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### Research article

**O. Susla, O. Bushtynska, I. Yakubyshyna, L. Logoyda,  
K. Symko, I. Mysula**

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### The potential mechanisms of cardiovascular calcification in patients with chronic kidney disease

I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

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**Abstract.** *Based on current literature data, the important potential role of calciprotein particles, matrix vesicles, and extracellular matrix degradation in cardiovascular calcification mechanisms in chronic kidney disease (CKD) can be confirmed. The involvement of advanced glycation end products, insulin resistance, microRNAs, iron metabolism disorders, fluid overload, and hemodialysis treatment in these processes is discussed. It was concluded that the above potential mechanisms of ectopic calcification, which are being actively explored, are directly or indirectly related to endothelial damage/dysfunction and metabolic disturbances in the nitric oxide system. It was concluded that further thorough scientific investigations and close collaboration between clinical and experimental nephrologists are useful to optimize programs for the early detection of cardiovascular calcification, develop new effective therapeutic strategies, and improve the prognosis of CKD patients.*

**Key words:** *chronic kidney disease, cardiovascular calcification, calciprotein particles, matrix vesicles, hemodialysis, endothelial dysfunction.*

Conflict of interest. The authors declare no conflict of interest.

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Correspondence should be addressed to Oleksandr Susla: [oleksandrsusla@ukr.net](mailto:oleksandrsusla@ukr.net)



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О. Б. Сусла, О. В. Буштинська, І. Г. Якубишина, Л. С. Логойда, К. Є. Симко, І. Р. Мисула

## Потенційні механізми кардіоваскулярної кальцифікації у хворих на хронічну хворобу нирок

Тернопільський національний медичний університет імені І. Я. Горбачевського  
МОЗ України, Тернопіль, Україна

**Резюме.** Узагальнення даних літератури дозволяють на сьогодні стверджувати про важливу потенційну роль частинок кальційпротеїну, везикул матриксу та деградації позаклітинного матриксу в механізмах кардіоваскулярної кальцифікації при хронічній хворобі нирок (ХХН). Дискутується залучення до цих процесів кінцевих продуктів глікування, інсулінорезистентності, мікроРНК, порушення метаболізму заліза, перевантаження рідиною та гемодіалізного лікування. Підкреслено, що зазначені потенційні механізми ектопічної кальцифікації, які активно досліджують, прямо чи опосередковано пов'язані з ушкодженням/дисфункцією ендотелію, розладами метаболізму в системі оксиду азоту. Зроблено висновок про доцільність проведення подальших ґрунтовних наукових досліджень, тісної співпраці фахівців клінічної та експериментальної нефрології з метою оптимізації програм ранньої діагностики кардіоваскулярної кальцифікації, впровадження нових ефективних терапевтичних стратегій та покращення прогнозу у хворих на ХХН.

**Ключові слова:** хронічна хвороба нирок, кардіоваскулярна кальцифікація, частинки кальційпротеїну, везикули матриксу, гемодіаліз, ендотеліальна дисфункція.

**Introduction.** It has been established that cardiovascular calcification is a critical complication of chronic kidney disease (CKD), an independent predictor of general and cardiovascular mortality, and a component of mineral and bone disorder syndrome (CKD-MBD). According to modern ideas, in patients with CKD, the implementation of both traditional and non-traditional risk factors, associated with a kidney function decline and dialysis treatment, initiates the processes of cardiovascular calcification, mainly by active mechanisms [1-3]. Many other interesting ways of formation and progression of cardiovascular calcification in CKD are currently widely discussed in scientific circles, and this review is devoted to their coverage and analysis.

**Calciprotein particles and matrix vesicles.** The scientific studies of recent years convincingly show that, in addition to the traditional mechanisms associated with endothelial damage/dysfunction and the osteoblastic transformation of vascular smooth muscle cells, passive calcification are closely associated with the interaction of calciprotein particles and matrix vesicles, can play an important role in the processes of cardiovascular calcification in CKD [1].

Protein-binding uremic toxins and hyperphosphatemia initiate the processes of calcification of the medial layer of arteries by active mechanisms, moreover, these compounds reduce the mineralization of osteoblasts and inhibit the release of extraosseous deposits of calcium and phosphate crystals [1-4]. Several local and systemic calcification inhibitors, including the Klotho secretory protein, matrix Gla protein (MGP), osteopontin, osteoprotegerin, fetuin-A, vitamin K, pyrophosphate and magnesium counteract the formation of vascular calcification [5-7]. The calciprotein particles, specifically, the calciprotein monomer and low-density lipoprotein particle, are a solid phase of calcium-phosphate compounds [8]. Interacting with fetuin-A and MGP, the calciprotein particles acquire, as a rule, a spherical shape [9]. Such calciprotein particles (primary forms) can be purified by the liver through the scavenger receptor A, which is expressed in its endothelial cells [8]. In the case of unregulated or insufficient fetuin-A or MGP levels, the primary forms of calciprotein particles are converted into secondary forms (crystallization nuclei) of the calciprotein particles that are not excreted by the liver. The primary calciprotein particles have a rounded shape with a diameter of 60-75 nm, and consist mainly of amorphous calcium phosphate, which is represented as a colloid. The secondary calciprotein particles include calcium hydroxyapatite, which is formed due to the crystallization of calcium phosphate, resides in the nucleus, and has a diameter of 120-150 nm. It is believed [1] that the cysteine-like domain D1 of fetuin-A promotes binding to the cal-

Oleksandr Susla  
oleksandrsusla@ukr.net

cium phosphate, preventing its growth, aggregation and precipitation. Compared to the primary forms of the calciprotein particles, the secondary forms have a lower content of fetuin-A, a lower surface charge, and a higher content of the apolipoproteins. Therefore, a significant number of the secondary calciprotein particles directly damages the endothelium, deepens the vascular calcification by attracting (activation of Toll-like type 4 receptors) the inflammatory cells, in particular the macrophages, induces the oxidative stress (OS) and modulates the activity of tumor necrosis factor- $(\text{TNF-}\alpha)$  [10]. Accumulated secondary calciprotein particles are deposited in the extracellular matrix and enhance ectopic calcification. It has been shown that an insufficient level of the serum calcification inhibitors is a predictor of vascular calcification [11]. Yamada et al. [12] have determined that the dietary restriction in CKD promotes insufficient formation of the calcification inhibitors, which correlates with the more pronounced vascular calcification. In addition, Chen et al. [11] have proved that the transformation of primary forms of the calciprotein particles into the secondary forms is accelerated in patients with CKD, and this transformation is probably related to the calcification of arterial media. A post-hoc analysis of a large-scale EVOLVE study [13] has shown that a shorter conversion time of the calciprotein particles is a predictor of mortality in CKD patients, who were taking calcimimetics. Moreover, the clinical consequences of vascular calcification in CKD reliably depend on the variability of primary and secondary forms of calciprotein particles [14]. In patients, who were treated with haemodialysis (HD) and who stopped taking calcimimetics for more than 12 months, had increased concentrations of intact parathyroid hormone (PTH) and crystalline calciprotein particles in serum [14]. In these individuals, the annual mortality rate was 19%. It should be noted, that quantitative change of calciprotein particles is associated with vascular calcification in patients with CKD [1]. Based on the above-indicated information, the transformation of calciprotein particles may play a critical role in the mechanisms of vascular calcification in CKD, so further research, according to Hou et al. [1], should be concerned with the study of the effects of modulation of their primary forms.

Matrix vesicles must be actively involved in the processes of vascular calcification in CKD, which is gradually released after the deposition of secondary calciprotein particles in the extracellular matrix [15]. Matrix vesicles are a subset of extracellular vesicles enclosed in a double membrane and consist of phosphatidylserine and annexin [1, 15]. Various cells release vesicles into the extracellular environment to inhibit cellular apoptosis [16]. Vascular hematopoietic cells, such as endothelial progenitor cells, monocytes, platelets and red blood cells, release extracellular vesicles. These extracellular vesicles interact with endothelial cells and induce endothelial dysfunction (ED) through the activation of free-radical processes,

the accumulation of intercellular adhesion molecules, in particular ICAM-1 and other chemokines [1]. In the medial layer of the arteries, excess phosphates enter the smooth muscle cells by endocytosis, and the calcium, which is released with lysosomes, activates osteogenic expression and secretes matrix vesicles to the extracellular environment [1]. In patients with CKD, hyperphosphatemia and extraosseous calcium penetrate the vascular smooth muscle cells, later induce the intracellular OS and endothelial reticular stress [17]. The OS increases the release of calcium and phosphate into the extracellular space through the matrix vesicles. It is known that in CKD, matrix vesicles contain less fetuin-A and Gla-rich proteins (GRP) and these vesicles are closely associated with a high degree of extraosseous mineralization [1]. In addition, it has been investigated that the extracellular vesicles in the serum of patients with CKD are prone to vascular calcification, since they carry a higher percentage of the markers associated with the calcification, in particular, the GRP [18]. Since hyperphosphatemia and unregulated calcium deposition directly activate vascular calcification, it is necessary to determine the character of further interventions aimed at the interaction of calciprotein particles and matrix vesicles, in order to prevent ectopic calcification in CKD conditions.

**Degradation of extracellular matrix.** It is believed [5, 19] that the degradation of the extracellular matrix, its rigidity, the connection of the extracellular matrix with competent cells, affect the cellular response in the process of vascular calcification. Data are provided [20] that lysosomal proteases – cathepsins – are actively involved in the degradation of the extracellular matrix, play a crucial role in various conditions that include such large biological systems as autoimmune diseases, heart recovery, cardiomyopathy, heart valve disease and atherosclerosis. Inhibition of cathepsin S can be an important tool to reduce cardiovascular risk, reduce inflammation activity and the formation of cardiovascular calcification in patients with CKD [20]. Moreover, the extracellular matrix can determine the signaling, mediator, and regulatory mechanisms of phenotypic cell transformation in CKD [19]. Increasing the stiffness of the extracellular matrix promotes osteogenic differentiation of mesenchymal stem cells, with the cytoskeleton playing an integrating role in this process [19]. The interaction of extracellular vesicles with abnormal collagen leads to the appearance of microcalcifications in the cover of the atherosclerotic plaque [15]. The matrix vesicles, just as the calcified extracellular vesicles, become the nucleus of calcification in the extracellular space of the tissues of the cardiovascular system [15]. Typically, the collagen degradation contributes to the accumulation of calcified extracellular vesicles in damaged cells [5, 15, 16]. Research on the synergistic effect of extracellular vesicles and matrix vesicles on the mechanisms of vascular calcification and bone mineralization is in an active phase [1], their solution will obviously be able to form the basis for the development of new therapeutic

strategies within the framework of the CKD-MBD. It is also interesting the matrix vesicles, regardless of their origin, reach the vessel matrix as circulating nuclear complexes and the formation of hydroxyapatite crystals in these vesicles is clearly associated with the balance of procalcifying factors and inhibitory agents [16].

**Advanced glycation end product accumulation and insulin resistance.** Of considerable interest today is the study of the pathogenetic role of advanced glycation end products (AGEs) and insulin resistance (IR) in the mechanisms of cardiovascular calcification in CKD [21-25]. The metabolic factors, primarily hyperglycaemia, are assigned a leading (inducing) role in the development of chronic micro- and macrovascular lesions [26] with further deepening of ED and progression of cardiovascular complications. Enhanced production of reactive oxygen species (ROS) by competent cells [27] in violation of carbohydrate metabolism is considered the main stage in the formation of cardiovascular diseases. According to the Brownlee theory [28], hyperglycaemia triggers a whole cascade of biochemical transformations that lead to damage in the vascular wall – the polyol pathway of glucose transformation into sorbitol and protein kinase C is activated, AGEs are formed, and free radical processes are intensified. Since glucose metabolism by a polyol mechanism occurs mainly in those organs and tissues that do not require the presence of insulin to move glucose into cells (nerve endings, retinal vessels, pericytes, renal interstitium cells and, importantly, the vascular endothelium), sorbitol accumulates in them due to this, which is an osmotically active substance [29]. This, in turn, causes the insufficient formation of antioxidants glutathione and tocopherol, preventing the generation of nitric oxide (NO) (due to the depletion of NADPH coenzyme). In diabetes mellitus (DM), activation of protein kinase C increases the permeability of the vascular wall, accelerates the processes of fibrosis and sclerosis of tissues, activates lipid peroxidation, and disrupts intraorgan hemodynamics. The excess formation of AGEs in long-term hyperglycaemia changes the structure and metabolism of the main proteins of the body (collagen, myelin, etc.). Scientists emphasize [27] that, the accumulation of glycated collagen in the myocardium can contribute to its increased stiffness. It is important, that AGEs are quite stable molecules, that accumulate in tissues and vessel walls [29]. The glycation of collagen can lead to structural deficiencies, consisting in deterioration of collagen cross-linking, increased matrix and tissue stiffness, inhibition of matrix transformations, disruption of cell-matrix interaction, as well as reduction of collagen fibril slip [30]. In addition to the structural changes, glycated proteins can be ligands for the multiligand receptor to AGEs (RAGE) [3, 25].

The spectrum of pathological effects of AGEs is broad: 1) by binding to the proteins of the basal membrane of blood vessels, they change its configuration and increase the permeability to proteins and other

components of plasma; 2) reduce the activity of the metabolism enzymes of the basal membrane of blood vessels, which leads to its thickening; 3) interacting with the receptors of macrophages and endothelium of blood vessels AGEs, increase the synthesis of cytokines (TNF- $\alpha$ , interleukin-1 (IL-1), growth factors), which, in turn, activate the processes of cell proliferation/hyperplasia (fibroblasts, smooth muscle, endothelial, etc.); 4) activate platelet aggregation [3, 22, 31]. In addition, AGEs affect lipid metabolism by promoting the oxidation of low-density lipoprotein cholesterol (LDL cholesterol) and other atherogenic lipoproteins. It is proved [29] that in patients with DM, AGEs are involved in the development of arterial hypertension, and its manifestation, violates the adequate sensitivity of the vessel wall to the influence of vasodilator substances, in particular NO. The formation of AGEs is irreversible, which explains the progression of micro- and macrovascular complications, even under conditions of sufficient compensation for carbohydrate metabolism [31].

AGEs, as generally recognized uremic toxins, are actively involved in the development of dysfunction of vascular smooth muscle cells, transforming their contractile phenotype to a secretory one [3]. The accumulation of AGEs is an integral mechanism of abnormal proliferation, migration, aging, apoptosis and calcification of smooth muscle cells in CKD [3]. Indeed, the interaction between AGEs and RAGE induces *in vitro* proliferation of rat vascular smooth muscle cells, as a function of time and dosage, by increasing the ROS production [32], followed by activation of the kappa  $\beta$  (NF- $\kappa\beta$ ) nuclear transcription factor system and the MAPK protein kinase pathway [3]. In addition, vascular smooth muscle cell proliferation is inhibited in homozygous RAGE-null mice compared to the *wild-type* animals [33]. In mice of the C57BL/6 line, the blockade of ligand binding to RAGE (through the introduction of soluble RAGE) is accompanied by a decrease in the proliferation of vascular smooth muscle cells [33]. Interestingly, the circulating levels of soluble RAGE in CKD are reduced in patients with aortic valve calcifying stenosis and vascular calcification [25]. In obese rats on the DM model, blocking the RAGE/ligand interaction inhibited the proliferation of vascular smooth muscle cells and the formation of neointima after arterial balloon injury [3]. In mice, ApoE-/- RAGE activation is closely related to aortic calcification in diabetes [34]. In this way, the AGE-RAGE axis can affect smooth muscle cell proliferation in vascular uraemia. Activation of RAGE with AGEs causes migration of smooth muscle vascular cells of rats, humans, and rabbits [3] and is associated with high expression of matrix metalloproteinases-2 and -9. In addition, the induction of endoplasmic reticulum stress through RAGE activation plays a key role in vascular smooth muscle cell apoptosis [35].

Recently, Belmokhtar et al. [25] have shown that enhanced rage signaling is a key (modulation of the

sodium-phosphate cotransporter PiT-1) mechanism for arterial media calcification in experimental animals in CKD. RAGE activation inhibits gene expression of smooth muscle cells by inhibiting the transactivating function of myocardin and is accompanied by the osteogenic differentiation of smooth muscle elements of the vascular wall [36]. In addition, *in vitro* results indicate that the induction of NADPH-oxidase by AGEs is involved in RAGE-dependent vascular calcification [3]. In experiments on mice, ApoE<sup>-/-</sup> RAGE-induced NADPH-oxidase contributed to the progression of atherosclerosis, intima calcification and the artery media [3]. In rats with DM, AGEs inhibitors prevented the development of vascular calcification [37]. Moreover, under these conditions, the acceleration of diabetes-associated vascular calcification processes was also prevented by antioxidants. The use of *high cut-off-dialysis* membranes, characterized by high clearance of removal of inflammatory mediators and AGEs is important and it can reduce dysfunction of vascular smooth muscle cells, and thus, slow the progression of vascular remodeling in HD patients [21, 38].

The following factors are important in the mechanisms of IR formation in CKD: 1) anemia; 2) PTH; 3) 1.25 (OH)<sub>2</sub> D<sub>3</sub> (calcitriol); 4) protein- or ketoacid analogues; 5) guanidine substrates; 6) exercise; 7) acidemia; 8) dyslipidemia; 9) chronic inflammation (adipocytokine, adiponectin); 10) renin-angiotensin-aldosterone system (RAAS); 11) fibroblast growth factor-23 (FGF-23); 12) ghrelin; 13) uremic toxins [23]. IR occurs in the early stages of CKD, it is observed in 90% of patients with type 2 DM [31]. The degradation of *insulin receptor substrate 1* (IRS-1) – substrate-1 to the insulin receptor has been proven to be a key molecular mechanism of IR in CKD [39]. High levels of insulin in the blood promotes the proliferation of vascular smooth muscle cells, increases the activity of the sympathetic nervous system, activates the RAAS, increases the sodium retention in the renal tubules [23, 39]. IR is a trigger for the disorders of carbohydrate (induction of hyperglycaemia) and lipid (increase in the content of triacylglycerols (TG), LDL cholesterol, decrease in high-density lipoprotein cholesterol) metabolism, hemodynamic disorders, hemostasis disorders, development of hyperglycaemia [29, 40].

The formation of insulin sensitivity disorders precedes (15 years or more) the development of type 2 DM, so it can be a predictor of this disease [23, 29]. In the experimental research [41], a defect in the insulin-stimulated glucose intake was demonstrated due to a decrease in the translocation of glucose transporters, in particular the GluT-4, which is responsible for the insulin-stimulated glucose uptake by the muscles, including cardiomyocytes. This condition is accompanied by ATP deficiency, and hypoxia of tissues, which arise because the necessary amount of glucose is unable to be transported in the mitochondria due to a defect in its transporters. It is believed [29] that one of the triggering mechanisms of the IR is the

increased expression of TNF- $\alpha$ , which reduces the activity of insulin receptor tyrosine kinase and tyrosine phosphorylation of IRS-1; under these conditions, GluT-4 remains intact. In addition, in the development of IR syndrome, a reduced number of the receptors to insulin is also important, due to the direct activity of TNF- $\alpha$  at the level of fat and muscle tissues [39]. There are hypotheses about the genetic predisposition to IR [31], about the involvement of mediators such as leptin, adiponectin, resistin, free fatty acids and TG in the formation of insulin sensitivity disorders [23, 29, 31, 41]. The important thing is that IR indirectly inhibits the production and/or bioavailability of NO, and contributes to the impaired endothelial function and activation of vasoconstrictor agents [23].

The notion of mechanisms for the development of ED in the context of the IR still remains debatable. Some researchers indicate that ED is a consequence of the influence of factors characterizing IR, namely: hyperglycaemia, hyperinsulinemia, dyslipidemia, arterial hypertension [42], others prove that endothelial damage/dysfunction is the cause of insulin sensitivity disorders [31]. There is also a suggestion that ED and IR are closely associated conditions, in which there is a decrease in insulin-mediated endothelial-dependent vasodilation [40, 42]. ED is considered an integrated IR syndrome, contributes to its deepening, increased vascular reactivity and leads to cardiovascular complications [43].

It is noted, that in the presence of type 2 DM, hyperglycaemia and AGEs accumulation are highlighted as the major factors causing the endothelial damage/dysfunction [22, 23, 40]. The OS caused by excessive production or insufficient utilization of ROS [26], as a result of prolonged hyperglycaemia, in addition to oxidative modification of lipids, proteins and nucleic acids, induces an impaired expression of endothelial NO synthase (eNOS), thus reducing the production and bioavailability of NO [23, 40, 44]. The accumulation of AGEs causes disruption of the barrier function of the vascular wall, increases the expression of adhesive molecules, stimulates the formation of ROS, an inducible NO synthase that blocks eNOS and reduces the bioavailability of NO, leading to the deepening of ED [22, 29]. AGEs can directly inhibit antioxidant enzymes [27], which enhances free-radical processes. By interacting with appropriate monocyte and macrophage receptors, AGEs induce the expression of pro-inflammatory cytokines by these cells, which are important factors in the activation of the inflammation processes and the development of atherosclerotic damage [3, 25].

It is important that TNF- $\alpha$  activates transcriptional pathways, enhancing the OS and inflammation, reduces the level of GluT-4 proteins – insulin-regulated glucose transporters, which are mainly found in adipocytes, skeletal muscle and myocardium [41], which ultimately contributes to the occurrence of IR and ED. In addition, TNF- $\alpha$  is a chemoattractant of macrophages and

Langerhans cells, a stimulant of angiogenesis, a potential activator of monocytes, adhesion molecules, enhances phagocytosis, the production of ROS, stimulates the production of proteins of the acute phase of inflammation [24]. The pro-inflammatory mediators, in particular TNF- $\alpha$ , promote cell degeneration, damage the endothelium, initiate inflammatory processes in the walls of arterial vessels and their calcification [5, 6, 20, 22, 24, 45-47]. It has been proven, that the multipotent cytokine TNF- $\alpha$  in CKD patients, affecting the endothelium, enhances the expression of cell adhesion molecules on it, significantly reduces the formation of basal NO and induces apoptosis and differentiation of endothelial cells [3, 24, 40, 48].

It is worth noting that the uremic toxins, in particular asymmetric dimethylarginine, in patients with CKD stage 5D, are significantly associated with the diabetic nephropathy and cardiovascular diseases [3, 29, 49], and can adversely affect the reduction in the number and dysfunction of endothelial progenitor cells, induce endothelial cell aging, promote neoangiogenesis and vascular calcification [3, 4, 6, 24, 40, 50]. Under the influence of provoking factors, microvascular inflammation of the endotheliocytes occurs, and the bioavailability of NO decreases, including in cardiomyocytes, which leads to their hypertrophy, the development of interstitial fibrosis [22, 29].

**Disorders of iron metabolism.** In recent years, the problem of the relationship between impaired iron metabolism and cardiovascular calcification in CKD has been widely discussed in scientific circles. Neven et al. [51] believe that iron plays an important role in numerous cellular processes and the mechanisms of ectopic calcification in chronic renal dysfunction. It has been shown that in patients with non-dialysis stage CKD, the regulatory protein hepsidin correlates closely with such markers of bone and mineral metabolism as intact PTH, phosphate and 25 (OH) D (calcidiol) [52]. In non-dialysis patients, 16 weeks of iron citrate administration, in addition to improving the iron balance in the body, led to a likely decrease in the serum content of phosphate and FGF-23 [53]. In another study [54], the use of iron oxyhydroxide complex as a new non-calcium-containing phosphate binder in patients with CKD stage 5D who received chronic HD, contributed to the effective reduction of phosphate content, an increase in Klotho morphogenetic protein concentrations and a decrease in C-reactive protein concentration. It is important that the administration of iron oxyhydroxide improves (by increasing  $T_{50}$ ) *serum calcification propensity* and reduces the incidence of cardiovascular complications in HD patients [55]. The efficacy and safety of iron-based phosphate binding agents in CKD-MBD has also been demonstrated in other clinical interventions [56, 57]. It has recently been found that iron citrate, affecting inflammatory mechanisms, growth factors and adhesive molecules, prevents the development of myocardial fibrosis and cardiac hypertrophy in rats with CKD [58].

The direct effects of iron on the cardiovascular calcification processes have been demonstrated *in vitro* [59, 60] and *in vivo* [61, 62]. It was found that the increased expression and ferritin heavy chain ferritin peroxidase activity prevents vascular calcification by inhibiting the osteoblast transformation of vascular smooth muscle cells [60]. In addition, the inhibition of vascular calcification of iron is also due to the prevention of apoptosis and increased autophagy [63]. These data indicate that the iron has a protective effect on calcium deposition in the vascular wall under phosphate excess. Moreover, in conditions of phosphate-induced vascular calcification, the phosphate binders containing iron completely prevent its progression [59]. It is experimentally proven [61] that excess iron prevents the activation of PiT-1 and the development of vascular calcification in CKD.

Studies by Ciceri et al. [59] have shown that iron citrate is able to influence the modification of the muscle component of the extracellular matrix, as an important mechanism of arterial stiffness and vascular calcification under conditions of CKD. Therapeutic doses of iron, in addition to blocking the formation of the osteochondrogenic phenotype of vascular smooth muscle cells and the additional deposition of acidic glycoproteins, can protect the aortic wall from the progression of fibrosis, restructuring of fibrils, blocking the intensive thickening of collagen fibrils caused by hyperphosphatemia. Interestingly, iron can also help improve the elastic structure of blood vessels by protecting the aortic wall from the progression of elastolysis, thus preventing it [2].

On the other hand, excess free iron, which is toxic, can lead to the generation of ROS, the development of OS, inflammation, ED, ectopic calcification and cardiovascular disease [51, 62]. Iron impairs osteoblastic differentiation and cell mineralization. The use of L-histidine, in addition to its active participation in haematopoiesis, is promising in terms of weakening free radical processes in the treatment of anemia in CKD [62].

**microRNAs.** Today, there is more and more scientific data [64-68] on the important role of microRNAs in the mechanisms of cardiovascular calcification in CKD. The results of these experimental and clinical studies have shown that microRNAs are a factor in the regulation of vascular calcification, an active participant in the processes of inflammation, ED, arterial stiffness and remodeling. *In vitro* studies indicate [65, 69] that microRNAs are critical for endothelial cell gene expression and function; microRNAs are found in atherosclerosis, cardiac hypertrophy, hypertension, coronary artery disease, DM, and inflammatory diseases. MicroRNAs control the endothelial cell senescence, angiogenesis, and vascular inflammation [69], and therefore can be targeted for therapeutic interventions on the endothelial functional activity, inflammation, and vascular remodelling.

**Hemodialysis treatment.** An important component of accelerating the processes of athero- and arteriosclerotic damage, cardiovascular calcification is considered to be the possible interventional effect of various methods of renal replacement therapy, in particular HD, on the mechanisms of inflammation, OS, endothelial damage/dysfunction [21, 38, 70-73]. Shifts in acid-base homeostasis and water-electrolyte disturbances associated with HD treatment may induce cardiovascular calcification in patients with CKD stage 5D. Seras et al. [74] note that the competitive combination of calcium load, progressive alkalisation of blood during the HD session, hyperphosphatemia in combination with hypomagnesemia is a “perfect storm” for the formation of vascular calcification in HD patients. The advantages in removing uremic toxins of expanded HD<sub>x</sub>, using dialysis membranes with middle cut-off and high cut-off points over high-flow, low-flow HD or hemodiafiltration, were presented [21, 38, 75, 76]. In addition, it has recently been established that high cut-off HD reduces the serum procalcifying activity in patients treated with HD [77].

**Fluid overload.** Interesting and promising are the studies on the association of hydration status with the

cardiovascular calcification, general and cardiovascular mortality in patients with non-dialysis and dialysis-dependent stages of CKD [78-83]. Specifically, Park et al. [81] noted that the excess extracellular fluid was likely and independently associated with coronary calcification in the dialysis patients. In a study by Mitsides et al. [82] it has been determined that the extracellular hyperhydration in HD patients is clearly associated with low-energy inflammation and ED markers, with elevated levels of cell adhesion molecules VCAM-1, IL-6, thrombomodulin, and reduced serum leptin levels reported in the group of individuals with extracellular fluid expansion. Moreover, in 2021, it had been proven, that vascular endothelial growth factor D is a new biomarker of fluid overload in dialysis patients and is characterized by the unique diagnostic and prognostic capabilities [83]. It is believed that adequate control of the body’s water sectors in the conditions of the CKD stage 5D can be one of the tools for a favorable prognosis in this category of patients.

Figure 1 provides a schematic view of the potential impact of new pathogenetic mechanisms on the processes of cardiovascular calcification in patients with CKD.

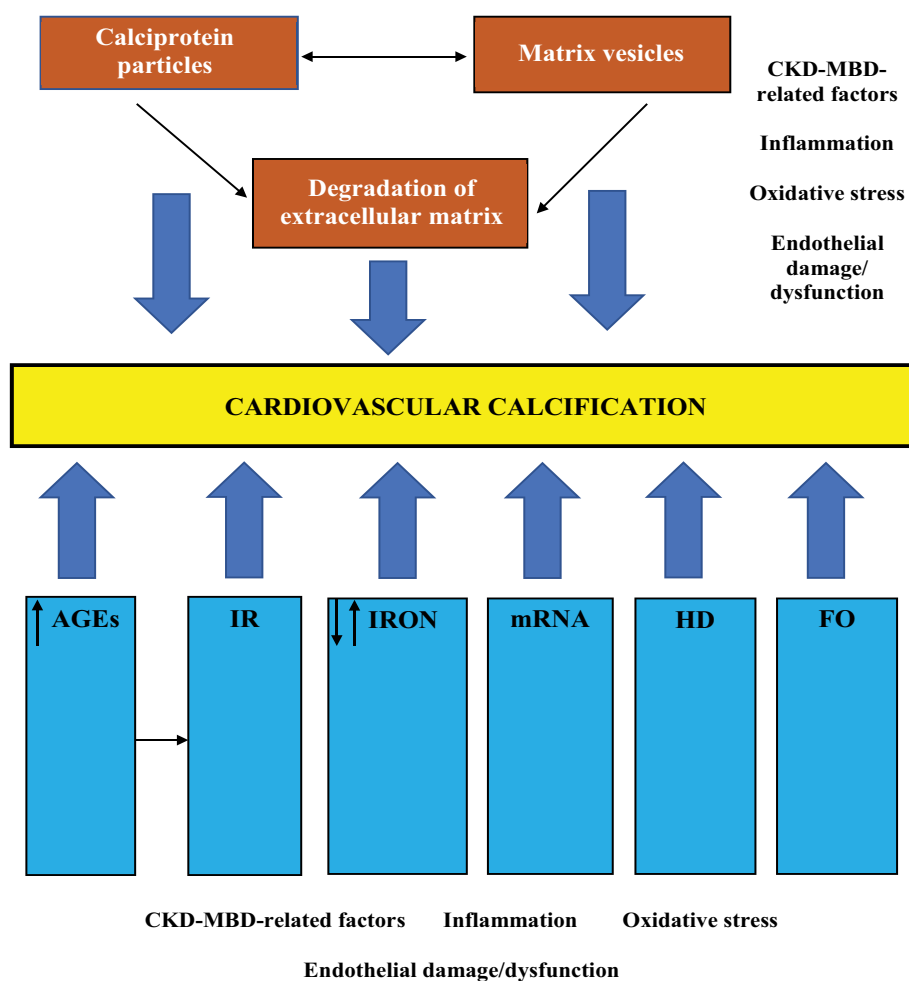


Fig 1. The potential mechanisms of cardiovascular calcification in CKD.

Notes: AGEs – advanced glycation end products, IR – insulin resistance, HD – hemodialysis, FO – fluid overload.

**Conclusions.** Thus, the scientific data presented in this article demonstrate once again the extreme complexity, multifaceted nature, and multifactoriality of the processes of cardiovascular calcification in CKD, with most of the pathogenetic and triggering mechanisms of this phenomenon currently under active investigation being directly or indirectly related to endothelial damage/dysfunction and metabolic disturbances in the system NO. The potential pathogenetic role of the interaction of calciprotein particles with matrix vesicles, extracellular matrix degradation, accumulation of AGEs, IR, microRNAs, HD treatment, hyperhydration in the mechanisms of development and progression of ectopic calcification in patients with CKD requires further scientific investigation, close collaboration between experts in experimental and clinical nephrology, which will ultimately allow the development of programs for early detection of this damage to the cardiovascular system, the development and implementation of new

effective therapeutic strategies, and the stratification of cardiovascular risk.

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**Authors contribution:**

**O. Susla:** concept of the paper, literature search, data analysis, manuscript writing and submission, supervision;

**O. Bushtynska:** literature search, data analysis, preparation of the manuscript for printing;

**I. Yakubyshyna:** literature search, data analysis, literary editing of the manuscript;

**L. Logoyda:** literature search, data analysis;

**K. Symko:** literature search, design of the text of the work;

**I. Mysula:** concept of the paper, formulation of conclusions.

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