Purpose of review
To provide an overview of the association between vitamin D deficiency and atherosclerosis.

Recent findings
Vitamin D exerts protective effects on atherosclerosis through multiple mechanisms. It has been shown to protect against endothelial dysfunction, vascular smooth muscle cell proliferation and migration, and modulation of the immune system, as well as the inflammatory response. In addition, vitamin D has been shown to have systemic effects on insulin resistance, dyslipidemia, and hypertension.

Summary
Vitamin D deficiency is widely prevalent in the United States and worldwide. Although deficiency of this fat-soluble vitamin is usually associated with musculoskeletal disorder, it is associated with a wide range of disease processes that include multiple organ systems. Recently, there has been mounting evidence linking vitamin D deficiency to cardiovascular disease and atherosclerosis.

Keywords
atherosclerosis, cardiovascular disease, vitamin D

INTRODUCTION
Vitamin D deficiency occurs in approximately 30–50% of the world’s population [1,2] and is particularly associated with the musculoskeletal system and characterized by rickets in children and osteomalacia or osteoporosis in adults. Although it is true that vitamin D receptors (VDR) are present on osteoblasts, they have also been found in various other sites, including brain, breast, immune cells, pancreas, parathyroid glands, and cardiomyocytes [3].

There is a growing body of evidence linking vitamin D deficiency with cardiovascular disease (CVD) [4,5] and progression of atherosclerosis [6]. Potential consequences of vitamin D deficiency are as follows:

1. musculoskeletal disease,
2. cardiovascular disease,
3. atherosclerosis,
4. inflammation,
5. increased insulin resistance,
6. worsening blood pressure parameters,
7. worsening lipid profile,
8. psychiatric disorders.

Vitamin D has been shown to exert multiple vasoprotective effects by improving endothelial dysfunction, inhibiting vascular smooth muscle cell (VSMC) proliferation and migration, downregulating the inflammatory or immune process [5] and indirectly protecting against atherosclerosis by decreasing insulin resistance, improving lipid profiles, and improving blood pressure parameters [1]. The goal of this manuscript is to provide an overview of the existing literature linking vitamin D deficiency and atherosclerosis.

VITAMIN D METABOLISM
Humans obtain a major portion of their vitamin D stores through sunlight exposure (Fig. 1). Vitamin
D3 (cholecalciferol) is endogenously produced in the skin by photochemical conversion of 7-dehydrocholesterol via the action of the sun’s ultraviolet (UV) B light [7,8]. The first hydroxylation occurs in the liver to produce 25-hydroxy Vitamin D (25(OH)D3), the main circulating metabolite that best reflects vitamin D stores [9]. The active metabolite, 1,25(OH)D3 (calcitriol), is produced after the second hydroxylation in the kidneys [10]. It should be noted that levels of 1,25(OH)D do not correlate with 25(OH)D levels and may remain normal despite low levels of 25(OH)D [11]. Dietary sources of vitamin D include D2 (ergocalciferol) found in plants and D3 found in animals [12]. However, vitamin D absorption through the gastrointestinal tract is only about 50% efficient, and relying on dietary intake may not be sufficient to protect against vitamin D deficiency [13].

Vitamin D functions to maintain serum calcium and phosphate levels by increasing absorption from the gastrointestinal tract and release from the bones [14]. The synthesis of 1,25(OH)D is tightly regulated primarily by serum parathyroid hormone (PTH), hypocalcemia, and hypophosphatemia. Conversely, hypercalcemia, hyperphosphatemia, and elevated fibroblast growth factor-23 inhibit production [15].

VITAMIN D’S EFFECTS ON THE ATHEROSCLEROTIC PROCESS

Atherosclerotic CVD is a major cause of morbidity and mortality in the United States [16]. Over the last few decades, vitamin D has been shown to affect...
atherogenesis through multiple mechanisms. Protective roles of vitamin D on atherosclerosis are as follows:

1. Increases endothelial nitric oxide,
2. Inhibits platelet and leukocyte aggregation and adhesion,
3. Decreases endothelial oxidative stress,
4. Influences vascular muscle tone,
5. Decreases release of vasoconstrictor metabolites,
6. Inhibits release of pro-inflammatory cytokines,
7. Modulates immune response,
8. Inhibits proliferation and migration of vascular smooth muscle cells.

The endothelium is a major regulator of vascular homeostasis and exerts its effects on vasoconstriction, vasodilation, smooth muscle proliferation, inflammation, thrombogenesis, and fibrinolysis [17,18]. As a result, any damage to this cell layer of the vasculature can result in endothelial dysfunction, which may eventually play a role in the development of atherosclerosis.

The endothelial cells in vessel walls express VDR that may exert a protective effect on the development and progression of endothelial dysfunction and subsequent atherosclerosis development through different mechanisms. VDR in endothelial cells expresses 1-alpha-hydroxylase, which allows them to convert 25(OH)D to 1,25(OH)D [19]. 1,25(OH)D acts as a direct transcriptional regulator of nitric oxide synthase in endothelial cells. Deficiency of vitamin D may lead to lower bioavailability of nitric oxide [20]. Furthermore, vitamin D has a direct effect on phosphatidylinositol 3 kinase in endothelial cells [21]. Phosphatidylinositol 3 kinase causes the activation of endothelial nitric oxide synthetase, which in turn catalyzes the production of nitric oxide from l-Arg [22]. Apart from its vasodilatory effects, nitric oxide is a potent inhibitor of platelet and leukocyte aggregation and adhesion, which plays a role in early atherosclerosis development. Vitamin D has also been shown to protect endothelial cells from oxidative stress by counteracting superoxide anion generation, thus suppressing reactive oxygen species and countering apoptosis [23].

There is also evidence to suggest that vitamin D3 inversely influences muscular–vascular tone independently of blood pressure [24]. One proposed mechanism involves a reduction in endothelium-dependent contractions associated with chronic vitamin D intake. An influx of calcium into the endothelial cells results in the activation of calcium-dependent phospholipase A2. Phospholipase A2 converts membrane phospholipids into arachidonic acid, which ultimately produces endothelial-derived contracting factors. There is evidence to suggest that vitamin D may decrease the release of these vasoconstrictor metabolites of arachidonic acid by interfering with the calcium-surring process [23].

Both cyclooxygenase (COX) isoforms, COX-1 and COX-2, play a vital role in the production of endothelial-derived contracting factors [25,26] by catalyzing the conversion of arachidonic acid into endoperoxides, which are then converted into prostaglandins and thromboxane A2 by their respective synthase enzymes, both of which play a role in vascular reactivity and inflammation. Nonselective COX inhibitors have been shown to eliminate endothelium-dependent contractions [27,28]. COX-2 is an inducible enzyme and expression of this isoform is induced by proinflammatory mediators, such as cytokines and reactive oxygen species [29]. While absent in normal tissue, COX-2 expression is prevalent in atherosclerotic vasculature [30,31]. Vitamin D3 has been shown to decrease the expression of COX-2 while increasing the expression of 15-hydroxyprostaglandin dehydrogenase, an enzyme that catalyzes the inactivation of prostaglandins [32]. Additionally, vitamin D3 has also been shown to downregulate the expression of prostaglandin receptors [32]. Similarly, vitamin D3 has been shown to increase the production of prostacyclin in VSMC through the COX pathway [33].

In addition to the endothelium, VSMC, which constitute the medial layer of the arterial wall, play a pivotal role in atherosclerosis through multiple mechanisms. VSMC are major producers of extracellular matrix, which can be modified in response to atherogenic stimuli to increase or decrease lipid content in developing plaques and to express receptors that affect lipid uptake, thereby participating in accumulation of plaque lipid through the production of foam-like cells. VSMC also express multiple adhesion molecules and produce many inflammatory molecules that contribute to the initiation and development of atherogenesis [34].

Similarly to vascular endothelial cells, VSMC also possess VDR, which express 1-alpha-hydroxylase [35]. Vitamin D has antiproliferative effects on VSMCs by inhibiting endothelin-dependent DNA synthesis and cell proliferation and by suppressing activation of cyclin-dependent kinase 2 [36]. Other genomic effects of vitamin D include elastogenesis and immunomodulation [37]. By interacting with the vitamin D response elements in various gene promoter regions, a number of genetic sequences related to arterial wall functions are influenced, including vascular endothelial growth factors, matrix metalloproteinase type 9, myosin, elastin, and type 1 collagen [38–40]. Furthermore, at physiologic doses,
vitamin D inhibits proinflammatory cytokine release, adhesion molecule release, and proliferation and migration of VSMCs, thereby suppressing mechanisms that lead to intimal and medial artery calcification [41]. It should be noted that excess vitamin D, although rarely seen in the general population, could lead to mediacalcinosis through VSMC growth, calcification, and migration [42]. VSMC can undergo osteoblastic differentiation in the presence of vitamin D, resembling embryonic endochondral osteogenesis, leading to vascular calcification [43]. While some studies have shown that vitamin D supplementation was associated with a microvascular calcification [44], others have found no significant effect [45]. In the Framingham Offspring Study, involving more than 1700 participants without a history of CVD, the relationship between serum 25(OH)D and CVD was found to be biphasic, with risk increasing at levels less than 15 and more than 30 ng/ml. This suggests that both vitamin D deficiency and vitamin D excess increase the risk for CVD [46].

Both innate and adaptive cell lines are involved in atherosclerosis. Along with cholesterol infiltrates, atherosclerotic lesions contain macrophages, T cells, and other cells of the immune response [47]. T-helper 1 subtype primarily drives the inflammatory proatherogenic response by producing cytokines, such as interferon-γ, tumor necrosis factor-α and tumor necrosis factor-β, and interleukin-2, which cause macrophage activation, inflammation, and vascular activation [48]. T-helper 2 cells secrete cytokines, such as interleukin-4, 5, 10, 13, and 33, and assist in antibody production by B cells. Although the role of T-helper 2 in atherogenesis is controversial, there is evidence to suggest that T-helper 2-based responses may provide atheroprotection due to downregulation of interferon-γ and due to T-helper 2-related cytokines interleukin-5 and interleukin-33, which appear to have antiatherogenic properties [49]. Vitamin D3 has been shown to inhibit T-cell proliferation, and decrease expression of interleukin-2 and interferon-γ, which play a major role in the T-helper 1 proatherogenic response [50–52]. Furthermore, there is evidence to suggest that vitamin D3 shifts the T-cell response from T-helper 1 to T-helper 2, which may limit the atherogenic response seen with T-helper 1 immunity [53]. Similarly, vitamin D3 may directly regulate B-cell proliferation and antibody production, also contributing to its overall immunomodulating properties [54,55]. However, it should be noted that this effect on B cells may be an indirect consequence of vitamin D3’s effect on T cells as mentioned previously.

Vitamin D3 also exerts inhibition on the nuclear factor kappa-B (NF-κB) signaling of proinflammatory cytokines, making it a potent anti-inflammatory agent [56,57]. In monocytes and macrophages, which eventually become foam-cells in atherosclerotic lesions, vitamin D may lower acetylated and oxidized low-density lipoprotein uptake, thus reducing foam-cell formation [58]. Vitamin D inhibits tumor necrosis factor-α, which also restricts monocyte migration and transformation into foam-cells [59]. Finally, by downregulating NF-κB activity, platelet activation may be decreased [60]. Vitamin D3 may also regulate platelet activation by reducing the expression of vascular adhesion molecule-1 and membrane type 1 matrix metalloproteinase in endothelial cells [61].

Vitamin D also indirectly protects against atherosclerosis by exerting a protective effect against a variety of systemic conditions, namely insulin resistance, dyslipidemia, and renin–angiotensin–aldosterone system (RAAS) hypertension. Vitamin D deficiency has been linked to type 2 diabetes mellitus and glucose intolerance by affecting insulin sensitivity and/or β-cell function [62]. Furthermore, VDRs are present in pancreatic β-cells and function as a transcription factor for normal insulin secretion when bound with 1,25(OH)D [63]. In fact, there may be some evidence to suggest that higher baseline 25(OH)D levels are an independent predictor of better β-cell function and better glucose control [64]. It has been shown that taking more than 800 IU vitamin D daily when compared with less than 400 IU may reduce the risk of type 2 diabetes mellitus [65]. Finally, vitamin D may protect β-cells from the immune system by regulating the effects of B-lymphocytes, a variety of T cells, macrophages, and dendritic cells [66].

An association between vitamin D deficiency and dyslipidemia was shown in a study that correlated vitamin D to increased high-density lipoprotein and total cholesterol [67]. Although vitamin D levels correlate to favorable lipid profiles in some studies, the translation into clinical results is yet to be determined.

The RAAS has a profound impact on the cardiovascular system. 1,26(OH)D3 is a negative endocrine regulator of renin production, and there is evidence that demonstrates an inverse relationship between 1,25(OH)D3 levels and plasma renin levels in hypertensive individuals [68,69]. In fact, in a large study (>3000 individuals), low serum levels of 25(OH)D and 1,25(OH)2D were independently associated with upregulated circulating RAAS [70]. One possible mechanism involves 1,25(OH)D3 inhibition of renin gene transcription by blocking the formation of the cyclic adenosine monophosphate

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response element in the renin gene promoter [71]. Vitamin D deficiency also results in high PTH levels, which may be related to elevated blood pressure [72]. In a small study of healthy individuals (10 males), physiologic-dose PTH infusions under hyperinsulinemic conditions lead to elevation in mean arterial pressures [73]. Despite this, the relationship between PTH and arterial hypertension is still unclear. Finally, vitamin D response elements may repress both renin and angiotensinogen on the transcription level by blocking the NF-κB pathway [74].

**VITAMIN D DEFICIENCY**

The most profound skeletal manifestations of vitamin D deficiency are rickets in children and osteomalacia in adults [75]. Individuals who live at a greater distance from the equator are at an increased risk for vitamin D deficiency due to atmospheric filtering of UVB radiation [76]. Furthermore, increased skin pigment greatly reduces the amount of UV-mediated synthesis of vitamin D3, and these individuals require substantially more sunlight exposure to synthesize the same amount of vitamin D3 when compared with individuals with less skin pigmentation [77]. Current recommendations recently published by the United States Institute of Medicine and the Endocrine Society Task Force are based on these skeletal outcomes, as the evidence for nonskeletal outcomes is insufficient to inform nutritional requirements [78]. The Institute of Medicine states that individuals with serum 25(OH)D concentrations less than 30 nmol/l are at risk for deficiency. Furthermore, at levels ranging from 30 to 50 nmol/l, some individuals are potentially at risk for inadequacy. At levels of at least 50 nmol/l, practically all individuals are at sufficiency [79]. Some argue that, because of the lack of randomized controlled trials, the recommendation for a daily intake over 1500 IU or serum 25(OH)D levels of 30 ng/ml or higher, as suggested by the Endocrine Society Task Force, is premature [80]. Finally, it has been shown that, when comparing 62–80-year-old individuals with 20–30-year-olds, there may be over a four-fold difference in serum cholecalciferol levels induced by standard UVB radiation exposure [81]. This suggests that supplementation requirements may vary by age as cutaneous synthesis of vitamin D decreases with increasing age [82].

**CONCLUSION**

Vitamin D exerts protective effects against atherosclerosis by protecting against endothelial dysfunction, VSMC proliferation and migration, and modulating the inflammatory or immune process. It also has systemic effects on insulin resistance, dyslipidemia, and hypertension. These favorable effects suggest potential therapeutic use for vitamin D in nonskeletal scenarios. However, further randomized controlled trials are needed to define clear clinical parameters regarding vitamin D supplementation in the setting of atherosclerosis and CVD.

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**Conflicts of interest**

Dr Menezes, Ms Lamb, and Dr Lavie have no conflicts of interest. Dr DiNicolantonio works for a company that sells vitamin D products but does not personally profit from their sales.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


