THE ROLE OF VITAMIN D IN ARTERIAL STIFFNESS

A potential target in the treatment of CKD-related cardiovascular complications

Master essay
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Abstract

Chronic kidney disease (CKD) is a major cause of mortality worldwide, largely due to an increased risk of cardiovascular disease (CVD). Abnormalities of bone and mineral metabolism are strongly involved in the increased cardiovascular and CKD mortality. Notably, vitamin D deficiency is frequently found in CKD and CVD patients. Another important predictor of all-cause mortality in CVD and patients undergoing kidney transplantation is arterial stiffness. Moreover, many risk factors of CKD and CVD, such as diabetes and hypertension are associated with arterial stiffness. Interestingly, several studies reported a link between vitamin D deficiency and arterial stiffness. Vitamin D supplementation has shown to improve endothelial function and arterial stiffness in animal models. In contrast, some studies demonstrated increased serum vitamin D levels to be associated with increased risk of hypertension and vascular stiffening. This suggests, although several studies investigated the association between vitamin D and arterial stiffness, the exact link and underlying mechanisms remain to be unraveled. Therefore, this review aimed to evaluate the currently-available literature on the link between vitamin D and arterial stiffness. Herein, we gave an overview of the most important studies concerning vitamin D supplementation and arterial stiffness. To further demonstrate the involved pathogenesis, we focused on the role of the endothelium.

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Introduction

CKD: a global health problem

Chronic kidney disease (CKD) is one of the major causes of mortality worldwide. Furthermore, the incidence of CKD is on the rise. According to the 'Global burden of disease study', age-standardized mortality as result of CKD has increased by 36.9% in the past 25 years. (*Rhee CM et al, 2015*). Early-stage CKD is often asymptomatic or less symptomatic, unlike advanced-stage CKD, but it accounts for about 90% of all cases (*Jha V et al, 2013*).

CKD is defined as kidney damage or decreased kidney function, for a period of at least three months, regardless of the underlying cause. Often, kidney damage results in proteinuria; the loss of protein in the urine(*Cozzolino M et al, 2015, Hemmelgarn BR et al, 2010*). Based on the degree of proteinuria and renal function, expressed as the Glomerular Filtration Rate (GFR), CKD is subdivided into five stages. The fifth stage is termed end-stage renal disease (ESRD), which requires renal replacement therapy such as dialysis or renal transplantation (*Ojo A et al, 2014*).

The high risk of mortality in CKD patients is largely due to an increased risk of cardiovascular disease (CVD). Cardiovascular mortality among late-stage CKD patients is 10 to 30 times greater than among the general population and CKD is more likely to result in death than in the progression to ESRD (*Jha V et al, 2013*). This link between CKD and CVD is partly explained by strong associations between CKD and traditional cardiovascular risk factors (*Kovesdy CP et al, 2013*). For example, hypertension and diabetes are major risk factors for CKD. Similarly, CKD is linked to high cholesterol and obesity (*Jha V et al, 2013*). Nevertheless, these traditional risk factors do not fully explain the high mortality rate in CKD.

Mineral and bone disorders in CKD

Surprisingly, despite the high prevalence of traditional CVD risk factors, common strategies to reduce cardiovascular risk in the general population have shown only limited benefits in CKD patients (*Fellström BC et al, 2009*). Hence, there is an ongoing search for new risk factors contributing to CVD in CKD. Notably, abnormalities of mineral and bone metabolism are strongly associated with increased cardiovascular and CKD mortality (*Kovesdy CP et al, 2013*). These abnormalities, such as hyperphosphatemia, hypo- and hypercalcemia are grouped together under the name CKD-related Mineral and Bone Disorders (CKD-MBD). Primarily, CKD-MBD is characterized by impaired phosphate homeostasis and changes in vitamin D metabolism (*Liu WC et al, 2015, Pavlovic D et al, 2015*).

Although the pathophysiology of CKD-MBD is complex, vitamin D appears to play an important role in this disorder (*Parikh C et al, 2015*). Indeed, vitamin D deficiency is frequently found among patients suffering from CKD and CVD (*Liu WC et al, 2015, Pavlovic D et al, 2015*). Low vitamin D levels are common in early stages of CKD and the deficiency tends to become more severe as kidney disease progresses. This link is important, because vitamin D deficiency affects more than one billion people worldwide (*Liu WC et al, 2015, Pavlovic D et al, 2015, Yuste C et al, 2015*).

Vitamin D physiology

Vitamin D is obtained from two different sources: through nutrition and through endogenous synthesis in the skin. When the skin is exposed to ultraviolet B radiation, 7-dehydrocholesterol is converted into previtamin D, followed by conversion of previtamin D to vitamin D3 (cholecalciferol). Vitamin D3 then binds to vitamin D binding protein (VDBP) and is transported to the liver by the circulatory system. Diet-obtained vitamin D, ergocalciferol (from plant origin) and cholecalciferol (from animal origin), is taken up from the gastrointestinal tract. Next, it is transported to the liver, where the metabolites are converted into 25-hydroxyvitamin D by vitamin D-25-hydroxylase (*Liu WC et al, 2015*). When the complex formed by 25-hydroxyvitamin D and VDBP reaches the glomerulus in

the kidneys, the complex is filtered. Next, the filtrated complex enters the renal proximal tubular cells via endocytosis through megalin receptor. The enzyme 1α-hydroxylase facilitates the conversion of 25-hydroxyvitamin D to calcitriol (1,25-dihydroxyvitamin D), the active form of vitamin D, which is then transported back into the circulation (*Liu WC et al, 2015, Liu WC et al, 2015*). This active form of vitamin D has a shorter half-life, between 8 to 12 hours. However, compared to 25-hydroxyvitamin D, its affinity for specific binding with the vitamin D receptor (VDR) is much stronger. Since the expression of 1α-hydroxylase is not restricted to the kidneys, the conversion of 25-dydroxyvitamin D to 1,25-dihydroxyvitamin D also occurs in extra-renal tissues, where it functions as both autocrine and paracrine hormone (*Liu WC et al, 2015*).

Vitamin D is an important regulator of mineral homeostasis. As such, it affects bone, the kidneys, as well as extra-renal organs. It does so through a complex feedback loop involving phosphate, calcium and parathyroid hormone (PTH). The intestinal epithelial cells contain transient receptor potential vanilloid channels type 5 and 6, which facilitate the absorption of phosphate and calcium by vitamin D. PTH regulates the synthesis of vitamin D through increasing the activity of 1α-hydroxylase, resulting in an increase of vitamin D levels in the kidneys. Alternatively, when vitamin D levels are elevated, this process is inhibited by a negative feedback loop in which vitamin D decreases the secretion of PTH (*Liu WC et al, 2015, Pavlovic D et al, 2015, Liu WC et al, 2015*). Conversely, hypocalcemia leads to the secretion of PTH to stimulate the reabsorption of calcium and the release of phosphate and 1α-hydroxylase, which enhances the synthesis of vitamin D. Thus, serum levels of PTH, phosphate and calcium all are involved in the regulation of vitamin D, phosphate and calcium homeostasis (*Liu WC et al, 2015*).

The effects of the active form of vitamin D are mediated by the binding to the VDR. This receptor consists of a ligand binding domain, DNA binding domain (DBD), retinoid X receptor (RXR) and regulating proteins. The ligand binding domain affects VDR conformation and induces its activation, whereas RXR acts as a co-receptor. The DBD modulates the interaction of VDR with DNA sequences located in vitamin D promoter region. The VDR regulating proteins function either as co-activators in order to promote gene transcription, or as co-repressors which involves down-regulation of transcription (*Liu WC et al, 2015*).

Vitamin D deficiency in CKD

Vitamin D deficiency is a notable problem in patients with CKD stage 3-5. Multiple factors contribute to the low level of vitamin D in CKD patients. Primarily, the progression of CKD is associated with a reduction in kidney mass. This reduction in functional kidney tissue leads to reduced bioavailability of 1α-hydroxylase, which affects the production of active vitamin D (*Liu WC et al, 2015, Pavlovic D et al, 2015*). However, several other factors contribute to vitamin D deficiency. These factors include the renin-angiotensin system (RAS) and fibroblast growth factor 23 (FGF23) (*Santoro D et al, 2015*).

In general, the RAS regulates blood pressure and blood volume homeostasis. The activation of RAS starts with renin, which is secreted by the kidney, followed by a cascade resulting in angiotensin-2 (Ang2) as the end product. Ang2 is the main effector hormone of the RAS and it acts on several organs, such as the kidneys, heart, brain, adrenal glands and the vascular system. Recent studies demonstrated that vitamin D interacts with RAS, including renal and systemic effects associated with hypertension and proteinuria (*Santoro D et al, 2015*).

FGF23 is normally produced by osteocytes and osteoblasts as a result of enhanced vitamin D levels to induce phosphaturia. However, in CKD, FGF23 rises to high levels early in the course of the disease. As a consequence, the activity of 1α-hydroxylase decreases, resulting in reduced production of active vitamin D. Additionally, elevated levels of FGF23 contribute to the pathologic state of CKD-MBD, by impairing calcium and phosphorus homeostasis, thus inducing secondary hyperparathyroidism, elevated bone remodelling and vascular calcification (*Liu WC et al, 2015, Cernaro V et al, 2016*).

Furthermore, increased FGF23 levels are linked to adverse cardiovascular outcomes in CKD patients, such as left ventricular hypertrophy, endothelial dysfunction, atherosclerosis and arterial stiffness (*Ding HY et al, 2015, Freundlich M et al, 2013*).

Arterial stiffness and vitamin D deficiency

Arterial stiffness is a major contributor to mortality among CKD and CVD patients. CKD patients have stiffer vessels than the general population, resulting in less arterial compliance (*Toussaint ND et al, 2007*). Arterial stiffness is a major risk factor for cardiovascular events and also an important predictor of all-cause mortality found among patients undergoing kidney transplantation. Several traditional risk factors, such as diabetes and hypertension, as well as non-traditional risk factors such as reduced GFR and systemic inflammation are associated with arterial stiffness (*Bargnoux AS et al, 2015*).

Vascular calcification is an important cause of arterial stiffness, through a process characterized by arterial remodelling and changes in the intrinsic properties of the arterial wall. CKD promotes uraemia, which in turn, induces architectural abnormalities. These abnormalities include increased extensive calcification, but also extracellular matrix and fibro-elastic intimal thickening. As a consequence, both the intimal and medial layers of the vasculature are affected. Calcification formed in the medial layer is the most important contributor to arterial stiffness (*Toussaint ND et al, 2007*).

Interestingly, vitamin D deficiency is linked to arterial stiffness (*Chang J et al, 2015*). Several studies reported that low serum vitamin D levels are associated with increased arterial stiffness. Clinical trials and animal models showed that vitamin D supplementation is associated with improved endothelial function and reduced arterial stiffness. However, some papers demonstrated vitamin D overload to be causative for vascular stiffening and increased risk for hypertension (*Giallauria F et al, 2012*).

Although several published studies have investigated the associations of vitamin D and arterial stiffness, the underlying mechanisms remain to be elucidated. In light of the high cardiovascular mortality in CKD patients, it is of great importance to gain further insight in this association. This review aims to evaluate the currently-available literature about the link between vitamin D and arterial stiffness. To do so, we have performed a literature survey using the pubmed database. Briefly, in this review, we will give an overview of studies regarding vitamin D supplementation and arterial stiffness. We will provide an overview of the most important results of these studies and will compare and discuss the findings.

Research findings

Early studies on arterial stiffness and vitamin D

One of the earliest study on arterial wall properties and vitamin D was conducted in dogs. This study examined the effect of vitamin D3-induced calcinosis on aortic elastic behaviour. Nine healthy dogs (between 48-72 months old) were instrumented with a pair of ultrasonic diameter dimension gauges and a pressure microtransducer in the thoracic aorta. Five of them received 1,25-dihydroxycholecalciferol and a normal diet for ten days, while the remaining four dogs were controls. At the end, aortic wall stiffness and pulse pressure were measured (*Cabrera Fischer EI et al, 1991*). In the experimental group, a decrease in aortic pulse pressure along with an increase in serum calcium concentration were found. Vitamin D treatment reduced aortic stiffness and this was correlated with serum calcium levels and aortic pulse pressure. The results show that high doses of vitamin D3 induced calcinosis but no aortic wall thickening. Surprisingly, although severe experimental calcinosis induces calcium deposition in the arterial walls, this was accompanied by reduced arterial rigidity, primarily due to alteration of vessel wall collagen elasticity (*Cabrera Fischer EI et al, 1991*).

Later, more clinical studies were conducted to gain more insights on the specific role of vitamin D in mineral metabolism and arterial disorders. A cross-sectional study, performed in fifty-two stable and uncomplicated ESRD patients on hemodialysis examined the relationship between arterial alterations and mineral metabolism parameters, including serum PTH and vitamin D. Aortic stiffness was measured using aortic pulse wave velocity (PWV). Additionally, brachial artery distensibility, flow-mediated dilation (FMD) and the presence of arterial calcification were determined in patients (*London GM et al, 2007*). After adjustment for blood pressure and age, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 were negatively correlated with aortic PWV, while positively correlated with brachial artery distensibility and FMD. This indicated that low vitamin D has been associated with increased arterial stiffness and impaired endothelial function. Furthermore, patients with higher arterial calcification scores had lower serum 25-hydroxyvitamin D, however, this association was abolished after adjustment for age. Based on the results, the authors conclude arterial dysfunction in ESRD patients is linked to vitamin D deficiency (*London GM et al, 2007*).

Vitamin D supplementation: inconsistent associations

Cholecalciferol supplementation is associated with improved endothelial function and reduced local vascular stiffness. A clinical study, conducted in forty-one pediatric CKD patients with low 25-hydroxyvitamin D levels, and twenty-four vitamin D-deficient healthy patients, investigated the impact of cholecalciferol on cardiac function, local arterial stiffness and endothelial function. All subjects were treated with a single, high dose of 300,000 IU cholecalciferol. After a follow up period of twelve weeks, endothelium-dependent FMD, common carotid arteries distensibility and arterial stiffness measurements were performed, along with blood parameters such as serum PTH and 25-hydroxyvitamin D levels (*Aytaç MB et al, 2015*). The baseline mean 25-hydroxyvitamin D level was similar in the patient and the control group. The patient group had lower distensibility values for carotid arteries, while arterial stiffness index was increased with respect to the control group. Cholecalciferol treatment induced increased serum vitamin D levels in both groups. However, cholecalciferol supplementation increased FMD and endothelium-independent FMD only in children with CKD. The patient group showed a rise in arterial distensibility and a fall in the arterial stiffness index after cholecalciferol treatment. Altogether, the study concludes that oral cholecalciferol treatment improves endothelial dysfunction and local vascular stiffness in children with CKD and low vitamin D (*Aytaç MB et al, 2015*).

Furthermore, vitamin D supplementation has been shown to suppress oxidative stress and inflammatory mediators of arterial stiffness. A double blind, randomized, placebo controlled study in 130 obese participants with hypertension and low serum vitamin D level was performed to

investigate whether high dose vitamin D supplementation affects inflammatory and oxidative mediators of arterial stiffness. Participants received a monthly dosage of 100,000 IU of vitamin D3 for three months, in which control subjects received placebo. Vascular function, through PWV and endothelial function, using FMD were assessed (*Martins D et al, 2014*). Vitamin D supplementation induced a decrease in serum PTH and urinary isoprostane (a marker of oxidative stress) (*Basarici I et al, 2008*). However, vitamin D supplementation reduced PWV only among participants in the highest tertile by level of urinary isoprostane. This finding indicates that the beneficial effects of a short-term, high dose vitamin D treatment on vascular function are more apparent in patients with excessive oxidative stress (*Martins D et al, 2014*).

Interestingly, a recent study analysed the effect of a high versus low dose vitamin D administration in untreated hypertensive and vitamin D-deficient individuals, with a follow up period of six months. In accordance with the previous study, high dosage of vitamin D supplementation lowered surrogate markers of arterial stiffness but not central PWV. Participants were randomized and supplemented either with a high dose (4000 IU/day) or low dose (400 IU/day) cholecalciferol (*Zaleski A et al, 2015*). Cardiovascular endpoints including blood pressure, PWV and augmentation index (AIx) (a measure for arterial stiffness) were determined. Mean blood pressure was not different between high dose and low dose groups. The group treated with a high dose vitamin D showed decreased AIx, only when not adjusted for heart rate, whereas no improvement of AIx was observed among participants in the low dose group. There was an association between PWV and serum 25-hydroxyvitamin D levels. Thus, the pleiotropic beneficial effect of vitamin D on arterial stiffness appears to be dose-dependent (*Zaleski A et al, 2015*).

Vitamin D supplementation does not improve arterial stiffness

In contrast to the beneficial effects of vitamin D supplementation shown in these previous studies, several other studies did not find effects of vitamin D treatment on arterial stiffness (*Gepner AD et al, 2012, Mose FH et al, 2014, Stricker H et al, 2012, Veloudi P et al, 2015,*). A randomized, placebo-controlled, double-blind study, in 56 patients on chronic dialysis showed that cholecalciferol treatment for six months did not improve arterial stiffness and cardiac function. Subjects received 3000 IU cholecalciferol or placebo every day for six months. Variables, such as blood pressure, PWV and AIx and structural parameters, such as left ventricular mass index (LVMI) and LV wall thickening were determined. Baseline blood pressure, PWV, AIx and LVMI were not different between the groups. Similarly, these parameters were not affected by vitamin D treatment. Thus, six months cholecalciferol supplementation in individuals on chronic dialysis does not improve arterial stiffness and cardiac function (*Mose FH et al, 2014*).

Vitamin D promotes arterial stiffening

Making the story even more intricate, some studies even report adverse outcomes of vitamin D supplementation (*Fortier C et al, 2014, Richart T et al, 2007*). An observational, longitudinal study in 85 individuals, undergoing chronic hemodialysis examined the impact of active vitamin D treatment on the progression of aortic stiffness with a follow-up of 1,2 years . Subjects were treated either with a low dose (<2µg/week) or a high dose (≥2µg/week) alfacalcidol. Results showed that the progression of PWV was remarkable in both groups (*Fortier C et al, 2014*). Individuals receiving a high dose alfacalcifedol therapy had accelerated progression of PWV. Furthermore, AIx was increased in both groups, and it increased to a greater extend in the group subjected to the high dose. Moreover, adjustments for changes in blood pressure and other factors including PTH, 25-hydroxyvitamin D and FGF23 showed that aortic stiffness was still more progressed in the group treated with high dose of alfacalcidol. This indicates that therapy with high dose of alfacalcifedol in hemodialysis patients is linked to an accelerated progression of aortic stiffening (*Fortier C et al, 2014*).

A report on renal versus extrarenal activation of vitamin D regarding atherosclerosis, arterial stiffening and hypertension gave more insights on the adverse effects of vitamin D in cardiovascular

parameters. Interestingly, the infiltration and accumulation of macrophages in atherosclerotic plaques can induce the activation of vitamin D. Extrarenally, in sites of inflammation, macrophages can express 1α-hydroxylase, leading to the activation of calcitriol. Calcitriol is a vasoactive and pro-oxidative substance when it comes to vascular smooth muscle cells (VSMCs). The activation of calcitriol might facilitate deleterious effects on the function and structure of the arterial media. This leads to arterial calcification, followed by arterial stiffening and hypertension (*Richart T et al, 2007*).

In vitro studies, using VSMCs described three mechanisms by which vitamin D involves the development of arterial lesions. Firstly, vitamin D stimulates the activation of L-type calcium channels. This results in an increased contractile response to vasoconstrictors and calcium deposition on elastin fibers. The second pathway occurs through the inhibition of PTH related peptide transcription and stimulation of osteopontin and vitamin D, which promotes arterial calcification. The third mechanism in which vitamin D is associated with arterial lesions, is through the activation of p38 mitogen-activated protein kinase and phosphatidyl-inositol kinase, that causes cell differentiation, cell migration and oxidative stress. This process is partly induced by growth factors and cytokines such as Ang2, finally, promoting structural disintegration and arterial wall stiffening (*Richart T et al, 2007*).

In spite of the inconsistent outcomes found in studies on vitamin D supplementation and arterial stiffness, many studies confirmed that the endothelium is a crucial component in maintaining vascular function. Moreover, studies showed that vitamin D exerts its cardiovascular effects through the preservation of endothelial function (*Mozos I et al, 2015*). With this in mind, we continue on the impact of vitamin D on endothelial function, to find any associations with arterial stiffness.

Vitamin D and endothelial function

Vitamin D and endothelium-dependent vasodilation

A recent study reported beneficial effects of vitamin D therapy on the cardiovascular system, through the endothelium. Paricalcitol (a synthetic activated form of vitamin D) treatment improves endothelium-dependent vasodilation in CKD patients. The paricalcitol and endothelial function in chronic kidney disease (PENNY) study, has tested the effect of active vitamin D on endothelium-independent and –dependent vasodilatation, in a double-blind, randomized, controlled trial setting. 89 Patients with stage 3 to 4 CKD were subjected to a daily dose of 2,0 μg paricalcitol or placebo for twelve weeks. Blood pressure and heart rate were assessed in all participants (*Zoccali C et al, 2014*). Vascular function was determined using endothelium-dependent and –independent vasodilation measurement. Biochemical parameters, such as plasma PTH and 1,25-dihydroxyvitamin D were assessed as well. Paricalcitol supplementation markedly reduced PTH and suppressed serum 1,25-dihydroxyvitamin D level. Vascular measurements showed increased FMD in paricalcitol-treated patients, but endothelium-independent vasodilation remained unaffected by paricalcitol. Thus, this study shows that paricalcitol treatment in CKD patients improves endothelium-dependent vasodilation without affecting endothelium-independent vasodilation (*Zoccali C et al, 2014*).

Vitamin D affects cardiovascular risk factors trough endothelial function

Vitamin D deficiency is known to be associated with endothelial dysfunction and arterial stiffness in healthy individuals as well as CKD patients (*Andrukhova O et al, 2013*). Vitamin D is described as an endothelium-protective agent (*Alyami A et al, 2014*). Vitamin D affects the vessel wall directly by the presence of the specific endothelial enzyme 1α-hydroxylase (*Brewer LC et al, 2011*). The endothelium is able to convert 25-hydroxivitamin D to the active form of vitamin D by 1α-hydroxylase (*Alyami A et al, 2014*). This conversion modulates the inhibition of antigen-induced cytokine-mediated endothelial cell activation and the expression of TNF-α adhesion molecule, accompanied with increased NO production, decreased oxidative stress, vascular cell adhesion molecules (VCAM), intracellular adhesion molecules (ICAM) and IL-6 (*Alyami A et al, 2014, Brewer LC et al, 2011*). This indicates that

the overall effects of vitamin D on cardiovascular risk factors might be achieved through the reduction of systemic inflammation and endothelial dysfunction (*Alyami A et al, 2014*).

The role of the endothelium in arterial stiffening

Endothelial function and vascular stiffness

The endothelium is a monolayer of cells that covers the luminal side of blood vessels. It functions as a structural barrier between blood and the vessel wall, in order to prevent adhesion and aggregation of platelets and leukocytes, control permeability to plasma compounds and to maintain blood flow (*Marti CN et al, 2012*). Nitric oxide (NO) is the predominant agent released by the endothelium, which diffuses to the vessel wall to induce VSM dilatation and myofibrillar relaxation. Additionally, the endothelium exerts anti-proliferative and anti-inflammatory effects (*Marti CN et al, 2012*). In pathologic conditions, the endothelium fails to mediate vasodilation responses, partially due to an imbalance between NO and vasoconstricting hormones (*Shirwany NA et al, 2010*). In such a state, mainly inflammatory responses promote atherogenesis, enhancing oxidative stress while reducing the production of NO. As a result, endothelial dysfunction triggers down regulation of eNOS and further stimulates oxidative stress. This all contributes to impaired arterial distensibility and vascular stiffness (*Marti CN et al, 2012*).

An important but complex feedback loop exists between the endothelium and arterial stiffness. Endothelial cells respond to alterations in arterial stiffness by structural and biochemical adaptations. However, these structural and biochemical adaptations of endothelial cells and VSMCs further contribute to cellular contractility, leading to vascular wall stiffening (*Huveneers S et al, 2015*). This vicious cycle explains that vascular stiffening impairs endothelial function and this in turn, promotes arterial stiffness (*Shirwany NA et al, 2010*).

VDR and NOS

Vitamin D-mediated VDR signalling plays an important role in the preservation of vascular function. VDR is widely expressed throughout the body and it has a broad spectrum of impacts on many different cell types, including VSMC and the endothelium (*Andrukhova O et al, 2013, Brewer LC et al, 2011*). In the vasculature, endothelial nitric oxide synthase-3 (NOS3) is one of the main sources of NO. Interestingly, in VDR mutant mice aortas, mRNA and protein expression of NOS3 are reduced. Wild-type aorta rings treated with vitamin D showed increased NOS3 expressions, but this effect was absent in aortas lacking VDR. Hence, the loss of VDR signalling impairs aortic NOS3 expression and endothelial NO production, leading to a reduction in NO bioavailability. As a result, the VDR mutant mice developed arterial stiffening and increased arterial pulse pressure. Thus, vitamin D influences endothelial function through a VDR- and NOS-dependent mechanism (*Andrukhova O et al, 2013*). In another *in vitro* study, the administration of VDR-specific ligands resulted to the activation of eNOS and NO (*Molinari C et al, 2011*). In addition to the effects of VDR on endothelial function, VDR knockout in mice is linked to elevated activation of RAS, by increased expression of renin and Ang2 production (*Brewer LC et al, 2011*). Vitamin D is directly involved in the negative endocrine regulation of the RAS (*Li YC et al, 2002*).

The effect of vitamin D on the RAS: an alternative pathway

Clinical studies show an inverse relationship between the activity of RAS and plasma vitamin D concentrations. Renal-protective effects of vitamin D or VDR activators are partially mediated by alterations in RAS. For example, VDR activators exert direct effects on the RAS, suppressing the gene transcription of renin (*Li YC et al, 2002, Yuan W et al, 2007*). Furthermore, vitamin D has shown to decrease renin levels through the suppression of renin gene promoter activity (*Li YC et al, 2002*). Moreover, clacitriol has shown to reduce angiotensin-1 receptor expression in endothelial cells, located in renal arteries of hypertensive patients. As a consequence, endothelial function was improved, accompanied with reduced ROS production. In this way, calcitriol is involved in restoring the endothelium-dependent relaxation in the kidney and normalizing proteins associated with

oxidative stress (*Mozos I et al, 2015*). Further elucidating the interaction between vitamin D and the RAS, mice treated with strontium to block the biosynthesis of 1,25-dihydroxyvitamin D had increased renin expression on mRNA levels. Supplementing those mice with 1,25-dihydroxyvitamin D decreased renal expression of renin. Since vitamin D also decreased renin expression in vitamin D-treated cultured cells, it is clear that the suppression of renin expression by vitamin D occurs directly (*Li YC et al, 2002*).

The potential role of RAS

Ang2 directly induces contraction of arteriolar smooth muscle, which contributes indirectly as well as directly to endothelial dysfunction. Ang2 modulates ROS in VSM and endothelial cells to regulate proliferative and hypertrophic responses, involved in inflammation. The suppression of eNOS and NO activation, an intergral component of endothelial dysfunction causes local synthesis of endothelin-1 (a potent vasoconstrictor) and enhances the effects of Ang2. Moreover, the main Ang2 receptors AT1-R and AT2-R trigger endothelial apoptosis. This Ang2-mediated apoptosis in the endothelium is induced by ROS and dephosphorylation of ERK1/2. Studies also demonstrated that aldosterone has an impact on the vascular tone by reducing the bioavailability of NO. Furthermore, aldosterone alters structural and mechanical properties of the vessels, leading to increased peripheral vascular resistance and stiffness (*Silva PM et al, 2010*).

Discussion

Arterial stiffness has emerged as a key risk factor in the pathophysiology of CVD and CKD. In this essay, we explored the link between vitamin D and arterial stiffness. Although vitamin D supplementation gave inconsistent results regarding arterial stiffness in patient with CKD, mounting evidence indicates that vitamin D has a beneficial impact on endothelial function, which is essentially involved in the preservation of vascular function and arterial stiffness. Studies demonstrated that vitamin D has an advantageous relationship with arterial stiffness through enhancing endothelial function, suppressing systemic inflammation and ROS.

Vitamin D supplementation with oral cholecalciferol improved endothelial dysfunction and local arterial stiffness in pediatric CKD patients with low vitamin D levels. The mechanisms underlying improved endothelial function in these patients can be explained by the effects of cholecalciferol on VSM and the endothelium. Cholecalciferol promotes the release of vasodilating agents, such as prostacyclin and decreases local inflammation. However, the number of subjects included in this study was relatively small with respect to other studies. Besides, the study period was only twelve weeks, which could be not long enough to investigate the long-term effects of oral cholecalciferol (*Aytaç MB et al, 2015*).

Interestingly, Martins et al demonstrated that a high dose vitamin D supplementation for a longer time period was associated with reduced expression and activation of oxidative stress and inflammatory mediators of arterial stiffness. However, vitamin D reduced PWV only in the group of participants with excessive urinary isoprostane. This might suggest that the cardiovascular benefit of vitamin D treatment is more evidently observable in high risk patients with enhanced ROS production. The lack of reduction in the PWV in the overall sample supplemented with vitamin D, might be due to an inadequate follow up period or due to insufficient raise of serum vitamin D levels obtained in this study (*Martins D et al, 2014*). In accordance with this, Zaleski et al showed that the beneficial effects of vitamin D on arterial stiffness are dose-dependent. Here, only high-dose vitamin D lowered arterial stiffness markers, while no similar effects were found in low-dose participants (*Zaleski A et al, 2015*).

The hypothesis that only high-dose vitamin D treatment affects arterial stiffness is further supported by another study in patients on chronic dialysis. This study showed that cholecalciferol treatment had not any effect on arterial stiffness and cardiac function. In this study, a daily dose of 3000 IU cholecalciferol was given, which is a lower dose compared to other studies. The absence of a potential effect on arterial stiffness and cardiac function could be explained by the insufficient raise of 1,25-dihydroxyvitamin D to activate VDR in cardiac tissue (*Mose FH et al, 2014*). However, some alternative explanations must be considered. Although PWV is accepted as a reliable measure for arterial stiffness, it can be affected by changes in volemic state. PWV tends to increase by volume expansion, which can be caused by hemodialysis. Thus, the beneficial effects of cholecalciferol might be concealed by volume expansion. Another possible explanation for the absence of any changes in PWV could be the continuation of usual medication and dialysis, which ensures optimal care. Furthermore, this study included patients irrespective of their plasma 25-hydroxyvitamin D, meaning that some patients had sufficient vitamin D levels. In this case, increasing vitamin D levels further may not induce changes in cardiovascular parameters (*Mose FH et al, 2014*).

In contrast to the data suggesting benefit, Fortier and colleagues showed adverse outcomes of vitamin D treatment regarding arterial stiffness in an observational study conducted in hemodialysis patients. Treating these patients with a high (pharmacological) dose of alfacalcidol ended in an accelerated progression of aortic stiffening (*Fortier C et al, 2014*). The role of vitamin D in the development of vascular calcification is still ambiguous in humans. According to Shroff et al, there

might be a U-shaped relationship between calcitriol and arterial calcification in a pediatric CKD population (*Shroff R et al, 2008*). The noticeable effect of high dose alfacalcidol on the progression of arterial stiffness is in line with the fact that high-dose active vitamin D can lead to toxic and procalcifying effects. Similarly, animal models of CKD showed that vascular calcification is enhanced after active vitamin D treatment (*Fortier C et al, 2014*). The administration of active vitamin D3 to rabbits and rats provided reproducible animal models of arterial stiffening and hypertension (*Richart T et al, 2007*). Moreover, a very recent study on the associations of 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D with hypertension has demonstrated that higher circulating 1,25-dihydroxyvitamin D levels are linked to a higher risk of hypertension. Possibly, vitamin D induces an increase in calcium absorption, leading to vascular calcification. This in turn, promotes arterial stiffness and a greater hypertension risk (*van Ballegooijen AJ et al, 2015*).

While there is still inconsistency among the available data on vitamin D treatment and arterial stiffening, the role of the endothelium seems to be crucial in maintaining vascular function. The PENNY study from 2014 reported beneficial outcomes of vitamin D therapy on cardiovascular system through endothelial function. Here, paricalcitol improved endothelium-dependent vasodilation without affecting endothelium-independent vasodilation (*Zoccali C et al, 2014*). Paricalcitol evoked a rise in the endothelium-dependent FMD in CKD patients with endothelial-dysfunction. This effect was specific, such that endothelium-independent vasodilation was unaffected. Interestingly, the PENNY trials also demonstrated that paricalcitol induced favourable changes in markers for atherosclerosis (*Zoccali C et al, 2014*). Some cross-sectional studies have demonstrated the association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and endothelium-dependent vasodilation in patients suffering from stage 3 to 4 CKD, which indicates that vitamin D deficiency or insufficiency may adversely affect the vascular system (*Chitalia N et al, 2011, Zoccali C et al, 2014*). Paricalcitol was also shown to improve survival in large-scale observational studies conducted in ESRD patients (*Wolf M et al, 2007*).

In addition to its fundamental role in vascular function, NO is involved in the regulation of LV diastolic function. When NO is released in coronary arteries, it accelerates LV relaxation and thereby increases LV distensibility (*Paulus WJ et al, 1999*). While the relationship between endothelium-dependent vasodilation and diastolic function is repeatedly confirmed, paricalcitol has shown to favourably affect LV diastolic function. The activation of VDR enhanced LV diastolic relaxation and induced advantageous effects on endothelium-dependent vasodilation in nephrectomized rats (*Zoccali C et al, 2014*). Although paricalcitol showed beneficial effect on endothelium-dependent vasodilation, which fits well with previous observational studies, the PENNY trial did not include any arterial stiffness measurements. Furthermore, a small proportion of the study population consists of participants with diabetes mellitus, which might influence vitamin D effects (*Zoccali C et al, 2014*). Expanding on the potential effect of vitamin D on the endothelium, several studies demonstrate the importance of the link between VDR and NOS. The lack of VDR is associated with impaired NO bioavailability and endothelial dysfunction (*Andrukhova O et al, 2013*).

Cross-talk between vitamin D and the RAS may offer an alternative explanation for the link between vitamin D and arterial stiffness. VDR signalling is directly linked to negative regulation of the RAS, and vitamin D or vitamin D activators appear to exert renal-protective effects partially through alterations in RAS (*Li YC et al, 2002, Yuan W et al, 2007*). Furthermore, associations have been found between vitamin D deficiency and inappropriately elevated renin levels, a potential link that may play a role in the progression of CKD and CVD (*Santoro D et al, 2015*). However, the interaction between vitamin D and the RAS is less well established and notably complex. In light of this, we were only able to give a brief overview regarding the possible role of RAS in the progression of arterial stiffness and vitamin D. Further research should expand our understanding of the role of RAS in CKD-related arterial stiffness.

CVD is a main cause of mortality in CKD and especially in ESRD (*Aytaç MB et al, 2015*). Multiple factors have been implicated in the development of cardiovascular abnormalities (*Aytaç MB et al, 2015*). Among these factors, arterial stiffness is considered an important risk factor for the high cardiovascular mortality (*Antonini-Canterin F et al, 2008*). Vitamin D deficiency, which is highly prevalent in this population is associated with increased arterial stiffness (*Giallauria F et al, 2012*). Vitamin D treatment seems to have a beneficial effect on endothelial function, in particular on endothelium-dependent vasodilation (*Zoccali C et al, 2014*). Furthermore, cardiovascular-protective effects of vitamin D are most likely achieved through the reduction of systemic inflammation and endothelial dysfunction (*Alyami A et al, 2014, Brewer LC et al, 2011*). Possibly, the latter involves VDR- and NOS-dependent mechanism and down regulation of the RAS (*Andrukhova O et al, 2013*).

While clinical studies showed the impact of vitamin D treatment on endothelial function and arterial stiffness in CKD patients, the potential beneficial effects of vitamin D appears to be dose-dependent. Studies that used a high dose vitamin D showed remarkable effects. Since vitamin D overload is used as a model of arterial stiffening, large trials investigating the posology and the effect of vitamin D supplementation on vascular structure are highly encouraged (*Giallauria F et al, 2012*). Similarly, patient characteristics such as comorbidity and the use of concomitant medications need to clarified to obtain the desired effect.

Altogether, although the present data concerning vitamin D supplementation and arterial stiffness remains mixed, accumulating evidence shows that vitamin D treatment has a beneficial impact on cardiovascular risk factors, including arterial stiffness. Vitamin D seems to exert these effects notably through the preservation of endothelial function. Additionally, vitamin D reduces oxidative stress, inflammation and the activity of RAS. Hence we conclude that vitamin D has an advantageous relationship with arterial stiffness and recommend further studies that assess long-term effects of vitamin D on cardiovascular risks in more specifically characterized populations.

References

Alyami A, Soares MJ, Sherriff JL, Zhao Y, Hallett J, Coombes F. A systematic review protocol examining the effect of vitamin D supplementation on endothelial function. BMJ Open. 2015 Jun 12;5(6):e006835. doi: 10.1136/bmjopen-2014-006835. Review. PubMed PMID: 26070793; PubMed Central PMCID: PMC4466623.

Andrukhova O, Slavic S, Zeitz U, Riesen SC, Heppelmann MS, Ambrisko TD, Markovic M, Kuebler WM, Erben RG. Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. Mol Endocrinol. 2014 Jan;28(1):53-64. doi: 10.1210/me.2013-1252. Epub 2013 Jan 1. PubMed PMID: 24284821.

Antonini-Canterin F, Carerj S, Di Bello V, Di Salvo G, La Carrubba S, Vriz O, Pavan D, Balbarini A, Nicolosi GL; Research Group of the Italian Society of Cardiovascular Echography (SIEC). Arterial stiffness and ventricular stiffness: a couple of diseases or a coupling disease? A review from the cardiologist's point of view. Eur J Echocardiogr. 2009 Jan;10(1):36-43. doi: 10.1093/ejechocard/jen236. Epub 2008 Sep 16. Review. PubMed PMID: 18799479.

Aytaç MB, Deveci M, Bek K, Kayabey Ö, Ekinci Z. Effect of cholecalciferol on local arterial stiffness and endothelial dysfunction in children with chronic kidney disease. Pediatr Nephrol. 2016 Feb;31(2):267-77. doi: 10.1007/s00467-015-3220-5. Epub 2015 Oct 2. PubMed PMID: 26432706.

Bargnoux AS, Vetromile F, Kuster N, Barberet J, Dupuy AM, Ribstein J, Mourad G, Cristol JP, Fesler P. Markers of bone remodeling are associated with arterial stiffness in renal transplanted subjects. J Nephrol. 2015 Dec;28(6):765-72. doi: 10.1007/s40620-015-0201-5. Epub 2015 Apr 29. PubMed PMID: 25920618.

Basarici I, Altekin RE, Demir I, Yilmaz H. Urinary 8-isoprostane levels can indicate the presence, severity and extent of angiographic coronary artery disease. Acta Cardiol. 2008 Aug;63(4):415-22. PubMed PMID: 18795577.

Brewer LC, Michos ED, Reis JP. Vitamin D in atherosclerosis, vascular disease, and endothelial function. Curr Drug Targets. 2011 Jan;12(1):54-60. Review. PubMed PMID: 20795937.

Cabrera Fischer EI, Armentano RL, Levenson J, Barra JG, Morales MC, Breitbart GJ, Pichel RH, Simon A. Paradoxically decreased aortic wall stiffness in response to vitamin D3-induced calcinosis. A biphasic analysis of segmental elastic properties in conscious dogs. Circ Res. 1991 Jun;68(6):1549-59. PubMed PMID: 1645231.

Cernaro V, Santoro D, Lacquaniti A, Costantino G, Visconti L, Buemi A, Buemi M. Phosphate binders for the treatment of chronic kidney disease: role of iron oxyhydroxide. Int J Nephrol Renovasc Dis. 2016 Feb 2;9:11-9. doi: 10.2147/IJNRD.S78040. eCollection 2016. Review. PubMed PMID: 26893577; PubMed Central PMCID: PMC4749089.

Chang J, Ye XG, Hou YP, Wu JL, Li SL, Sun QM. Vitamin D Level is Associated with Increased Left Ventricular Mass and Arterial Stiffness in Older Patients with Impaired Renal Function. Med Sci Monit. 2015 Dec 22;21:3993-9. PubMed PMID: 26691016; PubMed Central PMCID: PMC4692575.

Chitalia N, Recio-Mayoral A, Kaski JC, Banerjee D. Vitamin D deficiency and endothelial dysfunction in non-dialysis chronic kidney disease patients. Atherosclerosis. 2012 Jan;220(1):265-8. doi: 10.1016/j.atherosclerosis.2011.10.023. Epub 2011 Oct 25. PubMed PMID: 22071357.

Cozzolino M, Covic A, Martinez-Placencia B, Xynos K. Treatment Failure of Active Vitamin D Therapy in Chronic Kidney Disease: Predictive Factors. Am J Nephrol. 2015;42(3):228-36. doi: 10.1159/000441095. Epub 2015 Oct 7. PubMed PMID: 26439891.

Ding HY, Ma HX. Significant roles of anti-aging protein klotho and fibroblast growth factor23 in cardiovascular disease. J Geriatr Cardiol. 2015 Jul;12(4):439-47. doi: 10.11909/j.issn.1671-5411.2015.04.017. Review. PubMed PMID: 26347327; PubMed Central PMCID: PMC4554784.

Fellström BC, Jardine AG, Schmieder RE, Holdaas H, Bannister K, Beutler J, Chae DW, Chevaile A, Cobbe SM, Grönhagen-Riska C, De Lima JJ, Lins R, Mayer G, McMahon AW, Parving HH, Remuzzi G, Samuelsson O, Sonkodi S, Sci D, Süleymanlar G, Tsakiris D, Tesar V, Todorov V, Wiecek A, Wüthrich RP, Gottlow M, Johnsson E, Zannad F; AURORA Study Group. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. N Engl J Med. 2009 Apr 2;360(14):1395-407. doi: 10.1056/NEJMoa0810177. Epub 2009 Mar 30. Erratum in: N Engl J Med. 2010 Apr 15;362(15):1450. PubMed PMID: 19332456.

Fortier C, Mac-Way F, De Serres SA, Marquis K, Douville P, Desmeules S, Larivière R, Agharazii M. Active vitamin D and accelerated progression of aortic stiffness in hemodialysis patients: a longitudinal observational study. Am J Hypertens. 2014 Nov;27(11):1346-54. doi: 10.1093/ajh/hpu057. Epub 2014 Apr 2. PubMed PMID: 24695980; PubMed Central PMCID: PMC4263937.

Freundlich M, Li YC, Quiroz Y, Bravo Y, Seeherunvong W, Faul C, Weisinger JR, Rodriguez-Iturbe B. Paricalcitol downregulates myocardial renin-angiotensin and fibroblast growth factor expression and attenuates cardiac hypertrophy in uremic rats. Am J Hypertens. 2014 May;27(5):720-6. doi: 10.1093/ajh/hpt177. Epub 2013 Sep 26. PubMed PMID: 24072555; PubMed Central PMCID: PMC3978945.

Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH. A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. PLoS One. 2012;7(5):e36617. doi: 10.1371/journal.pone.0036617. Epub 2012 May 7. PubMed PMID: 22586483; PubMed Central PMCID: PMC3346736.

Giallauria F, Milaneschi Y, Tanaka T, Maggio M, Canepa M, Elango P, Vigorito C, Lakatta EG, Ferrucci L, Strait J. Arterial stiffness and vitamin D levels: the Baltimore longitudinal study of aging. J Clin Endocrinol Metab. 2012 Oct;97(10):3717-23. doi: 10.1210/jc.2012-1584. Epub 2012 Jul 5. PubMed PMID: 22767638; PubMed Central PMCID: PMC3674293.

Hemmelgarn BR, Manns BJ, Lloyd A, James MT, Klarenbach S, Quinn RR, Wiebe N, Tonelli M; Alberta Kidney Disease Network. Relation between kidney function, proteinuria, and adverse outcomes. JAMA. 2010 Feb 3;303(5):423-9. doi: 10.1001/jama.2010.39. PubMed PMID: 20124537.

Huveneers S, Daemen MJ, Hordijk PL. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. Circ Res. 2015 Feb 27;116(5):895-908. doi: 10.1161/CIRCRESAHA.116.305720. Review. PubMed PMID: 25722443.

Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, Saran R, Wang AY, Yang CW. Chronic kidney disease: global dimension and perspectives. Lancet. 2013 Jul 20;382(9888):260-72. doi: 10.1016/S0140-6736(13)60687-X. Epub 2013 May 31. Review. Erratum in: Lancet. 2013 Jul 20;382(9888):208. PubMed PMID: 23727169.

Kovesdy CP, Quarles LD. Fibroblast growth factor-23: what we know, what we don't know, and what we need to know. Nephrol Dial Transplant. 2013 Sep;28(9):2228-36. doi: 10.1093/ndt/gft065. Epub 2013 Apr 25. Review. PubMed PMID: 23625971; PubMed Central PMCID: PMC3769978.

Liu WC, Wu CC, Hung YM, Liao MT, Shyu JF, Lin YF, Lu KC, Yeh KC. Pleiotropic effects of vitamin D in chronic kidney disease. Clin Chim Acta. 2016 Jan 30;453:1-12. doi: 10.1016/j.cca.2015.11.029. Epub 2015 Dec 2. Review. PubMed PMID: 26656443.

Liu WC, Zheng CM, Lu CL, Lin YF, Shyu JF, Wu CC, Lu KC. Vitamin D and immune function in chronic kidney disease. Clin Chim Acta. 2015 Oct 23;450:135-44. doi: 10.1016/j.cca.2015.08.011. Epub 2015 Aug 17. Review. PubMed PMID: 26291576.

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 Jul;110(2):229-38. PubMed PMID: 12122115; PubMed Central PMCID: PMC151055.

London GM, Guérin AP, Verbeke FH, Pannier B, Boutouyrie P, Marchais SJ, Mëtivier F. Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. J Am Soc Nephrol. 2007 Feb;18(2):613-20. Epub 2007 Jan 3. PubMed PMID: 17202417.

Marti CN, Gheorghiade M, Kalogeropoulos AP, Georgiopoulou VV, Quyyumi AA, Butler J. Endothelial dysfunction, arterial stiffness, and heart failure. J Am Coll Cardiol. 2012 Oct 16;60(16):1455-69. doi: 10.1016/j.jacc.2011.11.082. Epub 2012 Sep 19. Review. PubMed PMID: 22999723.

Martins D, Meng YX, Tareen N, Artaza J, Lee JE, Farodolu C, Gibbons G, Norris K. The Effect of Short Term Vitamin D Supplementation on the Inflammatory and Oxidative Mediators of Arterial Stiffness. Health (Irvine Calif). 2014 Jun;6(12):1503-1511. PubMed PMID: 25505940; PubMed Central PMCID: PMC4260399.

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(6):661-8. doi: 10.1159/000330075. Epub 2011 Jun 17. PubMed PMID: 21691084.

Mose FH, Vase H, Larsen T, Kancir AS, Kosierkiewic R, Jonczy B, Hansen AB, Oczachowska-Kulik AE, Thomsen IM, Bech JN, Pedersen EB. Cardiovascular effects of cholecalciferol treatment in dialysis patients--a randomized controlled trial. BMC Nephrol. 2014 Mar 24;15:50. doi: 10.1186/1471-2369-15-50. PubMed PMID: 24661355; PubMed Central PMCID: PMC3994388.

Mozos I, Marginean O. Links between Vitamin D Deficiency and Cardiovascular Diseases. Biomed Res Int. 2015;2015:109275. doi: 10.1155/2015/109275. Epub 2015 Apr 27. Review. PubMed PMID: 26000280; PubMed Central PMCID: PMC4427096.

Ojo A. Addressing the global burden of chronic kidney disease through clinical and translational research. Trans Am Clin Climatol Assoc. 2014;125:229-43; discussion 243-6. Review. PubMed PMID: 25125737; PubMed Central PMCID: PMC4112688.

Parikh C, Gutgarts V, Eisenberg E, Melamed ML. Vitamin D and Clinical Outcomes in Dialysis. Semin Dial. 2015 Nov-Dec;28(6):604-9. doi: 10.1111/sdi.12446. Epub 2015 Oct 1. PubMed PMID: 26424141.

Paulus WJ, Shah AM. NO and cardiac diastolic function. Cardiovasc Res. 1999 Aug 15;43(3):595-606. Review. PubMed PMID: 10690331.

Pavlovic D, Katicic D, Gulin T, Josipovic J. Vitamin d in the patients with chronic kidney disease: when, to whom and in which form. Mater Sociomed. 2015 Apr;27(2):122-4. doi: 10.5455/msm.2015.27.122-124. Epub 2015 Apr 5. Review. PubMed PMID: 26005391; PubMed Central PMCID: PMC4404993.

Rhee CM, Kovesdy CP. Epidemiology: Spotlight on CKD deaths—increasing mortality worldwide. Nat Rev Nephrol. 2015 Apr;11(4):199-200. doi: 10.1038/nrneph.2015.25. Epub 2015 Mar 3. PubMed PMID: 25734769; PubMed Central PMCID: PMC4379111.

Santoro D, Caccamo D, Lucisano S, Buemi M, Sebekova K, Teta D, De Nicola L. Interplay of vitamin D, erythropoiesis, and the renin-angiotensin system. Biomed Res Int. 2015;2015:145828. doi: 10.1155/2015/145828. Epub 2015 Apr 27. Review. PubMed PMID: 26000281; PubMed Central PMCID: PMC4427087.

Shirwany NA, Zou MH. Arterial stiffness: a brief review. Acta Pharmacol Sin. 2010 Oct;31(10):1267-76. doi: 10.1038/aps.2010.123. Epub 2010 Aug 30. Review. PubMed PMID: 20802505; PubMed Central PMCID: PMC3078647.

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 Jun;19(6):1239-46. doi: 10.1681/ASN.2007090993. Epub 2008 Mar 12. PubMed PMID: 18337484; PubMed Central PMCID: PMC2396936.

Silva PM. From endothelial dysfunction to vascular occlusion: role of the renin-angiotensin system. Rev Port Cardiol. 2010 May;29(5):801-24. Review. English, Portuguese. PubMed PMID: 20866008.

Stricker H, Tosi Bianda F, Guidicelli-Nicolosi S, Limoni C, Colucci G. Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study. Eur J Vasc Endovasc Surg. 2012 Sep;44(3):307-12. doi: 10.1016/j.ejvs.2012.06.023. Epub 2012 Jul 24. PubMed PMID: 22831874.

Toussaint ND, Kerr PG. Vascular calcification and arterial stiffness in chronic kidney disease: implications and management. Nephrology (Carlton). 2007 Oct;12(5):500-9. Review. PubMed PMID: 17803475.

van Ballegooijen AJ, Gansevoort RT, Lambers-Heerspink HJ, de Zeeuw D, Visser M, Brouwer IA, Kema IP, de Borst MH, Bakker SJ, Joosten MM. Plasma 1,25-Dihydroxyvitamin D and the Risk of Developing Hypertension: The Prevention of Renal and Vascular End-Stage Disease Study. Hypertension. 2015 Sep;66(3):563-70. doi: 10.1161/HYPERTENSIONAHA.115.05837. Epub 2015 Jul 20. PubMed PMID: 26195480.

Veloudi P, Blizzard CL, Ding CH, Cicuttini FM, Jin X, Wluka AE, Winzenberg T, Jones G, Sharman JE. Effect of Vitamin D Supplementation on Aortic Stiffness and Arterial Hemodynamics in People With Osteoarthritis and Vitamin D Deficiency. J Am Coll Cardiol. 2015 Dec 15;66(23):2679-81. doi: 10.1016/j.jacc.2015.10.007. PubMed PMID: 26670070.

Wolf M, Thadhani R. Vitamin D in patients with renal failure: a summary of observational mortality studies and steps moving forward. J Steroid Biochem Mol Biol. 2007 Mar;103(3-5):487-90. Epub 2007 Jan 2. Review. PubMed PMID: 17197169; PubMed Central PMCID: PMC1865131.

Yuan W, Pan W, Kong J, Zheng W, Szeto FL, Wong KE, Cohen R, Klopot A, Zhang Z, Li YC. 1,25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. J Biol Chem. 2007 Oct 12;282(41):29821-30. Epub 2007 Aug 9. PubMed PMID: 17690094.

Yuste C, Quiroga B, de Vinuesa SG, Goicoechea MA, Barraca D, Verdalles U, Luño J. The effect of some medications given to CKD patients on vitamin D levels. Nefrologia. 2015;35(2):150-6. doi: 10.1016/j.nefro.2015.05.016. Epub 2015 Jun 23. PubMed PMID: 26300508.

Zaleski A, Panza G, Swales H, Arora P, Newton-Cheh C, Wang T, Thompson PD, Taylor B. High-Dose versus Low-Dose Vitamin D Supplementation and Arterial Stiffness among Individuals with Prehypertension and Vitamin D Deficiency. Dis Markers. 2015;2015:918968. doi: 10.1155/2015/918968. Epub 2015 Sep 16. PubMed PMID: 26451070; PubMed Central PMCID: PMC4588343.

Zoccali C, Curatola G, Panuccio V, Tripepi R, Pizzini P, Versace M, Bolignano D, Cutrupi S, Politi R, Tripepi G, Ghiadoni L, Thadhani R, Mallamaci F. Paricalcitol and endothelial function in chronic kidney disease trial. Hypertension. 2014 Nov;64(5):1005-11. doi: 10.1161/HYPERTENSIONAHA.114.03748. Epub 2014 Aug 4. Erratum in: Hypertension. 2015 Jun;65(6):e48. PubMed PMID: 25259743 .