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THE ROLE OF CALCIUM PHOSPHATE MICROCRYSTALS AND CALCIPROTEIN PARTICLES (CPPs) IN CHRONIC KIDNEY DISEASE (CKD) PROGRESSION AND ASSOCIATED INFLAMMATION

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კალციუმის ფოსფატის მიკროკრისტალებისა და კალციპროტეინის ნაწილაკების (CPPs) როლი თირკმელების ქრონიკული დაავადების (CKD) პროგრესირებაში

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რეზიუმე

თირკმლის ქრონიკული დაავადება (CKD) ჯანმრთელობის გლობალურ პრობლემას წარმოადგენს, რომელიც ხასიათდება მინერალური მეტაბოლიზმის დარღვევებით, ანთებითი პროცესებით და ქსოვილების დაზიანებით. ფოსფატების გამოყოფის დაქვეითება და კალციუმის დისრეგულაცია ხელს უწყობს მინერალების დაგროვებას თირკმლებსა და სისხლძარღვებში, რაც დაკავშირებულია ანთებით და ფიბროზულ პროცესებთან. კალციუმის ფოსფატის მიკროკრისტალები და კალციპროტეინის ნაწილაკები (CPP) წარმოიქმნება, როდესაც კალციუმ-ფოსფატის ნანოფაზები სტაბილიზდება შრატის ცილებით და ცირკულირებს როგორც CPP-I ან უფრო აქტიური კრისტალური CPP-II ფორმებით. კვების შემდგომ ფოსფატების მომატება და ძვლის რემოდელირება ხელს უწყობს მათ წარმოქმნას. CPP იწვევს ენდოთელურ დისფუნქციას, NLRP3 ინფლამასომის აქტივაციას, ჟანგვით სტრესს და პროანთებით პროცესებს, ხოლო ფოსფატები აძლიერებენ თირკმლის ფიბროზს GM-CSF, MCP-1/CCR2 და Akt/mTORC1 გზების მეშვეობით.

კლინიკურად, CPP ხელს უწყობს სისხლძარღვთა კალციფიკაციას და გულ-სისხლძარღვთა დაავადებების რისკს. ადრეული ინტერვენცია, მათ შორის ფოსფატების დამაკავშირებლები, როგორიცაა საქაროზა-ფერის ოქსიჰიდროქსიდი, ამცირებს CPP აქტივობას და ანთებას, რაც ხაზს უსვამს CPP-ების სამიზნე ტრანსლაციური პოტენციალის არსებობას. CPP-ებით გამოწვეული დაზიანების მოლეკულური მექანიზმების დადგენა კრიტიკულად მნიშვნელოვანია CKD-ის სამომავლო თერაპიისთვის.

OBJECTIVE. Chronic kidney disease (CKD) is marked by mineral metabolism disturbances, vascular calcification, and systemic inflammation, with calciprotein particles (CPPs) emerging as key mediators. This review aims to synthesize current evidence on CPP biology, including formation, maturation, and tissue sources, their cellular and molecular effects, and their contribution to CKD progression, vascular calcification, and cardiovascular risk. It also evaluates clinical biomarkers of CPP burden and potential therapeutic strategies to mitigate CPP-induced inflammation and calcification, providing a comprehensive framework for understanding and managing CPP-driven CKD–MBD.

METHODS. A focused literature search was conducted in PubMed, Scopus, and Web of Science for studies on calcium phosphate microcrystals and calciprotein particles (CPPs) in CKD and related inflammation. Search terms included “calciprotein particles,” “CPP,” “calcium phosphate,” “fetuin-A,” “vascular calcification,” “chronic kidney disease,” “CKD,” “inflammation,” and “biomarker.” Human

studies published between 2019 and 2025 were included, while conference abstracts, editorials, and case reports were excluded. Out of 50 screened studies, 17 met inclusion criteria. Data on CPP formation, inflammatory mechanisms, vascular calcification, biomarkers, clinical outcomes, and therapeutic strategies were extracted. Reference lists of key papers were also reviewed. Evidence was synthesized narratively, emphasizing mechanistic and translational insights.

RESULTS: Calciprotein particles (CPPs) are circulating calcium and phosphate nanoparticles associated with the development of vascular calcification (VC) in chronic kidney disease (CKD).

1. CPP FORMATION AND MATURATION. CPPs are generated when CaP precipitates are bound and stabilized by fetuin-A, preventing immediate crystal growth. This produces primary CPPs (CPP-I), amorphous and relatively inert complexes. Over time, particularly under conditions of reduced magnesium, diminished pyrophosphate, low citrate, and reduced fetuin-A, CPP-I transitions into secondary CPPs (CPP-II) containing crystalline hydroxyapatite cores. This transition is accelerated in uremic serum and reflects systemic calcification stress [1].

2. LIKELY MICROENVIRONMENTS FOR CPM/CPP FORMATION. CPPs form in localized microenvironments, with circulating particles representing a fraction that "leaks" into the blood. In the intestine, post-prandial phosphate surges (>30 mM) promote formation of CPP-I–like ACP nanoparticles, which enter circulation via Peyer's patches. In bone, high-turnover or pathological remodeling releases calcium–phosphate, which binds fetuin-A to form CPPs. Other sites, such as saliva (~4 mM) and bile (~5 mM), carry minor nucleation risk but lack fetuin-A, producing less stable particles [1]. Overall, the intestine dominates CPP formation postprandially, while bone contributes more under conditions of active remodeling.

3. INFLAMMATION-MEDIATING PATHWAYS. Calcium phosphate microcrystals and CPPs activate multiple inflammatory pathways in renal and vascular cells. In VSMCs and endothelial cells, CPPs trigger NF-κB, increasing IL-6, TNF-α, oxidative stress, and impairing NO bioavailability [2,3]. CRIC study data linked elevated phosphate with NF-κB markers (CRP, IL-6) predicting cardiovascular events [4], and phosphate binders or CPP-lowering therapies (sevelamer, sucroferric oxyhydroxide) reduced IL-6/IL-8/VCAM-1 and hsCRP [5]. CPPs also engage TLR4/NF-κB in macrophages and VSMCs, activating the NLRP3 inflammasome and IL-1β/IL-18 release [6]; in tubular cells, CPP uptake via CaSR induces lysosomal damage, cathepsin B release, NLRP3 activation, GM-CSF release, macrophage recruitment, TGF-β1 signaling, and fibrosis [4,7]. Additional pathways include Ras/MAPK, ERK, and Akt/ERK1/2-Mnk1, promoting IL-6/IL-1β, oxidative stress, apoptosis, vascular stiffness, and inflammation [4,8]. Collectively, CPPs act as DAMPs, increasing ROS, leukocyte adhesion, cytokine release, CRP, and FGF23, correlating with CKD progression and clinical outcomes. The key molecular pathways described above, their cellular targets, mechanisms, downstream effects, and supporting clinical or experimental evidence are summarized in **Table 1** for clarity and reference.

4. VASCULAR CALCIFICATION AND CPPs. CPPs accumulate in VSMCs, inducing osteogenic transdifferentiation and vascular calcification, correlating with arterial stiffness, cardiovascular risk, and mortality in CKD [9]. They trigger a contractile-to-osteochondrogenic switch, upregulating Runx2, BMP-2, osteocalcin, and alkaline phosphatase, while downregulating inhibitors like matrix Gla protein and fetuin-A, and release matrix vesicles that nucleate hydroxyapatite [9]. Experimental studies show CPP injection in rats causes medial calcification, and CPP-II exposure in vitro forms calcified nodules, preventable by magnesium or pyrophosphate; CPP-II induces stronger inflammatory and calcifying responses via TLR4/NF-κB, oxidative stress, and NLRP3 inflammasome signaling [9]. At the endothelium, CPPs impair NO, increase VCAM-1, ICAM-1, and E-selectin, and promote stiffness, while macrophage

uptake induces IL-1β release and foam cell–like changes. Clinically, higher CPP-II and shorter T50 associate with vascular calcification, arterial stiffness, inflammation, and adverse CKD outcomes [9].

TABLE 1. MOLECULAR PATHWAYS OF INFLAMMATION TRIGGERED BY CALCIUM PHOSPHATE PARTICLES (CPPs) AND HIGH PHOSPHATE LEVELS IN CKD

Pathway	Cellular Targets	Mechanism of Activation	Downstream Effects	Clinical/Experimental Evidence
NF-κB Pathway	VSMCs, endothelial cells	CPPs activate NF-κB; oxidative stress reduces NO bioavailability, worsening dysfunction	↑ IL-6, ↑ TNF-α → osteogenic transdifferentiation of VSMCs; endothelial dysfunction	CRIC cohort: phosphate correlated with NF-κB–driven CRP & IL-6 predicting CV events (Scialla 2021). Phosphate binders ↓ CPPs, IL-6, IL-8, VCAM-1, hsCRP [3,5]
TLR4/NF-κB Pathway	Macrophages, VSMCs, tubular cells	CPPs bind TLR4 → NF-κB activation + NLRP3 inflammasome; CaP crystals enter via CaSR macropinocytosis, destabilize lysosomes	Caspase-1 activation → ↑ IL-1β, ↑ IL-18 secretion	In vitro: CPPs induce inflammasome activation in macrophages & tubular cells [6]
NLRP3 Inflammasome	Renal tubular cells, VSMCs	High phosphate directly activates NLRP3	Pyroptosis & ↑ IL-1β; tubular injury → GM-CSF release → macrophage MCP-1 → CCR2+ recruitment → ↑ TGF-β1 → fibrosis	NLRP3 activation correlates with tubular injury, fibrosis markers, and ESKD progression (Scialla 2021). GM-CSF/MCP-1 axis drives fibrosis [7]
MAPK/ERK Pathways	Tubular cells, bronchial epithelial	Phosphate activates Ras/MAPK and ERK1/2	↑ Cytokine secretion, ↑ oxidative stress, apoptosis	Inhibition reduces inflammation in vitro. ERK activation linked to anemia & inflammation in CKD [4,8]
Akt/ERK1/2-Mnk1 Pathway	Vascular cells, lung epithelial cells	Phosphate stimulates Akt/ERK1/2-Mnk1 cascade	Promotes senescence and chronic inflammation	CKD patients: Akt activation correlates with vascular stiffness & ↑ inflammatory burden [8]

5. ENDOTHELIAL AND VASCUAL CELLULAR RESPONSES TO CPPs. Proteomic analysis of HCAEC and HITAEC exposed to CPP-P and CPP-S revealed compartment-specific stress responses, including mitochondrial and ER activation, ROS generation, calcium dysregulation, and apoptosis. Nuclear and cytosolic pathways showed downregulation of transcription, RNA metabolism, and cell cycle processes, with upregulation of cytokine/chemokine signaling. Lysosomal calcium release contributed to mitochondrial overload and intrinsic apoptosis. CPP-S induced stronger mitochondrial stress and apoptosis than CPP-P, while both caused ER stress and lysosomal acidification. Western blots confirmed

caspase-3 cleavage, downregulation of endothelial markers CD31 and ERG, and altered kinase signaling [10]. In VSMCs, CPPs promoted phenotypic switching with reduced contractile proteins (ACTA2, SMTN) and increased collagen expression (COL1A1, COL1A2), likely mediated by endothelial IL-6, IL-8, and MCP-1 release. CPP-S triggered stronger oxidative stress, TLR4 activation, apoptosis, and calcification than CPP-P, while both induced senescence-associated secretory phenotype (SASP) pathways [10].

6. CPP-P VS CPP-S PATHOLOGY. Earlier hypotheses suggested CPP-P were relatively harmless while CPP-S were more toxic. Current findings indicate that both induce pro-inflammatory responses, though CPP-S drives stronger apoptosis and vascular calcification. Distribution differs, with CPP-P internalized by liver sinusoidal endothelial cells and CPP-S recycled by liver and spleen macrophages. At the organelle level, CPP-S promotes cytosolic and nuclear oxidative stress and TLR4 signaling, whereas CPP-P induces lysosomal and ER pH stress. Lysosomal CPP dissolution causes Ca^{2+} overload and apoptosis, while ER stress complements lysosomal and mitochondrial dysfunction. Cytosolic and nuclear proteomes show downregulation of housekeeping pathways, reflecting reduced endothelial resilience [10].

7.CKD–MBD AND CARDIOVASCULAR DISEASE. CKD–MBD links disturbances in phosphate, calcium, PTH, FGF23, α-klotho, and CPPs to vascular calcification, bone fragility, and cardiovascular disease. CPP-I act as protective chaperones, whereas CPP-II promotes vascular smooth muscle cell osteogenic transformation, inflammation, and systemic calcification. Clinical evidence associates CKD–MBD mediators with increased cardiovascular morbidity and mortality, but therapies such as phosphate binders, vitamin D receptor activators, calcimimetics, and parathyroidectomy show mixed effects, underscoring the need for well-designed randomized trials [11].

8. BIOMARKERS FOR CPP DETECTION AND CHARACTERIZATION IN CKD. Biomarkers focus on circulating CPP levels, particle morphology (CPP-I vs. CPP-II), and associated proteins. Circulating CPPs are detected with fluorescent bisphosphonate probes binding crystalline calcium-phosphate, while CPP-II size and crystallinity strongly predict inflammation, vascular risk, and mortality [12]. Fetuin-A levels, typically measured by ELISA, serve as indicators of mineral stress, with reduced levels linked to unstable CPPs and microcrystal formation [13]. Gla-Rich Protein (GRP), an inhibitor of CPP maturation, also functions as a biomarker for vascular calcification, measured via immunoassays [14]).

9. DIAGNOSTIC TOOLS AND CLINICAL RELEVANCE OF CPPs. Nano-flow cytometry (nano-FC) is currently the most specific method for measuring calciprotein particles, allowing clear distinction between primary CPP-I and secondary CPP-II. Among these, only CPP-II quantified by nano-FC has shown independent associations with mortality and CKD progression, making it a clinically significant marker. Another approach, the T50 test, assesses serum crystallization propensity by evaluating the transition from amorphous to crystalline phases. While a shortened T50 indicates faster CPP-II formation and correlates with vascular calcification risk, it does not directly reflect in vivo CPP levels. Importantly, T50 values and circulating CPP counts are not interchangeable, emphasizing the need for precise, particle-based quantification to inform patient risk and outcomes [1].

10. THERAPEUTIC IMPLICATIONS. Magnesium supplementation has been shown to inhibit the transition of CPP-I to CPP-II, prolonging T50 and reducing vascular calcification [15]. Pyrophosphate directly prevents hydroxyapatite crystallization [16], while phosphate binders such as sevelamer and lanthanum lower phosphate load and decrease CPP formation [17]. Calcimimetics, including cinacalcet, reduce calcium–phosphate imbalance and indirectly limit CPP accumulation [5].

Under normal conditions, CPPs are cleared by Kupffer cells in the liver; however, clearance is impaired in CKD, leading to their systemic buildup [1]. Strategies aimed at enhancing reticuloendothelial clearance,

together with interventions that stabilize CPP-I and delay CPP maturation, are being explored as potential approaches to reduce inflammation and vascular calcification in CKD [1,10].

CONCLUSION. CPPs link mineral dysregulation, inflammation, and vascular pathology in CKD. CPP-I forms from calcium-phosphate stabilized by fetuin-A and can transition to crystalline CPP-II, which drives endothelial dysfunction, oxidative stress, NLRP3 inflammasome activation, vascular smooth muscle cell osteogenic transformation, renal fibrosis, and arterial stiffness. Proteomic studies show compartment-specific stress, apoptosis, and senescence. Clinically, elevated CPP-II and shortened T50 predict cardiovascular events. Interventions like phosphate binders, magnesium, pyrophosphate, calcimimetics, and CPP stabilization, reduce CPP burden and inflammation. CPPs thus act as pathogenic mediators and biomarkers, highlighting targets for future CKD–MBD therapies.

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SUMMARY

Chronic kidney disease (CKD) is a global health burden marked by disturbances in mineral metabolism, inflammation, and tissue injury. Impaired phosphate excretion and calcium dysregulation promote mineral deposition in the kidney and vasculature, linking to inflammatory and fibrotic pathways.

Calcium phosphate microcrystals and calciprotein particles (CPPs) form when calcium-phosphate nanophases are stabilized by serum proteins, circulating as CPP-I or the more active crystalline CPP-II. Postprandial phosphate surges and bone remodeling favor their formation. CPPs induce endothelial dysfunction, NLRP3 inflammasome activation, oxidative stress, and pro-inflammatory signaling, while phosphate amplifies renal fibrosis through GM-CSF, MCP-1/CCR2, and Akt/mTORC1 pathways.

Clinically, CPPs contribute to vascular calcification, arterial stiffness, and cardiovascular risk. Early interventions, including phosphate binders such as sucroferric oxyhydroxide, reduce CPP activity and inflammation, highlighting the translational potential of targeting CPPs. Understanding the molecular mechanisms of CPP-induced injury remains critical to guide future therapies in CKD.

Keywords: Chronic kidney disease, Vascular calcification, Inflammation, Calciprotein particles