Molecular Mechanisms of Vascular Calcification in Diabetes Mellitus: Insights from Human Aortic Smooth Muscle Cells, A Systematic Review

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Abstract. Vascular calcification is a significant contributor to cardiovascular complications in diabetes mellitus (DM), particularly affecting the prognosis of patients. This review conducts a comprehensive analysis of the molecular mechanisms underlying vascular calcification in DM, with a focus on insights from human aortic smooth muscle cells (HASMCs). The search was conducted following the PRISMA guidelines, utilizing databases such as MEDLINE/PubMed, Science Direct, and Google Scholar. The search focused on articles published within the last 5 years that discussed the molecular mechanisms of vascular calcification in DM, specifically in HASMCs. Five selected reviews were found in a total of 637 articles. DM significantly accelerates vascular calcification in HASMCs through the upregulation of osteogenic markers and activation of the Wnt/β-catenin signaling pathway. Other identified mechanisms include inflammation, ferroptosis, and endothelial dysfunction, contributing to the complex interplay of factors that drive vascular calcification in diabetic patients. It is concluded that DM significantly accelerates vascular calcification in enhanced expression of osteogenic markers and activation of the Wnt/β-catenin signaling pathway in human aortic smooth muscle cells. Patients with diabetes are more likely to have cardiovascular issues as a result of this pathological process.

Keywords: Dapagliflozin, Diabetes Mellitus, Metformin, Efficacy.

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1. Introduction

Diabetes mellitus (DM) and its consequences represent a significant hazard to people's well-being and have recently become an issue in public health throughout the world [1]. More than 90% of individuals with diabetes have type 2 diabetes mellitus (T2DM) [2]. The presence or absence of peripheral vasculopathy, retinopathy, cardiopathy, encephalopathy, and nephropathy is a defining feature of diabetic complications [3]. DM elevates the likelihood of all these problems, and multiple vasculopathy correlates with a worse prognosis [4]. Recent rigorous research on diabetes complications has greatly enhanced the comprehension of the disease's pathophysiology. Nonetheless, the growing segmentation of medical knowledge into distinct subspecialties has led to a propensity for concentrating on localized lesions rather than synthesizing comprehensive information. Therefore, a comprehensive examination of diabetes complications affecting various systems and distinct angiopathies is required. The pathophysiology of diabetes complications has significant similarity at the vascular level, mostly presenting as endothelial dysfunction and atherosclerosis [5].

Patients with DM face several vascular comorbidities, which greatly affect their prognosis and treatment options. This condition is referred to as “panvascular disease” [6]. Polyvascular atherosclerotic disease, which includes both coronary and non-coronary atherosclerosis, mainly peripheral arterial and cerebrovascular illnesses [7] has been defined since the late 20th century, when the concept of a “vessel tree” was first proposed.

This concept signifies that thorough therapy of multivessel illness is clinically crucial for enhancing outcomes and prognoses. This concept, however, overlooks microvascular illness, particularly in essential organs, as well as interdisciplinary integration. We propose the notion of diabetic panvascular disease (DPD) to enhance this definition. This clinical condition features a prevalent pathology of atherosclerosis in internal and external blood vessels in diabetics, including those in their hearts, brains, kidneys, eyes, and extremities. Major complications include heart attacks and strokes, and a better prognosis might be achieved with aggressive treatment of metabolic abnormalities. Both macrovascular and microvascular disease and complications classified by organ affected are common ways to classify diabetic complications. These concepts are integrated via DPDs [8].

Hypertension and coronary artery disease are both exacerbated by the hardness and occlusion of arteries caused by pathological calcification in vascular tissues, especially the aorta and coronary arteries [9]. Similar to how calcific aortic stenosis develops when valve tissue mineralizes, it blocks function. Several investigations have shown that valve calcification occurs independently of calcium deposition in vascular tissues [10], and only 25–40% of those with calcific aortic stenosis have significant arterial disease, even though the risk factors and related molecular pathways are identical [10]. Consequently, it is posited that these unique biological traits may exhibit greater diversity than previously recognized.

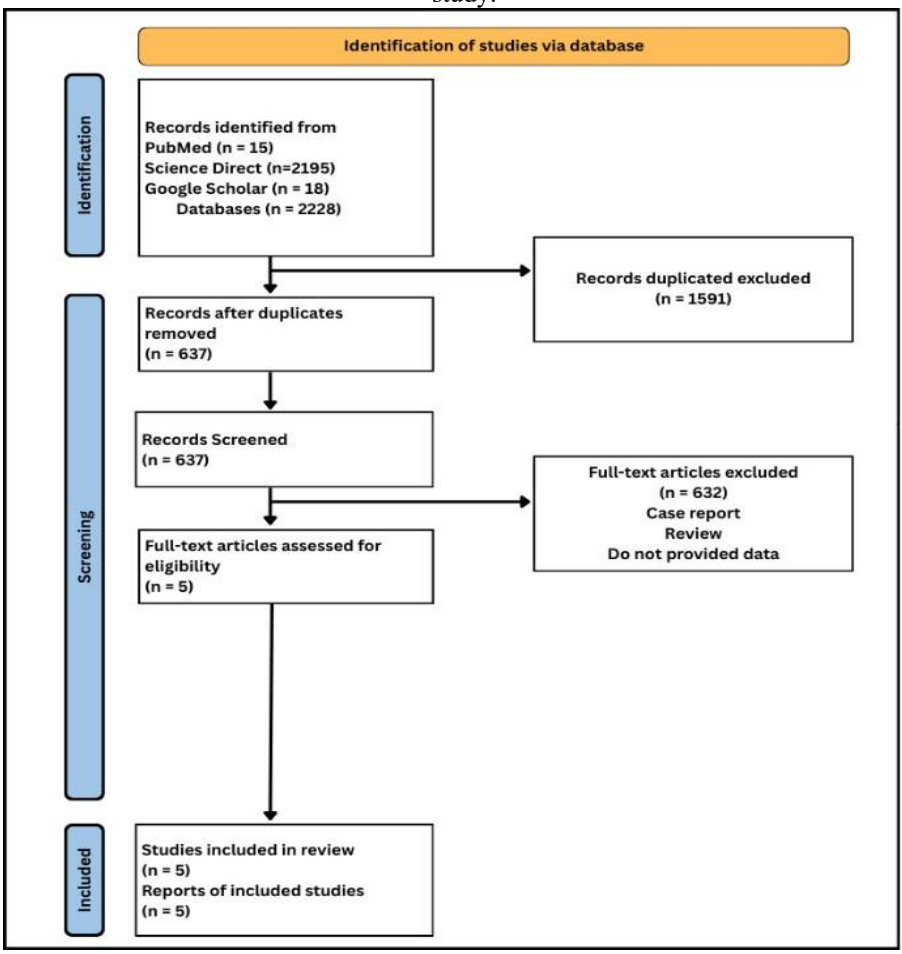
Where mineralization occurs in the body is the main differentiator between calcific aortic stenosis and calcification-induced coronary artery disease. Aortic heart valves are trilaminar structures that are painstakingly organized according to blood flow dynamics and comprised of layers of collagen, proteoglycans, and elastin fibers. Spread out throughout the valve cusp, a heterogeneous population of fibroblast-like valve interstitial cells (VICs) and an extracellular matrix (ECM) that is supported by an endothelial layer are present. Similar to the valve, the artery walls connective tissue is stratified, but it's structure is different. In the adventitia layer, collagen and fibroblasts are abundant. In the medial layer, vascular smooth muscle cells (VSMCs) and aligned elastin fibers make up the middle layer. Adjacent to laminar blood flow, there is a monolayer of endothelial cells and sub-endothelial connective tissue from the intimal layer. VICs undergo osteogenic changes, such as increased expression of pro-calcification markers and suppression of inhibitors, which lead to calcification of the valve, which mainly occurs in the collagen-dense fibrosa layer found in regions of oscillatory blood flow. On the flip side, local VSMCs may have similar molecular alterations when arterial calcification occurs in either the medial or intimal layers [11].

VICs and VSMCs share molecular and hemodynamic mechanisms that lead to calcification, according to a review of in vitro and in vivo studies [12]. Nevertheless, there is an increasing body of data supporting the existence of separate mechanisms as well [13]. Although both calcific aortic stenosis and arterial calcification are more common in those above the age of 65, age is still a major risk factor for both types of cardiovascular calcification (2–13% compared to 31–55%) [14]. In addition, studies show that VSMCs, as opposed to VICs, have a stronger calcification response to osteogenic stimuli, as seen by elevated levels of alkaline phosphatase activity and Alizarin Red staining. This suggests that vascular mineralization takes longer than valve calcification or that VSMCs are more prone to calcification than VICs [12]. This work sought to conduct a comprehensive literature analysis on the molecular underpinnings of vascular calcification in DM, specifically focusing on insights from human aortic smooth muscle cells.

2. Method

The PRISMA approach was used to conduct this systematic review. Articles pertaining to the suggested subject were sought for in the August 2024 time frame using the Google Scholar, Science Direct, and the National Library of Medicine's electronic databases (PubMED/MEDLINE). Aortic smooth muscle, "molecular mechanism," "vascular classification," "diabetes mellitus," and combinations thereof were used in the searches. Descriptive and review publications published in English and accessible in their entirety online since 2018 were included in the study. This review only included studies that met these inclusion criteria and discussed molecular processes of arterial calcification in DM. After two writers had finished analyzing the chosen articles, a third writer would decide whether or not to include the piece in the review in the event of a dispute.

Fig. 1. Flowchart derived from PRISMA 2009 for the identification of articles in the study.



The process for selecting the articles began with the identification and selection of potential articles, proceeded to the eligibility phase where inclusion and exclusion criteria were applied, and concluded with the final inclusion of the selected articles. A comprehensive search of the databases yielded a total of 2228 scientific articles. Among these, 15 were in MEDLINE/PubMED, 2195 in Science Direct, and 18 in Google Scholar. However, there were some duplicate articles in the databases, resulting in an initial total of 637 articles. After this step, a thorough analysis of the articles was conducted through readings of the titles and abstracts, followed by a full-text review of the pre-selected articles, resulting in a total of 5 articles chosen for this systematic review. A flowchart was created (Fig. 1) to illustrate the stages involved in selecting the articles. The findings indicated that DM notably increases vascular calcification in human aortic smooth muscle cells by elevating osteogenic marker expression and activating the

Wnt/β-catenin signaling pathway.

Table 1. A detailed overview of the articles that were included and analyzed, featuring a presentation of the authors, the type of study conducted, and the outcomes observed.

Author / year	Type of study	Outcome
Li et al., 2023 [8]	Descriptive study/review	The management of DPD requires an integrated, cross-disciplinary approach that combines aggressive glycemic control with early and comprehensive vascular interventions to address the complex and interconnected nature of macrovascular and microvascular complications in diabetes.
Kessler et al., 2023 [23]	Descriptive study/review	Cardiovascular calcification in vascular and valvular structures involves distinct molecular and cellular mechanisms, emphasizing the need for personalized therapeutic approaches despite shared risk factors.
Sutton et al., 2023 [26]	Descriptive study/review	There exists a multifaceted interaction of genetic, environmental, and molecular factors in age-associated vascular calcification, underscoring the need for deeper insights to create approaches for encouraging healthy vascular aging.
Pan et al., 2022 [30]	Descriptive study/review	There is a complexity of VC and the potential of targeting molecular mechanisms such as inflammation, ferroptosis, and endothelial dysfunction to develop new therapeutic strategies
Durham et al., 2018 [33]	Descriptive study/review	Vascular calcification, influenced by the phenotypic switching of VSMCs as a reaction to local environmental signals, is a dynamic and active process that offers potential therapeutic targets for preventing arterial calcification, which remains untreatable.

3. Discussion

Endothelial cells (ECs), smooth muscle cells (SMCs), pericytes, fibroblasts, and a host of other cell types make up the vasculature, according to Li et al. Pathologies often seen in systemic vascular disease include damage to the endothelial barrier, loss of pericytes, thinning of capillaries, and angiogenic diseases. Vascular nerve bundles consist of a network of interconnected blood vessels, nerves, lymphatic vessels, and connective tissue membranes8. Blood vessel function may alter due to differences in perivascular tissues, vascular nerve bundles, and intravascular building blocks. An imbalance in homeostasis, characterized by abnormalities in glucose and lipid metabolism, triggers the activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS). The activation process causes widespread damage to the body's blood vessels, which in turn contributes to the development of panvascular problems in

people with DM [15]. The SNS plays a crucial role in vasoconstriction, while the RAAS is responsible for regulating vascular tone and blood pressure and volume [16]

Opposite tissues accurately govern microvascular units by physical contacts and signal transduction pathways, which in turn regulate the vascular system in target organs. Patients with advanced DM are more likely to develop vascular problems and undergo various pathological changes, such as microcirculatory abnormalities, atherosclerosis (AS), and endothelial dysfunction. All these things work together to cause DPD. Microangiopathy and macroangiopathy are the two main types of diabetic vasculopathy. The atherosclerosis of medium- and large-sized arteries, such as the aorta, coronary, renal, basilar, and peripheral arteries, is known as macroangiopathy. Microangiopathy, on the other hand, is caused by endothelial damage to the arteries that are located between the main arterioles and the venules. This endothelial damage causes the vascular basement membrane to thicken, microthrombosis to occur, and platelets and red blood cells to aggregate, leading to microcirculatory disorders [8].

Macroangiopathy and microangiopathy exhibit distinct pathological manifestations due to variations in hemodynamics, vascular structure, and the cells involved. Atherosclerosis frequently develops in areas of hemodynamic disturbances, particularly within elastic arteries. It presents as foaming macrophages, endothelial cell lesions, and mesangial smooth muscle cell lesions. Microvessels exhibit greater hemodynamic stability, characterized by a limited number of cell layers and a rich plexus. Conversely, variations in energy metabolic status and organ-specific growth factors or cytokines in distinct target organs are significant factors contributing to these differences. Diabetic macrovasculopathy and microvasculopathy impact most target organs, including the heart, brain, and peripheral vasculature; however, the retina and kidney are primarily influenced by microvasculopathy [8].

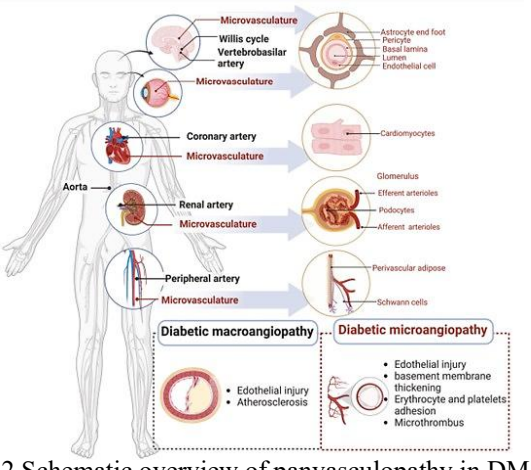


Fig. 2 Schematic overview of panvasculopathy in DM [8].

Fatty acid and triglyceride concentrations in the blood are elevated in insulin resistance because the resistance increases hepatocyte lipid production and adipocyte li-

polysis. Fatty acid-induced lipotoxicity and lipid buildup may affect fatty acid oxidation mechanisms in myocardium, trigger stress in the endoplasmic reticulum, autophagy, and apoptosis, and cause remodeling of the ventricles [17]. All things considered, advanced glycation end products (AGEs) are diabetic glycotoxin's most crucial byproducts. The oxidative stress/inflammatory cascade is initiated when these end-products connect to AGEs receptors (RAGE) on cells, which in turn promotes the formation of reactive oxygen species (ROS), nuclear factor κB (NF-κB), and proinflammatory cytokines such IL-1β, IL-6, IL-18, and TNF-α [18]. AGEs are the primary metabolites of diabetic glycotoxin. The end-products bind to cellular receptors of RAGEs, which stimulate the production of ROS, NF-κB, and proinflammatory cytokines, including IL-1β, IL-6, IL-18, and TNF-α. This process induces significant intracellular ROS production and initiates a cascade of inflammation and oxidative stress. Myocardial structural changes are brought about by AGEs and RAGE. Endothelial cells, macrophages, and smooth muscle cells are all triggered by inflammatory signals that are activated by advanced glycation end-products via RAGEs. Increased reactive ROS generation and decreased nitric oxide syntheses are the outcomes of this activation [19]. Hyperglycemia leads to a reduction in the expression of the JunD, in addition to free radical scavenger's aldehyde dehydrogenase 2 and superoxide dismutase 1. Inflammatory mediators such as IL-6, MCP-1, TNF-α, and NF-κB are enhanced in expression via its mediation [20]. Hyperglycemia enhances the expression of lncDACH1, subsequently leading to mitochondrial oxidative stress and apoptosis via the augmented ubiquitination-mediated degradation of sirtuin 3 (SIRT3) [21]. Protein kinase C (PKC) functions as an effector within the G protein-coupled receptor system, while SMC is responsible for maintaining vascular tone. ROS, AGEs, and diacylglycerol (DAG) can activate PKC, leading to impaired VSM function. Hyperglycemia activates traditional inflammatory pathways and induces oxidative stress. Hyperglycemia upregulates the expression of membrane cofactor MCP-1 and NLR family pyrin domain containing NLRP3 [22].

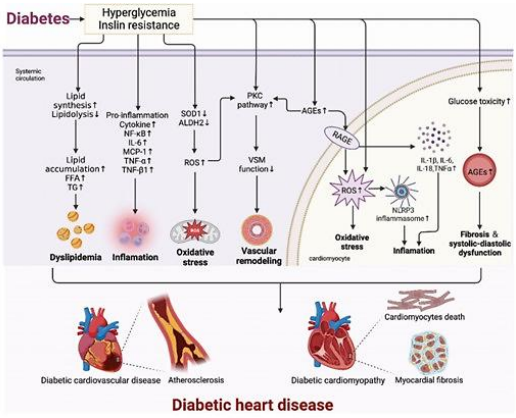


Fig. 3 Pathological and molecular mechanisms of DHD [8].

The pathogenic processes involving VSMCs and VICs are important, according to different research by Kessler et al., and although the terms are used interchangeably when discussing cardiovascular calcification, there are distinct morphological, physiological, and clinical distinctions between the two. In this work, we further demonstrate that AVSMCs experience calcification at an earlier stage compared to AVICs, with the calcification being more pronounced in high phosphate (as opposed to OM) environments. Findings from transcriptome analysis further reflect these cell- and calcific stimulispecific variations. Among them, you may find a wide variety of differentially expressed genes (DEGs) linked to biological processes known as “ossification” or “bone mineralization.” Additionally, there is a downregulation of mRNAs related to "actin" that is unique to VIC and a role for P13K/AKT signaling. It is worth noting that both cell types showed distinct molecular responses to OM treatment, but no modifications specific to high phosphate were seen [23].

Cardiovascular calcifications impacting heart valves and arteries exhibit several shared risk factors and are occasionally regarded as a singular illness. Nonetheless, several lines of evidence indicate that CAVD and CAC are complex illnesses. In most instances, CAVD occurs in isolation without arterial calcification, with about 25–40% presenting both CAVD and CAC. Moreover, research indicates that CAVD occurs in around 2–5% of those aged 65 and older, while other studies reveal an incidence of CAC in roughly 28.4% at the age of 50 [24]. By showing that AVSMC and AVIC responses to phosphate-dependent and phosphate-independent calcific stimuli are not uniform, this study lends credence to clinical findings. The reason for these distinctions is still unclear, although in living organisms, valvular and vascular structures differ in anatomy and structure, and different in vivo settings contribute to these disparities. It is possible that differences in the main cell types involved and their origins in development account for some of the observed phenotypic diversity [25].

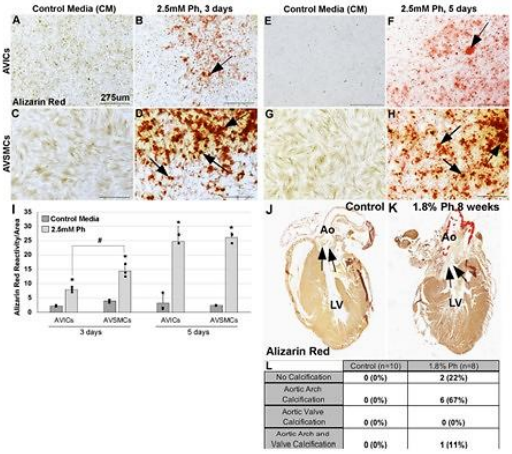


Fig. 4 Calcification of AVIC and AVSMC produced by elevated phosphate levels [23].

The research conducted by Sutton et al reveals the existence of DNA damage, suggested by gH2AX and 8-oxo-Dg in the calcified arteries of individuals with CKD and diabetes, together with senescence markers such as the cell cycle regulators p21 and p16 [26]. Recent studies have established a connection between protein O-GlcNAcylation and the upregulation of Runx2, as well as smooth muscle cell calcification in diabetes [27]. Moreover, The DNA damage response involves Runx2. An rise in calcium and phosphate levels causes Runx2 to be poly-ADPribosylated, which in turn activates its downstream osteogenic targets [28]. Metabolic changes may influence vascular calcification via epigenetic alterations to DNA or histones, another route. Additionally, DNA damage signaling and aging often intersect along these pathways. A family of histone deacetylases known as sirtuins regulates vascular smooth muscle cell senescence and the DNA damage response; this, in turn, affects osteogenic differentiation and calcification. Diabetic blood arteries have reduced levels of sirtuin 1 (SIRT1), which, when activated, restores the normal phenotype of vascular smooth muscle cells and promotes successful DNA repair [29].

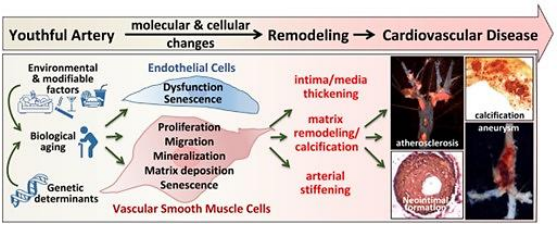


Fig. 5 Vascular aging and the progression of vascular disease associated with aging [26].

According to Pan et al., VSMC loss due to different types of cell death can cause the fibrous cap to shrink and necrotic core and calcification to occur [30]. These days, apoptosis, necroptosis, pyroptosis, autophagy, ferroptosis, necroptosis, necroptosis, and so on are all part of PCD [31]. It was shown that irisin's molecular defense mechanism against VC includes NLRP3-mediated VSMC pyroptosis [32]. It was found that following AGEs induction, the calcification of HASMCs persisted in the Atg7-/- animal model. The fact, that Atg7 is an inhibitor of autophagy suggest that autophagy plays a significant role in VC caused by diabetes.

Durham et al. found that calcification likely causes the same VSMC phenotypic shift in both medial and intimal calcification. One thing that stands out is that the causes that cause calcification at the two sites can be very different. Medial calcification occurs more quickly in patients with renal failure, however this is not always associated with an increased atherosclerotic burden. The media and intima can calcify more quickly in some diseases, such diabetes, although the impacted vascular beds can be entirely separate. Among the many medical disorders that might cause arterial medial calcification (AMC) is DM. In contrast to atherosclerotic calcification, it doesn't always include inflammatory cell infiltration or lipid buildup. Also, atherosclerosis isn't the only illness that may cause medial calcification; there are many more, indicating that several pathways promote VSMC change [33].

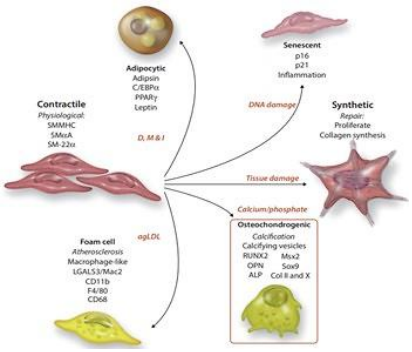


Fig. 6 Hierarchy of potential VSMC phenotypes and the underlying cellular, disease-related, or organotypic factors [33].

Both VSMCs and chondrocytes generate MGP, which inhibits BMP-2. Some have postulated that when MGP is lost, BMP-2 is free to express itself, which in turn causes VSMC phenotypic alterations and calcification. A lack of MGP activity causes the uncommon autosomal recessive illness known as Keutal syndrome. Patients with this disorder experience a number of maladies, such as malformed cartilage and vascular calcification. Similarly, severe arterial calcification causes the death of mgp-knock-out mice after 6 weeks. It has been suggested that unopposed BMP-2 driving AMC could result from decreased MGP levels seen in diabetic individuals.34

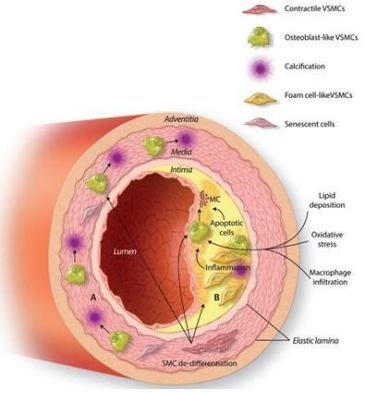


Fig. 7 Formation of VSMCs during medial and intimal calcification [33]

Study limitations

This study relies on in vitro models, which may not fully capture the complex in vivo environment of diabetic mellitus by calcifying blood vessels. Using HASMCs, or human aortic smooth muscle cells, provides valuable insights, but these findings may not be entirely generalizable to other vascular cell types or tissues. Additionally, the study's focus on the Wnt/β-catenin signaling pathway and osteogenic markers limits the exploration of other potentially significant molecular mechanisms. Furthermore, the exclusion of clinical data means that the findings might not directly translate to patient outcomes, necessitating caution when interpreting the results in the context of broader

clinical applications. Finally, the limited sample size of reviewed studies and potential publication bias could also impact the robustness of the conclusions.

4. Conclusion

This study's findings are important because they contribute to the understanding of how DM exacerbates vascular calcification, which is an important factor in the development of cardiovascular disease in diabetes patients. This study highlights the need for further research to explore additional molecular pathways involved in vascular calcification and to investigate potential therapeutic targets to mitigate this process. Integrating these findings into clinical practice may improve the management of vascular complications in diabetes, ultimately improving patient outcomes.

5. Conflict of Interest

The authors declared no conflict of interest.

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