Supplement A

Table 1 Human diseases associated with medial arterial calcification (examples).

Disease	**Main Feature**	**Non-medial tissue calcifications Phenotype**	**Reference**
Diabetes mellitus	Disorders of insulin metabolism	No	1
Chronic kidney disease	Excretory and metabolic renal dysfunction	No	2
Aging	Unknown; "Wear and tear"; elastic fibers?	No	3
Primary Medial Mönckeberg sclerosis	Unknown; genetic disorder?	No	4
Vitamin K deficiency	Inhibition of antiinflammatory properties mainly mediated through NF-κB signaling pathway; inhibition of carboxylation of Matrix Gla protein (MGP), a major inhibitor of soft tissue calcification	No	5
Vitamin D disorders Hypervitaminosis; Hypovitaminosis?	Stimulation of renal calcium resorption; synergistic effect with parathormon on bone resorption?	No	6
Atherosclerosis	Calcifications of the intima	Yes	7
Pseudoxanthoma elasticum	Defect of the ABCC6 gene ATP binding cassette subfamily C member 6	Yes	8
Rheumatoid arthritis?	Susceptibility by external factors and genetic patterns including those or the human leukocyte antigen (HLA) major histocompatibility complex	Yes	9

	(MHC), cytokine promotors, T-cell singalling genes etc.		
β-Thalassemia	Mutations that affect β genes resulting in low or no β-globin production. About 300 β-thalassemia alleles characterized	Yes	10
Calciphylaxis	Unknown	Yes	11
Kawasaki disease	Unknown; generalized inflammatory disease secondary to infection in genetically predisposed children?	Yes	12
Singleton-Merten Syndrome and other type I interferonopathies	Genetic mutations associated with activation of type I interferon (IFN1) responses	Yes	13
Parathyroid hormone disorders (hyper- and hypoparathyroidism)	Disorders of parathormon metabolism	Yes	14
Generalized Arterial Calcification of Infancy (GACI)	Mutations of the gene encoding for ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) which cleaves ATP to generate inorganic pyrophosphate (PP_i) and adenosine monophosphate (AMP) extracellularly	Yes	15
Arterial Calcification due to CD73 Deficiency (ACDC)	the ecto-5'-nucleotidase (*NT5E*) gene, which encodes CD73, is mutated causing defective transformation of adenosine monophosphate (AMP) into adenosine	Yes	16
Idiopathic Basal Ganglia	Associated with an impaired extracellular transport of inorganic	Yes	17

Calcification (IBGC)	phosphate, mutations in *SLC20A2*, *PDGFRB*, *PDGFB*, *XPR1*, *MYORG* genes		
Scleroderma?	Autoimmun disease most commonly associated with genetic constellations of the human leukocyte antigen (HLA) complex	Yes	18
Hutchinson-Gilford progeria syndrome	Single nucleotide substitution in the *LMNA* gene	Yes	19

Table 2

Comparison of human diseases associated with medial arterial calcification and animal models with calcification confined to the media layer of the vessel wall.

Human disease	**Animal model**	**Comments**	**Reference**
Diabetes mellitus	Streptozotocin-induced diabetes (+high-fat and VitD3 diet)	High-fat diet induces also intimal calcification	1,20, 21
Chronic renal disease	Kidney reduction Adenine diet Phosphate diet (±VitD3 diet) Cy+ rat Lewis polycystic kidney disea se	Models vary in calcification progression, surgical reduction of kidney mass often go hand in hand with acute kidney injury, death rate increase, higher variability in calcification progression; high VitD3 dose leads to acute hypercalcemia, hyperphosphatemia, physical impairment and weight loss	2,20
Aging	Klotho knockout model FGF-23 knockout model	Klotho deficiency results in higher FGF23 levels	3,20,22,23

Primary medial calcification	DBA2 mice	Female DBA2 mice are more prone to develop calcification	4,20, 24
Vitamin K deficiency	MGP knockout model	MGP gene deletion is lethal within 2 months in mice due to extensive vascular calcification. In contrast, humans with Keutel syndrome have no vascular calcifications	5,20,25, 26
Vitamin D disorders	FGF-23 knockout model Klotho knockout model Tcal/Tcal mice	Phosphate-deficient diet prevent calcification in FGF-23 knockout mice, Tcal/Tcal mice have a missense mutation in the Galnt3 gene leading to extensive ectopic calcification	6,20,21,22, 27,28

Legend: FGF-23: fibroblast growth factor 23, MGP: matrix Gla protein, VitD3: Vitamin D3.

Experimental models; Comments

Until now, various experimental models exist to study mechanisms and pathways of vascular calcification (VC) such as *in vitro* models with various cell types, *ex vivo* aortic tissue protocols and *in vivo* animal models – all with its strengths and shortcomings (20). The models are also used to identify biomolecules resident or foreign to the medial layer with the pathogenetically significance for CaP precipitation (20).

In vitro models reduce the complexity at the cost of losing tissues' context. In perfused aortic tissue *ex vivo* models the context is partly preserved and physiologic conditions partially restored (29). To preserve the whole body conditions, *in vivo* rodent models are available. The calcification patterns observed in the experimental models depend on the inducers' types. By employing the genetic models of the lipoprotein disorders or by feeding the animals with high-fat/cholesterol-rich diet, the predominant localization of calcification is intimal (20). While overlaps in pathogenesis between intimal and medial calcifications may exist, the medial arterial calcification (MAC) phenotype is not sufficiently mirrored.

MAC progressing in humans over decades is impossible to duplicate in animal models, even more so as the animals are less prone to calcification compared with humans. Therefore, biomolecules and pathways identified in experimental models and their putative mechanism of action must comply with the laws of thermodynamics to demonstrate their pathogenetic significance in humans.

For example: the knockout of MGP in mice, an inhibitor of hydroxyapatite (HAP), suffer from extensive ectopic calcification and is lethal in mice within two months, while in patients a clear association of MGP with VC still remains to be demonstrated (26). On the contrary, MAC seen in CD73 inactivating mutations in patients (16) are not found in the murine CD73-deficient model (30). These observations are in line with other commentary noting significant differences between murine and human forms of MS (31).

To obtain reproducible and replicable data in models, experimental conditions must be standardized, maintained and closely monitored. Development of animal models accurately replicating MAC in humans should provide access to encyclopedic explorations of diseases' signatures. Furthermore, studies on vascular tissues' samples from patients with MAC maintained *in vivo* conditions hold promise in targeting medial layer's cellular and molecular interactions.

References

1. Lehto S, Niskanen L, Suhonen M, Rönnnemaa T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1996;16:978-983.
2. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 2003;18:1731-1740.
3. Pescatore LA, Gamarra LF, Liberman M. Multifaceted mechanisms of vascular calcification in Aging. Arterioscler Thromb Vasc Biol. 2019;39:1307-1316.
4. Lanzer P. Mediakalzinose Mönckeberg. Z Kardiol 1998;87:586-593.
5. Wen L, Chen J, Duan L, Shuzhuang Li S.Vitamin K-dependent proteins involved in bone and cardiovascular health (review). Mol Med Rep 2018;18:3-15.
6. Wang J, Zhou JJ, Robertson GR, Lee VW. Vitamin D in vascular valcification: A Double-edged sword? Nutrients 2018, 10, 652; doi:10.3390/nu10050652.

7. StaryHC, Chandler AB,Dinsmore RE, Fuster V,Glagov S, Insull Jr W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995;92:1355-74.
8. Li Q, van de Wetering K, Uitto J. Pseudoxanthoma elasticum as a paradigm of heritable ectopic mineralization disorders: Pathomechanisms and treatment development. Am J Pathol 2019;189:216-225.
9. Ruscitti, P., Cipriani, P., Liakouli, V. et al. Subclinical and clinical atherosclerosis in rheumatoid arthritis: results from the 3-year, multicentre, prospective, observational GIRRCS (Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale) study. Arthritis Res Ther 21, 204 (2019). https://doi.org/10.1186/s13075-019-1975-y
10. Aessopos A, Samarkos M, Voskaridou E, Papaioannou D, Tsironi M, Kavouklis E, Vaiopoulos G, Stamatelos G, Loukopoulos D. Arterial calcifications in β-Thalassemia. 1998;49:137-43.
11. Moe SM, Chen NX. Calciphylaxis and vascular calcification: a continuum of extra-skeletal osteogenesis. Pediatr Nephrol 2003;18:969–975.
12. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association. On behalf of the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Cardiovascular Surgery and Anesthesia; and Council on Epidemiology and Prevention. Circulation 2017;135;17:e927-e999.
13. Feigenbaum A, Müller C,Yale C, Kleinheinz J, Jezewski P, Kehl HG, MacDougall M, Rutsch F, Hennekam RCM. Singleton-Merten Syndrome: An autosomal dominant disorder with variable expression. Am J Med Genet A 2013;161A:360-70.
14. Goettsch C, Iwata H, Aikawa E. Parathyroid hormone; Critical bridge between bone metabolism and cardiovascular disease. Arterioscler Thromb Vasc Biol 2014;34:1333–1335.
15. Wang C, Li Y, Shi L, Ren J, Patti M, Wang T, de Oliveira JR, Sobrido MJ, Quintans B, Baquero M, Cui X, Zhang XY, Wang L, Xu H, Wang J, Yao J, Dai X, Liu J, Zhang L, Ma H, Gao Y, Ma X, Feng S, Liu M, Wang QK, Forster IC, Zhang X, Liu JY. Mutations in slc20a2 link familial idiopathic basal ganglia calcification with phosphate

homeostasis. Nat Genet 2012;44:254-256.

16. St Hilaire C, Ziegler SG, Markello TC et al. Nt5e mutations and arterial calcifications. N Engl J Med 2011;364:432-442.
17. Rutsch F, Ruf N, Vaingankar S, et al. Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification. Nat Genet 2003;34:379-81.
18. Cannarile F, Valentini V, Mirabelli G, Alunno A, Terenzi R, Luccioli F, Gerli R, Bartoloni E. Cardiovascular disease in systemic sclerosis. Ann Transl Med. 2015 Jan; 3(1): 8.doi: 10.3978/j.issn.2305-5839.2014.12.12.
19. Kreienkamp R, Gonzalo S. Metabolic dysfunction in Hutchinson–Gilford Progeria syndrome. Cells 2020, 9, 395; doi:10.3390/cells9020395.
20. Herrmann J, Babic M, Tolle M, van der Giet M, Schuchardt M. Research Models for Studying Vascular Calcification. Int J Mol Sci 2020; **21** (6).
21. Ren X, Wei Q, Shao H, Sun Z, Liu N. A rat model of diabetic artery calcification. J Endocrinol Invest 2012;35):497-503.
22. Kuro-O M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E et. al. Mutations of the mouse klotho gene leads to a syndrome resembling aging. Nature 1997; 390, 45.51
23. Razzaque MS, Sitara D, Taguchi T, St-Arnaud R, Lanske B. Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. Faseb J Off Publ Fed Am Soc Exp Biol 2006, 20, 720-722
24. Rings RW, Wagner JE. Incidence of cardiac and other soft tissue mineralized lesions in DBA-2 mice. Lab Anim Sci 1972;22:344-52.
25. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997; 386:78-81
26. Barrett H, O'Keeffe M, Kavanagh E, Walsh M, O'Connor EM. Is Matrix Gla Protein Associated with Vascular Calcification? A Systematic Review. Nutrients 2018; **10** (4).
27. Stubbs JR; Liu S, Tang W, Zhou J, Wang Y, Yao X, Quarles LD. Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblast growth factor 23 null mice. J Am Soc Nephrolo JASN 2007, 18, 2116-2124
28. Esapa CT, Head RA, Jeyabalan J, Evans H, Hough TA, Cheeseman MT, McNally EG, Carr AJ, Thomas GP, Brown MA et.al. A mouse with an N-Ethyl-N-nitrosourea (ENU)

induced Trp589Arg Galnt3 mutation represents a model for hyperphosphateemic familial tumoural calcinosis. PLoS ONE 2012, 7, e43205

29. Schuchardt M, Siegel NV, Babic M, Reshetnik A, Lutzenberg R, Zidek W, van der Giet M, Tolle M. A Novel Long-Term ex vivo Model for Studying Vascular Calcification Pathogenesis: The Rat Isolated-Perfused Aorta. J Vasc Res 2019:1-7.
30. Joolharzadeh P and St. Hilaire C.CD73 and the differences between mice and humans. Arterioscler Thromb Vasc Biol 2019; 39: 339-348
31. Nelson AJ, Raggi P, Wolf M, Gold AM, Chertow GM, Roe MT. Targeting Vascular Calcification in Chronic Kidney Disease. JACC Basic Transl Sci 2020; 5:398-412.

Supplement B

The laboratory tests and typical biomarkers for MAC have been summarized in the Table 1.

Biomarkers	
Routinely used in clinical practice	**Laboratory Data**
Diabetes mellitus type 2	fasting glucose ≥ 126 mg/dL (2x) or HbA1c ≥7% or postprandial glucose >198 mg/dL
Chronic renal disease	Glomerular filtration rate ≥90 mL/min /1.73 m^2; albumin excretion in urine <2 mg/L or <80 mg/24h
Parathyroid gland disorders	Parathyroid hormone <10 – 55 pg/mL
Vitamin D disorders	25-hydroxyvitamin D < 20 ng/mL (50 nmol/L) and level between 21–29 ng/mL (52.5–72.5 nmol/L, respectively; 1,25-dihydroxyvitamin D: <20 or 45 pg/mL (<48 or >108 pmol/L)
Electrolyte disorders	Calcium (total) [<8.5 or > 10.2 mg/dL], phosphorus [< 2.4 or > 4.1 mg/dL], magnesium [<1.8 or >3.6 mg/dL]
Not routinely used in clinical practice	Fibroblast growth factor-23, Klotho, Fetuin-A, Bone morphogenetic proteins, Matrix Gla protein, Osteocalcin, Osteoprotegerin, Osteopontin, Osteonection, Pyrophosphates, Fibrillin, Smads, Carbonic anhydrase, Calcium-sensing receptor, Sclerotin

References

1. Lanzer P. Primary media sclerosis Mönckeberg: Diagnostic criteria. Cor et Vasa 2018;60:2205-e208.
2. Roumeliotis, S., Roumeliotis, A., Dounousi, E., Eleftheriadis, T, Liakopoulos V. Biomarkers of vascular calcification in serum. Adv Clin Chem 2020 doi:10.1016/bs.acc.2020.02.004.

3. Smith ER, Hewitson TD, Holt SG. Diagnostic tests for vascular calcification. Adv Chronic Kidney Dis 2019;26:445-463.

Supplement C

Brief review of the guiding thermodynamic principles of calcium phosphate (CaP) precipitation is provided. Thus, while promoters decrease the critical supersaturation of Ca and P ions required for nucleation of amorphous calcium phosphate (ACP) and subsequent hydroxyapatite (HAP) crystal growth, inhibitors increase the critical supersaturation of Ca and P ions and restrain nucleation and crystallization. Precipitation occurs when the solution becomes supersaturated with respect to the solid phase. In the absence of a solid phase (no CaP precipitate in tissue) additional energy is required to overcome the nucleation barrier. Based on experimental evidence the CaP precipitation proceeds from ACP formation to HAP crystallization where the former represents the reversible and the latter largely irreversible stages of the calcification process. Thus, preventive therapy should target primarily ACP formation while therapy of established disease should target both ACP prevention and HAP crystal growth.

Promoters reduce the critical level of supersaturation (S) by a local accumulation of Ca^{2+} at sites of the abundant Ca^{2+} -ligand groups such as phosphonate, phosphate, carboxylate and sulfonate (1, 2) potentially inducing the precipitation in the media that in their absence would continue to remain in a metastable state. Ca^{2+} ligand macromolecules in the arterial media include collagen and elastin, carboxy-glutamic-rich proteins, sulfate-containing glycosaminoglycans, phosphate-rich proteins and phospholipids also present in matrix vesicles and exosomes (3-7).

Inhibitors include inorganic pyrophosphate (PPi) and Gla-proteins, both abundantly present in the media (8). The bulk of PPi is produced extracellularly by hydrolysis of nucleotides via the ectonucleotide pyrophosphatase/phosphodiesterase (ENPP). PP is hydrolyzed by tissue-nonspecific alkaline phosphatase (TNAP), an enzyme that also releases phosphates from other sources, including phospholipids. This enzyme's activity appears to be necessary for medial calcification (8). PPi appears to retard CaP nucleation and to reduce HAP crystal growth by blocking sites on the surfaces of growing crystals (9). Other polyphosphates such as phytate originally shown to have inhibitory effect on oxalate renal calculi (10) and later on HAP

crystal growth (11) have been proposed as therapeutic drug in patients with CKD and are now undergoing clinical trials.

The number of promoters and inhibitors of CaP precipitation appears to be, at least in theory, proportional to the abundance of biomolecules with CaP moieties. To identify the most relevant among many potential candidates, understanding their influence on the fundamental principles of thermodynamics of CaP precipitations under physiologic conditions will be of utmost importance. However, while *in vitro* direct energetic measurements may be considered feasible, *in vivo*, given the complexity of the CaP ion homeostasis at the tissue and cellular level (12), biomolecular heterogeneity of the matrisome (13), the intra- and extracellular compartmentalization (14) and the macromolecular complexations and sequestrations of CaP moieties (15) impose considerable challenge. Addressing this thermodynamic complexity, systemic modelling based preferably on *in vivo* data will be required rather than building hypothesis based exclusively on experimental *in vitro* analogies.

To achieve progress in prevention and treatment of medial arterial calcification (MAC), the pathophysiology of the wide range of unrelated disorders associated with MAC (Supplement A; Table 1) will need to be understood. Thus, experimental models replicating human MAC associated disorders and standardization of protocols (Supplement A, Table 2), development of genetic analysis protocols and employment of omics technologies and systemic biology interdisciplinary approaches are needed.

References

1. Zhao W, Wang Z, Xu Z, Sahai N. Osteocalcin facilitates calcium phosphate ion complex growth as revealed by free energy calculation. *Phys. Chem. Chem. Phys* . 2018;20:13047-13056. https://doi.org/10.1039/C8CP01105B.

2. Millán A, Lanzer P, Sorribas V. The thermodynamics of medial vascular calcification. Front. Cell Dev. Biol. (2021) 9:633465.

3. Greengard O, Sentenac A, Mendelsohn N. Phosvitin the iron carrier of egg yolk. Biochim Biophys Acta. 1964;90:406-407.https://doi.org/10.1016/0304-4165(64)90208-9.

4. Sarem M, Lüdeke S, Thomann R, et al. Disordered Conformation with Low Pii Helix in Phosphoproteins Orchestrates Biomimetic Apatite Formation. *Adv Mater*. 2017;29:1701629. https://doi.org/10.1002/adma.201701629.

5. Felix R, Fleisch H. The role of matrix vesicles in calcification. *Calcif. Tissue Res.* 1976;21 Suppl:344-348. https://doi.org/10.1007/BF02546474.

6. Zhang C, Zhang K, Huang F, et al. Exosomes, the message transporters in vascular calcification. *J Cell Mol Med.* 2018;22:4024–4033. https://doi.org/10.1111/jcmm.13692.

7. Bäck M, T, Cancela ML, Carracedo M et al., Endogenous Calcification Inhibitors in the Prevention of Vascular Calcification: A Consensus Statement From the COST Action EuroSoftCalcNet. Front Cardiovasc Med, 2019;5:196. doi: 10.3389/fcvm.2018.00196. eCollection 2018.

8. Lomashvili KA, Garg P, Narisawa S, Millan JL, O'Neill WC. Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. Kidney Int. 2008;73:1024–30. doi: 10.1038/ki.2008.26.

9. Fleisch H, Russell RG, Straumann F. Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature 1966;212:901–3. doi: 10.1038/212901a0.

10. Millan A. Theoretical and analytical aspects of calcium oxalate urolithiasis. Doctoral thesis University of Balearic Islands, Spain, 1990.

11. Perelló J, Gómez M, Ferrer MD, Rodríguez NY, Salcedo C, Buades JM, Pérez MM, Torregrosa JV, Martín E, Maduell F. SNF472, a novel inhibitor of vascular calcification, could be administered during hemodialysis to attain potentially therapeutic phytate levels. J Nephrol. 31, 287–296, 2018.

12. Shaker JL, Leonard Deftos L. Calcium and Phosphate Homeostasis. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, Grossman A, Hershman JM, Hofland HJ, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE, New M, Purnell J, Singer F, Stratakis CA , Trence DL, Wilson DP. eds. South Dartmouth (MA): MDText.com, Inc.; 2000. https://www.ncbi.nlm.nih.gov/books/NBK279023/.

13. Hynes RO, Naba A. Overview of the matrisome – extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol 2012, doi: 10.1101/cshperspect.a004903

14. Hyman AA, Weber CA, Jülicher F. Liquid-liquid phase separation in biology. Annu Rev Cell Dev Biol 2014;30:39-58.

15. Kisiel M, Klar AS, Ventura M, Buijs J, Mafina M-K, Cool SM, Hilborn J. Complexation and sequestration of BMP-2 from an ECM mimetic hyaluronan gel for improved bone formation. PLoS One 2013 Oct 22;8(10):e78551. doi: 10.1371/journal.pone.0078551. eCollection 2013.

Supplement D

Calcium phosphate mineralization in the artery walls is a multistep process, and different types of "inhibitors" have the capacity to intervene at any of the steps slowing down the process. A full description of the whole thermodynamic process can be found in the literature (1). In short, the process is initiated when the amounts of **free** Ca^{2+} and PO_4^{3-} (ion activity product) are increased over the solubility product of the first calcium phosphate precipitating phase, which is amorphous calcium phoshate (ACP) (and not hydroxyapatite [HAP] that is formed at a later stage as a result of a slow solid state crystalline re-arrangement) or using a thermodynamical expression, when the medium is supersaturated with respect to ACP. The causes of this increase of the free Ca^{2+} and PO_4^{3-} ion activities could correspond to metabolic or ion transport abnormalities, because in blood ACP is undersaturated, as it is shown (1). On the other hand, as it was also pointed out in that reference, a factor that might increase the activity of PO_4^{3-} ions without any variation in the total phosphate content is a local increase of pH.

Before a supersaturation of ACP is reached, several substances with a capacity to sequester Ca^{2+} or PO_4^{3-} ions may intervene reducing the availability of free ions, and consequently inhibiting MAC (i.e. pyrophosphate, citrate, Ca-binding proteins in the case of Ca^{2+}, and Mg^{2+} in the case of PO_4^{3-}). Even when a supersaturation has been built up, some substances, so called nucleation inhibitors, have the capacity to slow down the nucleation of ACP. There is little experimental information about ACP nucleation inhibitors, but pyrophosphate has proved to be one of them (2).

Once ACP nuclei, consisting of a disordered arrangement of CaP clusters, are formed, a number of phosphoproteins and glycoproteins may actuate by encapsulating the ACP nucleus and preventing solidification and densification, consequently restraining the progress of MAC. Fetuin A is one example of glycoprotein that has been shown to cause this effect (3), and as it is highly present in blood and in extracellular matrix it could be an important factor in MCA inhibition. A phosphoprotein, OPN, has shown a similar effect (4).

In the next stage of MAC development, ACP converts into HAP (which is far more insoluble and difficult to revert). Again, there is another kind of inhibitor (i.e. Mg^{2+} and pyrophosphate) intervening at this stage by retarding this conversion and thus being effective in inhibiting MAC.

Finally, crystal growth inhibitors have the ability to block the HAP crystal growth sites at the surface, thus decreasing the HAP crystal growth rate. Examples of endogenous HAP crystal

growth inhibitors are pyrophosphate and citrate (5), and to a lesser extent Mg^{2+} (6). Among exogenous inhibitors, phytate has proven to be extraordinary efficient as a HAP growth inhibitor (7).

References

1. Millan A, Lanzer P, Sorribas V. The thermodynamics of medial vascular calcification. Front. Cell Dev. Biol. 2021; 9:633465.
2. Fleish H, Neuman WF. Mechanisms of calcification: role of collagen polyphosphates and phosphatase. Am J Physiol 1961; 200:1296-300.
3. Laura Brylka L, Jahnen-Dechent W. The Role of Fetuin-A in Physiological and Pathological Mineralization. Calcif Tissue Int 2013 93:355-64.
4. Lenton S. , Wang O, Nylander T, Teixeira S, Holt C. Structural biology of calcium phosphate nanoclusters sequestered by phosphoproteins. Crystals 2020, 10, 755; doi:10.3390/cryst10090755.
5. Mekmene O, Rouillon T, Quillard S, Pilet P, Bouler JM, Pezennec S, et al. Effects of citrate and NaCl on size, morphology, crystallinity and microstructure of calcium phosphates obtained from aqueous solutions at acidic or near-neutral pH. J Dairy Sci 2012; 79:238–248.
6. Boskey AL, Posner AS. Magnesium stabilization of amorphous calcium phosphate: a kinetic study.Mater Res Bull 1974; 9: 907–916.
7. Thomas WC, Tilden MT. Inhibition of mineralization by hydrolysates of phytic acid. Johns Hopkins Med J 1972; 131:133-142.