, Bernal-Mizrachi C. Vitamin D suppression of endoplasmic reticulum stress promotes an antiatherogenic monocyte/macrophage phenotype in type 2 diabetic patients. *J Biol Chem*. 2012;287:38482–38494.
92. Müller K, Haahr PM, Diamant M, Rieneck K, Kharazmi A, Bendtzen K. 1,25-Dihydroxyvitamin D3 inhibits cytokine production by human blood monocytes at the post-transcriptional level. *Cytokine*. 1992;4:506–512.
93. Koyama T, Shibakura M, Ohsawa M, Kamiyama R, Hirosawa S. Anticoagulant effects of 1alpha,25-dihydroxyvitamin D3 on human myelogenous leukemia cells and monocytes. *Blood*. 1998;92:160–167.
94. Aihara K, Azuma H, Akaike M, Ikeda Y, Yamashita M, Sudo T, Hayashi H, Yamada Y, Endoh F, Fujimura M, Yoshida T, Yamaguchi H, Hashizume S, Kato M, Yoshimura K, Yamamoto Y, Kato S, Matsumoto T. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. *J Biol Chem*. 2004;279:35798–35802.
95. Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile: 1,25-dihydroxyvitamin D(3) works as anti-inflammatory. *Diabetes Res Clin Pract*. 2007;77:47–57.
96. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. *Arch Intern Med*. 2008;168:1340–1349.
97. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr*. 2006;83:754–759.
98. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB Sr, Dawson-Hughes B, Ordovas JM, O'Donnell CJ, Kathiresan S, Keaney JF Jr, Vasan RS, Benjamin EJ. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. *Am J Epidemiol*. 2008;167:313–320.
99. Pittas AG, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan DM, Hu FB, Dawson-Hughes B; Diabetes Prevention Program Research Group. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. *Diabetes Care*. 2012;35:565–573.
100. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr*. 2004;79:820–825.
101. Kayaniyil S, Retnakaran R, Harris SB, Vieth R, Knight JA, Gerstein HC, Perkins BA, Zinman B, Hanley AJ. Prospective associations of vitamin D with β-cell function and glycemia: the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study. *Diabetes*. 2011;60:2947–2953.
102. Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, Hewison M. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol*. 2004;89-90:121–125.
103. Cheng Q, Boucher BJ, Leung PS. Modulation of hypovitaminosis D-induced islet dysfunction and insulin resistance through direct suppression of the pancreatic islet renin-angiotensin system in mice. *Diabetologia*. 2013;56:553–562.
104. Ding C, Gao D, Wilding J, Trayhurn P, Bing C. Vitamin D signalling in adipose tissue. *Br J Nutr*. 2012;108:1915–1923.
105. Dunlop TW, Väisänen S, Frank C, Molnár F, Sinkkonen L, Carlberg C. The human peroxisome proliferator-activated receptor delta gene is a primary target of 1alpha,25-dihydroxyvitamin D3 and its nuclear receptor. *J Mol Biol*. 2005;349:248–260.
106. Reusch JE, Begum N, Sussman KE, Draznin B. Regulation of GLUT-4 phosphorylation by intracellular calcium in adipocytes. *Endocrinology*. 1991;129:3269–3273.
107. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. *Bone*. 2012;50:568–575.
108. Ponda MP, Huang X, Odeh MA, Breslow JL, Kaufman HW. Vitamin D may not improve lipid levels: a serial clinical laboratory data study. *Circulation*. 2012;126:270–277.
109. Skaaby T, Husemoen LL, Martinussen T, Thyssen JP, Melgaard M, Thuesen BH, Pisinger C, Jørgensen T, Johansen JD, Menné T, Carlsen B, Szecsi PB, Stender S, Fenger RV, Fenger M, Linneberg A. Vitamin D status, filaggrin genotype, and cardiovascular risk factors: a mendelian randomization approach. *PLoS One*. 2013;8:e57647.
110. Querfeld U, Hoffmann MM, Klaus G, Eifinger F, Ackerschott M, Michalk D, Kern PA. Antagonistic effects of vitamin D and parathyroid hormone on lipoprotein lipase in cultured adipocytes. *J Am Soc Nephrol*. 1999;10:2158–2164.
111. Huang Y, Li X, Wang M, Ning H, A L, Li Y, Sun C. Lipoprotein lipase links vitamin D, insulin resistance, and type 2 diabetes: a cross-sectional epidemiological study. *Cardiovasc Diabetol*. 2013;12:17.
112. Shirts BH, Howard MT, Hasstedt SJ, Nanjee MN, Knight S, Carlquist JF, Anderson JL, Hopkins PN, Hunt SC. Vitamin D dependent effects of APOA5 polymorphisms on HDL cholesterol. *Atherosclerosis*. 2012;222:167–174.
113. Werner C, Pöss J, Böhm M. Optimal antagonism of the renin-angiotensin-aldosterone system: do we need dual or triple therapy? *Drugs*. 2010;70:1215–1230.
114. Vaidya A, Forman JP. Vitamin D and hypertension: current evidence and future directions. *Hypertension*. 2010;56:774–779.
115. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*. 2002;110:229–238.
116. Deb DK, Chen Y, Zhang Z, Zhang Y, Szeto FL, Wong KE, Kong J, Li YC. 1,25-Dihydroxyvitamin D3 suppresses high glucose-induced angiotensinogen expression in kidney cells by blocking the NF-{kappa}B pathway. *Am J Physiol Renal Physiol*. 2009;296:F1212–F1218.
117. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol*. 2004;89-90:387–392.
118. Tomaschitz A, Pilz S, Ritz E, Grammer T, Drechsler C, Boehm BO, März W. Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Clin Chim Acta*. 2010;411:1354–1360.
119. Fleck A. Latitude and ischaemic heart disease. *Lancet*. 1989;1:613.
120. Melamed ML, Michos ED, Post W, Astor B. 25-Hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med*. 2008;168:1629–1637.
121. Bolland MJ, Bacon CJ, Horne AM, Mason BH, Ames RW, Wang TK, Grey AB, Gamble GD, Reid IR. Vitamin D insufficiency and health outcomes over 5 y in older women. *Am J Clin Nutr*. 2010;91:82–89.
122. Semba RD, Houston DK, Bandinelli S, Sun K, Cherubini A, Cappola AR, Guralnik JM, Ferrucci L. Relationship of 25-hydroxyvitamin D with all-cause and cardiovascular disease mortality in older community-dwelling adults. *Eur J Clin Nutr*. 2010;64:203–209.
123. Zhao G, Ford ES, Li C, Croft JB. Serum 25-hydroxyvitamin D levels and all-cause and cardiovascular disease mortality among US adults with hypertension: the NHANES linked mortality study. *J Hypertens*. 2012;30:284–289.
124. Karakas M, Thorand B, Zierer A, Huth C, Meisinger C, Roden M, Rottbauer W, Peters A, Koenig W, Herder C. Low levels of serum 25-hydroxyvitamin D are associated with increased risk of myocardial infarction, especially in women: results from the MONICA/KORA Augsburg case-cohort study. *J Clin Endocrinol Metab*. 2013;98:272–280.
125. de Boer IH, Kestenbaum B, Shoben AB, Michos ED, Sarnak MJ, Siscovick DS. 25-Hydroxyvitamin D levels inversely associate with risk for developing coronary artery calcification. *J Am Soc Nephrol*. 2009;20:1805–1812.
126. Lim S, Shin H, Kim MJ, Ahn HY, Kang SM, Yoon JW, Choi SH, Kim KW, Song JH, Choi SI, Chun EJ, Shin CS, Park KS, Jang HC. Vitamin D inadequacy is associated with significant coronary artery stenosis in a community-based elderly cohort: the Korean Longitudinal Study on Health and Aging. *J Clin Endocrinol Metab*. 2012;97169–178.
127. Grandi NC, Breitling LP, Brenner H. Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. *Prev Med*. 2010;51:228–233.

128. Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med*. 2010;152:307–314.
129. Avenell A, MacLennan GS, Jenkinson DJ, McPherson GC, McDonald AM, Pant PR, Grant AM, Campbell MK, Anderson FH, Cooper C, Francis RM, Gillespie WJ, Robinson CM, Torgerson DJ, Wallace WA; RECORD Trial Group. Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D(3) and/or calcium (RECORD trial). *J Clin Endocrinol Metab*. 2012;97:614–622.
130. LaCroix AZ, Kotchen J, Anderson G, Brzyski R, Cauley JA, Cummings SR, Gass M, Johnson KC, Ko M, Larson J, Manson JE, Stefanick ML, Wactawski-Wende J. Calcium plus vitamin D supplementation and mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomized controlled trial. *J Gerontol A Biol Sci Med Sci*. 2009;64:559–567.
131. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ*. 2003;326:469.
132. Thadhani R, Appelbaum E, Pritchett Y, Chang Y, Wenger J, Tamez H, Bhan I, Agarwal R, Zoccali C, Wanner C, Lloyd-Jones D, Cannata J, Thompson BT, Andress D, Zhang W, Packham D, Singh B, Zehnder D, Shah A, Pachika A, Manning WJ, Solomon SD. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. *JAMA*. 2012;307:674–684.
133. Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A, Erwin PJ, Hensrud DD, Murad MH, Montori VM. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2011;96:1931–1942.
134. Manson JE, Bassuk SS, Lee IM, Cook NR, Albert MA, Gordon D, Zaharris E, Macfadyen JG, Danielson E, Lin J, Zhang SM, Buring JE. The VITamin D and OmegA-3 TriaL (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. *Contemp Clin Trials*. 2012;33:159–171.
135. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Manson JE, Mayne ST, Ross AC, Shapses SA, Taylor CL. IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab*. 2012;97:1146–1152.
136. Parikh S, Guo DH, Pollock NK, Petty K, Bhagatwala J, Gutin B, Houk C, Zhu H, Dong Y. Circulating 25-hydroxyvitamin D concentrations are correlated with cardiometabolic risk among American black and white adolescents living in a year-round sunny climate. *Diabetes Care*. 2012;35:1133–1138.
137. Williams DM, Fraser A, Sayers A, Fraser WD, Hypponen E, Smith GD, Sattar N, Lawlor DA. Associations of childhood 25-hydroxyvitamin D2 and D3 and cardiovascular risk factors in adolescence: prospective findings from the Avon Longitudinal Study of Parents and Children. *Eur J Prev Cardiol*. DOI:10.1177/2047487312465688. http://cpr.sagepub.com/content/early/2012/11/26/2047487312465688.full.pdf+html. November 26, 2012.
138. Williams DM, Fraser A, Sayers A, Fraser WD, Hingorani A, Deanfield J, Davey Smith G, Sattar N, Lawlor DA. Associations of 25-hydroxyvitamin D2 and D3 with cardiovascular risk factors in childhood: cross-sectional findings from the Avon Longitudinal Study of Parents and Children. *J Clin Endocrinol Metab*. 2012;97:1563–1571.

KEY WORDS: atherosclerosis ■ endothelial cells ■ inflammation ■ smooth muscle cells, vascular ■ vitamin D