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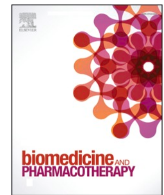

Review

Roles of Lysyl oxidases (LOX(L)) in pathologic calcification

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ABSTRACT

Calcification of tissues involves the formation and deposition of calcium-containing crystals in the extracellular matrix (ECM). While this process is normal in bones, it becomes pathological when it occurs in cardiovascular and musculoskeletal soft tissues. Pathological calcification (PC) triggers detrimental pathways such as inflammation and oxidative stress, contributing to tissue damage and dysregulated tissue biomechanics, ultimately leading to severe complications and even death. The underlying mechanisms of PC remain elusive. Emerging evidence suggests a significant role of lysyl oxidases (LOX(L)) in PC. LOX(L) are a group of five enzymes involved in collagen cross-linking and ECM maturation. Beyond their classical role in bone mineralization, recent investigations propose new non-classical roles for LOX(L) that could be relevant in PC. In this review, we analyzed and summarized the functions of LOX(L) in cardiovascular and musculoskeletal PC, highlighting their deleterious roles in most studies. To date, specific inhibitors targeting LOX(L) isoforms are under development. New therapeutic tools targeting LOX(L) are warranted in PC and must avoid adverse effects on physiological bone mineralization.

1. Introduction

Pathologic calcification (PC) refers to the formation and deposition of calcium-containing crystals in soft tissues, such as cartilage, arteries, and kidneys, contributing to the progression of several diseases. This condition is widespread, debilitating, and lacks effective treatments. Currently, only symptomatic treatments are available and no curative treatment exists to prevent or reverse calcium-containing crystal formation [1,2].

Tissue PC results from a two-step active mechanism. The initial phase involves crystal nucleation, where calcium-containing crystal precursors form within the extracellular matrix (ECM). This process is driven by various mechanisms, including cell hypertrophy, mitochondrial autophagy, and apoptosis. Once formed, these precursors grow within collagen fibers of the ECM [1,2]. Despite the incomplete understanding of the molecular mechanisms underlying PC, recent research has identified lysyl oxidase enzymes (LOX(L)) as key players in its development.

Initially described in physiological calcification, LOX(L) refers to a family of five enzymes (LOX and LOX-like proteins LOXL1–4) that support bone mineralization. However, emerging evidence suggests that LOX(L) enzymes also contribute significantly to pathologic calcification across a variety of soft tissues and diseases. This review aims to provide a comprehensive overview of the role of LOX(L) enzymes in calcification, offering new insights into their contribution to the pathogenesis of PC.

2. Lysyl oxidases LOX(L)

2.1. LOX(L) structure

LOX(L) are a group of five enzymes, including lysyl oxidase (LOX) itself and four lysyl oxidase-like enzymes (LOXL1–4). These enzymes share a catalytic site in their C-terminal domain, which contains a copper-binding site, a tyrosylquinone cofactor, and a cytokine receptor-like domain (Fig. 1). In contrast, the N-terminal domain is variable, subdividing LOX(L) into two groups. The first group comprises the pro-enzymes LOX and LOXL1, which contain a pro-peptide sequence in their N-terminal domain [3–6]. Pro-LOX undergoes post-translational modifications, such as N-glycosylations, and contains at least three disulfide bonds in the C-terminal domain [5,7,8]. After secretion into the extracellular matrix (ECM), LOX and LOXL1 become catalytically active once their pro-peptide is cleaved by bone morphogenetic protein 1 (BMP1) and, in the case of LOX, matrix metalloproteinase 2 (MMP2) [3,4,6,7]. The second group includes LOXL2, LOXL3, and LOXL4, whose N-terminal domains consist of four scavenger receptor cysteine-rich (SRCR) domains. Although the function of the SRCR domains is not well described, it is known that two SRCR domains from LOXL2 can be

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processed extracellularly by proprotein convertase [9], enhancing LOXL2's interaction with collagen type IV without altering its amine oxidase activity [10]. Additionally, these domains appear necessary for LOXL2 to regulate ECM protein deposition [5,7]. LOXL2–4 also undergo N-glycosylation, which is crucial for LOXL2 secretion into the ECM, although the specific function of this modification requires further investigation [5].

2.2. LOX(L) roles

Proper LOX(L) activity is essential for collagen cross-linking, which maintains extracellular matrix (ECM) homeostasis by stabilizing collagen and elastin fibrils. This cross-linking supports physiological bone mineralization, influencing biomechanical properties. A key event in bone mineralization is the nucleation of calcium-containing crystals, which starts in gaps adjacent to cross-linking sites [11–13].

Collagen cross-linking is initiated by LOX(L), which converts hydroxylysine (Hyl) and lysine (Lys) residues at the telopeptide (terminus) region of a collagen molecule into hydroxyallysine (H-Allys) and allysine (Allys), respectively, with concomitant generation of H_2O_2 [6,13,14]. These modifier residues then spontaneously interact with Hyl, Histidine (His), or Lys residues in the triple helical region of another collagen molecule to form immature cross-links such as deH-dihydroxylysinonorleucine (deH-DHLNL), deH-hydroxylysinonorleucine (deH-HLNL), deH-hydrohistidinohydroxymerodesmosine (deH-HHMD) and deH-lysinonorleucine (deH-LNL) (Fig. 2, red lines). At the telopeptide region, these molecules can react spontaneously with H-Allys, Allys or His on another collagen molecule to form mature cross-links, including hydroxylysylpyridinoline (HP); pyrrole (PRL); lysylpyridinoline (LP); deoxypyrrol (d-PRL) and histidinohydroxylysinonorleucine (HHL) (Fig. 2, blue lines) [3,4,6,15–17].

Interestingly, extracellular LOX(L) enzymes can re-enter cells to exert intracellular functions. For instance, LOX and LOXL2 can translocate into nuclei to modify histones and act as transcriptional regulators [5]. Specifically, LOX modifies histone H1 proteins and increases the expression of its natural substrates such as COL1, COL3, and ELN (elastin) [5,6,18,19]. Notably, LOX can also upregulate its own expression in human lung tissue culture [18]. LOXL2, similarly localized in the nucleus, exerts transcriptional repression by oxidizing or interacting with several transcription-related proteins, including H3K4me3 histones and the transcription factor TAF10 (part of the general transcription factor IID complex) [5,6]. In addition to its nuclear roles, LOX has been implicated in scleroderma, where cytosolic LOX induces inflammation by upregulating the expression of the c-Fos transcription factor and promoting its nuclear translocation, subsequently inducing interleukin 6 (*IL-6*) expression [18]. LOX has been also associated with intracellular ROS generation; for instance, arteries from TgLOX mice exhibited increased vascular H_2O_2 levels while BAPN, a pan-inhibitor of LOX(L), prevented the higher oxidative stress in hypertensive models [20]. Additionally, extracellular LOX(L) have been involved in various cellular processes chemotaxis [21], cell adhesion [22] and protein-protein interaction [23].

2.3. Regulation of LOX(L)

LOX(L) can be regulated at various levels, including transcription, maturation, and activity, by molecules relevant for pathologic calcification (Fig. 3). Notably, hypoxia-inducible factors (HIFs) can induce LOX(L) transcription. HIFs have been shown to trigger calcification in valve interstitial cells (VICs) *in vitro* by promoting chondro-osteogenic differentiation (increased *Sox9*, *Runx2*), and by enhancing ROS production [24,25]. Studies on the loss and gain of function of HIF-1α in human aorta smooth muscle cells cultured in calcification medium (CM) have demonstrated that HIF-1α promoted calcification by upregulating *Pit1* [26]. Additionally, HIFs can bind to HIF-response elements in the promoters of LOX and LOXL2 to activate their transcription [27–29].

In human aortic smooth muscle cells, advanced glycation end-products (AGEs) triggered calcification through different pathways, notably via mTOR-mediated autophagy and apoptosis [30] as well as p38 MAPK phosphorylation-mediated osteoblast differentiation through AGE receptor (RAGE) stimulation [31]. Interestingly, findings have shown that AGEs upregulated the phosphorylated forms of extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK), known to activate nuclear factor-κB (NF-κB) and activator protein 1 (AP-1). These transcription factors can bind to LOX promoter to activate its transcription [32,33]. In aged osteoarthritis (OA) mice

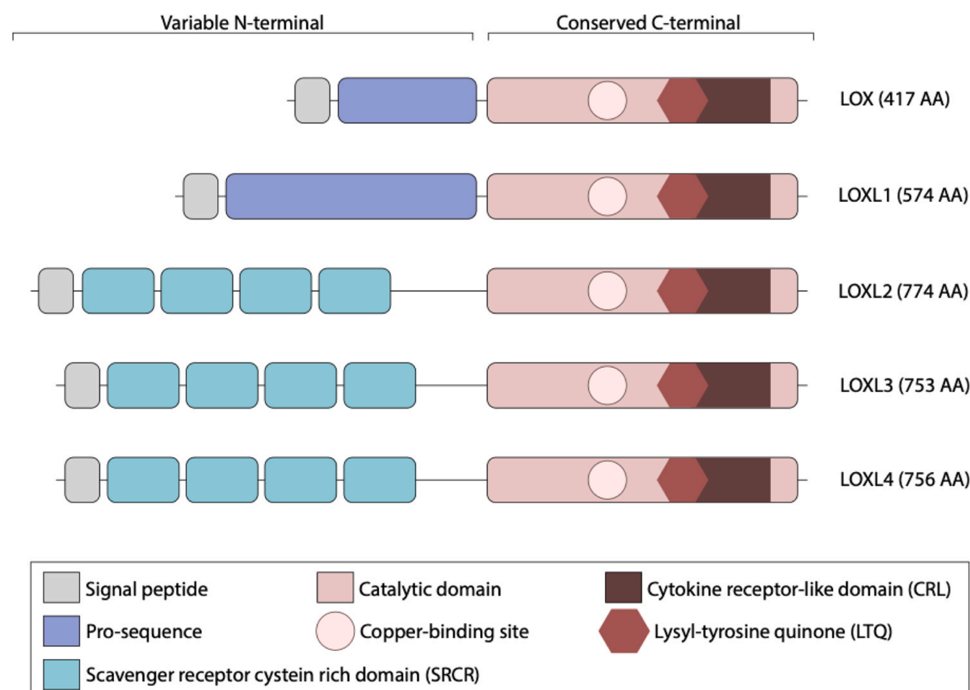

Fig. 1. Structures of lysyl oxidase (LOX) and LOX-like (LOXL1, LOXL2, LOXL3, and LOXL4) proteins.

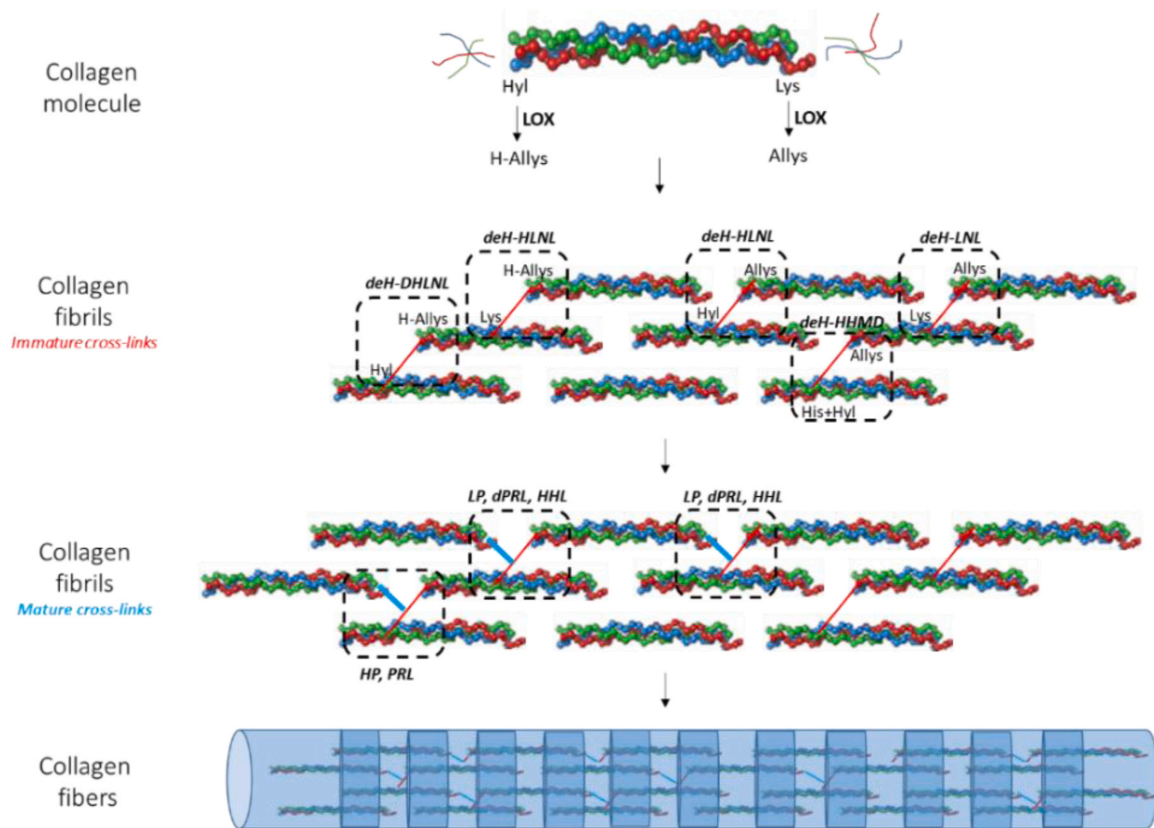

Fig. 2. Schematic representation of collagen fiber formation and LOX(L) mediated cross-links. Triple-helix collagen molecules assemble into collagen fibrils. LOX(L) converts hydroxylysine (Hyl) and lysine (Lys) into hydroxyallysine (H-Allys) and allysine (Allys) to initiate collagen immature (red) and mature (blue) cross-links.

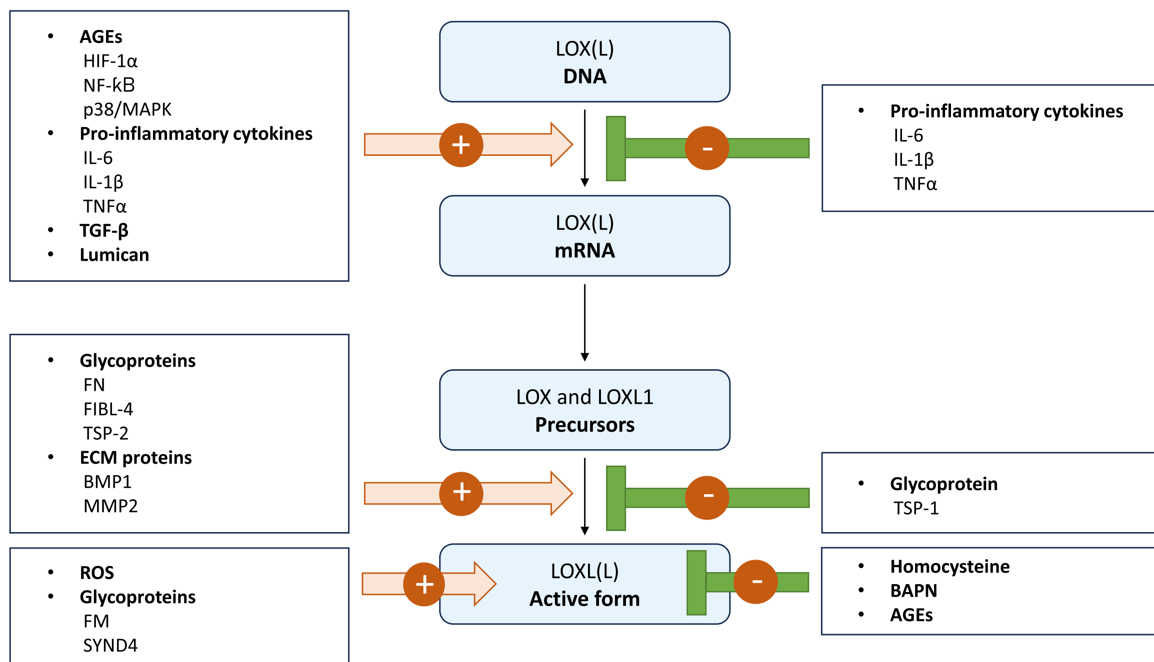

Fig. 3. Potential positive and negative regulatory mechanisms of LOX(L), involved in pathologic calcification. LOX(L) can be regulated at three levels: 1) transcription; 2) activation of precursors; 3) enzymatic activity.

cartilage, AGEs induced matrix stiffening by increasing *Lox* expression, contributing to OA progression [34]. Furthermore, primary rat vascular smooth muscle cells (VSMCs) stimulated with AGEs promoted HIF-1α nuclear translocation and its expression, enhancing calcification [35]. This suggests a link between AGEs, LOX(L) and calcification, mediated by different signaling pathways.

Pro-inflammatory cytokines, particularly IL-6, are well-known triggers of PC. Several reports have demonstrated IL-6's key role in vascular

calcification by inducing the differentiation of vascular smooth muscle cells (VSMCs) into osteoblasts through the activation of TNFα [36] or STAT3 pathways [37]. In line, IL-6 enhances calcification in murine chondrocytes stimulated by calcification medium. Conversely, the presence of calcium-containing crystals can activate *Il-6* expression via Syk kinase, PI3 kinase, Jak2 and Stat3 signaling [38]. Other pro-inflammatory cytokines, such as IL-1β and TNF-α, have also been shown to trigger vascular progenitor cell calcification [39,40]. Interestingly, LOX(L) have been reported to be influenced by these pro-inflammatory cytokines, although with contrasting results [41].

The role of transforming growth factor β (TGF-β) in calcification remains controversial. Several reports have described TGF-β as a promoter of calcification in VSMCs, valve interstitial cells (VICs), and human osteoblasts *in vitro*. Inhibition of TGF-β has been shown to ameliorate muscle and tendon pathologic calcification (PC) *in vivo* [42–46]. In contrast, other studies indicated that TGF-β may attenuate valvular calcification and chondrocyte hypertrophy, which precedes calcification [47,48]. Regarding LOX(L), TGF-β has been shown to upregulate LOX and LOXL1 in human synovial fibroblasts in the context of OA [49]. Additionally, TGF-β induces the expression of all LOX(L) isoforms in human trabecular meshwork cells in the context of glaucoma and in human fibroblasts from injured knee ligaments [50,51].

ECM composition significantly impacts cell homeostasis and phenotype, primarily regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Dysregulation of ECM composition can disrupt matrix turnover, contributing to PC. Various ECM components, such as collagen-binding receptor tyrosine kinases (DDRs), integrins, the elastin receptor complex, osteopontin, fibronectin, matrix Gla protein, and thrombospondin-1 (TSP-1), have been associated with PC, either promoting or inhibiting the process [52–58]. ECM proteins and proteoglycans can modulate LOX(L) maturation and activity. For instance, fibronectin and fibulin-4, known as positive regulators of calcification, are crucial for LOX activation by BMP1 [6,59,60]. ECM proteins such as BMP1 and MMP2 contribute to the activation of LOX and LOXL1 by processing their precursors, with MMP2 specifically targeting LOX [6,61–63]. Conversely, negative calcification regulator TSP-1 inhibits LOX activation by BMP1, while TSP-2 positively modulates LOX [6,64,65]. Proteoglycans also play a role; for example, lumican is involved in LOX expression [66] and fibromodulin and syndecan-4 induce LOX activity [6,67,68]. Finally, oxidative stress, which is closely linked to PC, has been shown to induce LOX-mediated collagen crosslinks in human fibroblasts [69–73]. Taken together, these data suggest a potential role for LOX(L) in pathologic calcification. Consequently, LOX(L) enzymes have been extensively investigated in the context of PC, particularly in cardiovascular and musculoskeletal tissues. The following section will summarize these studies and their findings (Fig. 4).

3. Evidence of LOX(L) roles in pathologic calcification

3.1. Calcification mechanisms

Pathologic calcification (PC) refers to the formation and deposition of calcium-containing crystals in tissues that normally do not undergo calcification. PC can occur in all soft tissues, including vascular and musculoskeletal tissues, as well as organs such as the eye, breast, kidney, heart, and brain.

Once activated, the process of pathologic calcification is regulated by a two-step mechanism. First, crystal nucleation forms precursors of calcium-containing crystals, called amorphous calcium phosphate (ACP) or amorphous calcium pyrophosphate (ACPP). These precursors then mature and grow in the extracellular matrix (ECM). Several deleterious pathways, including inflammation, catabolism, oxidative stress, and apoptosis, trigger this process.

One of the primary mechanisms involved in PC is trans-differentiation of cells, such as vascular smooth muscle cells or tenocytes, into chondrocyte-like cells and osteoblast-like cells. During this process, typical cell markers are replaced by chondro-osteogenic markers such as Runt-related transcription factor 2 (RUNX2), the transcription factor for Collagen type X (COLX), and alkaline phosphatase (ALPL) [2,74,75]. Inflammation, particularly interleukin-6 (IL-6) [38] and oxidative stress, through reactive oxygen species (ROS) production [72] are well-known triggers of this differentiation process.

Following cell trans-differentiation, a complex machinery of transporters and enzymes present in cell membrane is activated to process phosphate and calcium ions, leading to the formation of ACP and ACPP. Once formed, these amorphous crystals are released into the ECM by matrix vesicles. Interestingly, dysfunctional mitochondria can also lead to ACP formation by sequestering calcium (Ca^{2+}) and phosphate (P_i) ions. Mitophagy, a form of mitochondria autophagy, lead to the release of ACP into the ECM [76]. Additionally, apoptosis can result in ACP formation, as the apoptotic bodies exhibit key membrane enzymes that produce and concentrate P_i and Ca^{2+} ions. In non-vascularized tissues like cartilage, the absence of phagocytes prevents the elimination of apoptotic bodies, thereby allowing the release of ACP into the ECM [77]. Once deposited into the ECM, crystal precursors are proposed to first grow within collagen fibrils and then outside them [78]. Mice over-expressing BMP-4, or local injections of BMP-2 or BMP-4, are widely

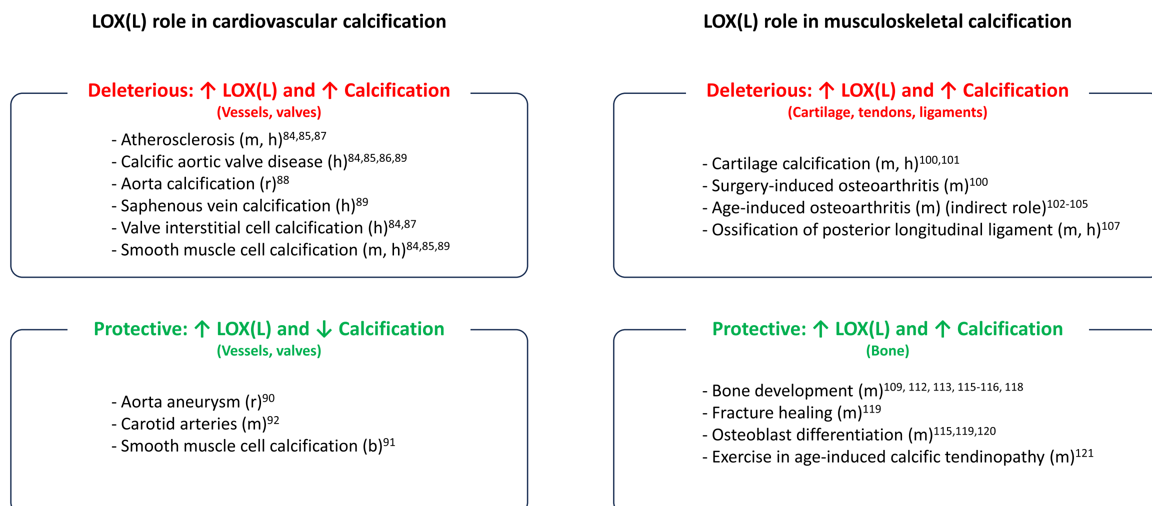

Fig. 4. Summary of known roles of LOX(L) in calcification. Red boxes are pathologic roles in calcification; Green boxes are protective role in calcification. Models: m=mouse, h=human, r=rat, b=bovine.

used models of pathologic calcification [79]. To note, mice overexpressing BMP-4, or local injection of BMP-2 or −4 are widely used models of pathologic calcification [80]. Several reviews provide more detailed mechanisms of PC in vascular and cartilage tissues [2,74,75].

Once deposited, ACP and ACPP mature into two forms of crystals: basic calcium phosphate (BCP) and calcium pyrophosphate dihydrate (CPP), with CPP appearing to be restricted to joints. BCP encompasses various forms, including hydroxyapatite (HA), octacalcium phosphate (OCP), carbonated apatite (CA), tricalcium phosphate (TCP), and magnesium whitlockite crystals [81].

3.2. Cardiovascular calcifications

Pathologic calcification in the vascular system is strongly associated with cardiovascular diseases such as atherosclerosis and is linked to high mortality risks [82]. Patients with chronic kidney disease (CKD) experience cardiovascular PC at rates 2–5 times higher than healthy age-matched individuals, with manifestations occurring 10–20 years earlier than in the general population [74,83]. In cases of end-stage renal disease, the progression of these cardiovascular conditions can be accelerated up to tenfold [83]. Cardiovascular PC occurs in both the intimal and medial leaflets of the arteries walls and in heart valves, contributing to tissue deterioration and affecting their mechanical properties [75].

For many years, cardiovascular calcifications were believed to be a passive process resulting from the precipitation of calcium and phosphate associated with aging [75]. However, recent studies have shown that vascular PC is an active process involving the transdifferentiation of vascular VSMCs into osteoblast-like cells.

3.2.1. Deleterious role of LOX(L) in cardiovascular calcification

3.2.1.1. Role of ECM and cytoskeleton. Calcified human atherosclerotic lesions and calcified valve leaflets have demonstrated increased LOX(L) expression [84,85]. Specifically, increased LOX expression has been observed in human calcific aortic valve disease, which induces ECM rearrangements [86]. Consistent with this, stimulation of VICs with CM resulted in increased deposition of collagen type I and higher secretion of LOX, accompanied by calcification (see Fig. 3 in [84]).

Similarly, CM-stimulated murine vascular smooth muscle cells (VSMCs) exhibited increased LOX expression and elevated osteogenic genes (*Runx2, Bmp2*). Overexpression of LOX in CM-stimulated murine VSMCs and thoracic aortas led to greater calcification deposition compared to WT conditions, coupled with higher expression of osteogenic markers BMP2, RUNX2, and OCN [85]. This suggests an aggravation of atherosclerosis and vascular calcification [84]. Inhibition of LOX(L) activity by BAPN or specific knockdown of LOX reduced calcification induced by CM in human VSMCs and human VICs through attenuated collagen I deposition (see figure 6 in [85] and 4 in [84,87]).

In a rat model designed to induce vascular calcification (WVK diet), aorta microcalcifications were detected within collagen type I and along the elastic fibers surrounding VSMCs. Rats treated with BAPN before calcification induction (preventive treatment) exhibited less calcification, though not significantly, compared to the control group. However, BAPN administration during calcification progression (curative treatment) significantly reduced vascular calcification, with lower LOX and collagen type I production compared to the control group [88]. However, the reduction of calcification by BAPN in CM-stimulated VSMCs was not reproducible *in vitro* [88].

Human saphenous veins cultured in a LOX-conditioned medium showed increased tissue stiffness and calcification. Mechanistically, LOX-induced matrix stiffness generated actin stress fibers, leading to reduced levels of nuclear actin monomers. This reduction failed to repress YAP-dependent activation of RUNX2. Once activated, RUNX2 triggered the expression of several VSMC calcification genes (*Bmp4, Bmp6, Sox9, Alpl,* and others). In this experimental setting, BAPN or adenoviral delivery of nuclear targeted polymerization defective actin inhibited LOX-induced calcification (see Fig. 5 in [89]).

3.2.1.2. Role of ROS. Beyond its role in ECM remodeling, LOX has been shown to contribute to vascular calcification through increased oxidative stress production. Calcified regions of human atherosclerotic lesions and calcific aortic valves exhibited elevated LOX and LOXL2 production alongside higher levels of oxidative stress [87]. Overexpression of LOX enhanced both ROS production and calcification in atherosclerosis-induced mice and human VICs (see figure 8 in [87]). Treatment with antioxidants or BAPN reduced both ROS and mineralization in human VICs, indicating that ROS generated by LOX supports VIC calcification [87]. Additionally, H_2O_2 byproduct of LOX activity has been shown to enhance oxidative stress and vascular stiffness through p38 MAPK activation, while BAPN prevented this effect [20].

3.2.2. Protective role of LOX(L) in cardiovascular calcification

In vitro experiments have demonstrated that supplementation of hyaluronan oligomers, either alone or with TGF-β1, increased LOX expression and concurrently reduced calcific deposits. This suggests a protective role of LOX in pathologic calcification associated with aortic aneurysms [90]. Stimulation of bovine aortic smooth muscle cells with calcification stimuli (beta-glycerophosphate), triggered calcification and decreased LOX expression [91]. Additionally, LOX levels were observed to be decreased in aged calcified carotid arteries of mice, which further supports the notion that reduced LOX expression may correlate with vascular calcification in aging [92].

In valvular heart diseases, heart valves can be replaced by bioprosthetic heart valves (BHVs). Over time, those BHVs can lead to structural damage and PC. Interestingly, the incorporation of exogenous LOX has been shown to provide enhanced protection against calcification deposition and improve the mechanical properties of these valves [93].

3.3. Musculoskeletal calcifications

Pathologic calcification has been found in different musculoskeletal diseases. Osteoporosis, a chronic metabolic bone disease, is characterized by reduced bone mineral density and bone mass, as well as changes in bone structure and strength. In 2017, over 200 million people were affected by osteoporosis [94,95]. Osteoarthritis (OA) is a degenerative rheumatologic condition and impacts more than 500 million individuals worldwide [96]. Remarkably, cartilage calcification is found in 100 % of patients undergoing joint replacement surgery [97], with calcium-containing crystals playing a critical role in the development and progression of OA [2]. In tendons, pathologic calcification can occur, especially in the shoulder's rotator cuff, leading to calcific tendinopathy. This type of calcification has been reported in up to 42 % of adults with shoulder pain [98]. Finally, ossification of the posterior longitudinal ligament (OPLL) involves calcification of the spinal posterior ligament, affecting up to 25 % of adults [99].

3.3.1. Deleterious role of LOX(L) in musculoskeletal calcifications

In cartilage, LOX(L) has a crucial role in PC in the context of osteoarthritis. LOX(L) activity was induced by calcification stimuli in primary mouse and human chondrocytes [100]. Additionally, inhibition of LOX(L) diminished chondrocyte calcification *in vitro* (Fig. 5A-C) and *in vivo* (Fig. 5D). This effect could be accounted for by modulation of calcification genes, decreased matrix cross-linking, diminished chondrocyte hypertrophy (*Runx2*, *Col10*), fibrosis (*Col1*, *Col3*), inflammation (IL6), and oxidative stress [100]. In line, CM-stimulated CD11b KO chondrocytes showed higher calcification partially caused by increased LOX activity and matrix cross-links [101]. In line, cartilage of aged OA mice revealed increased LOX expression which induced higher matrix

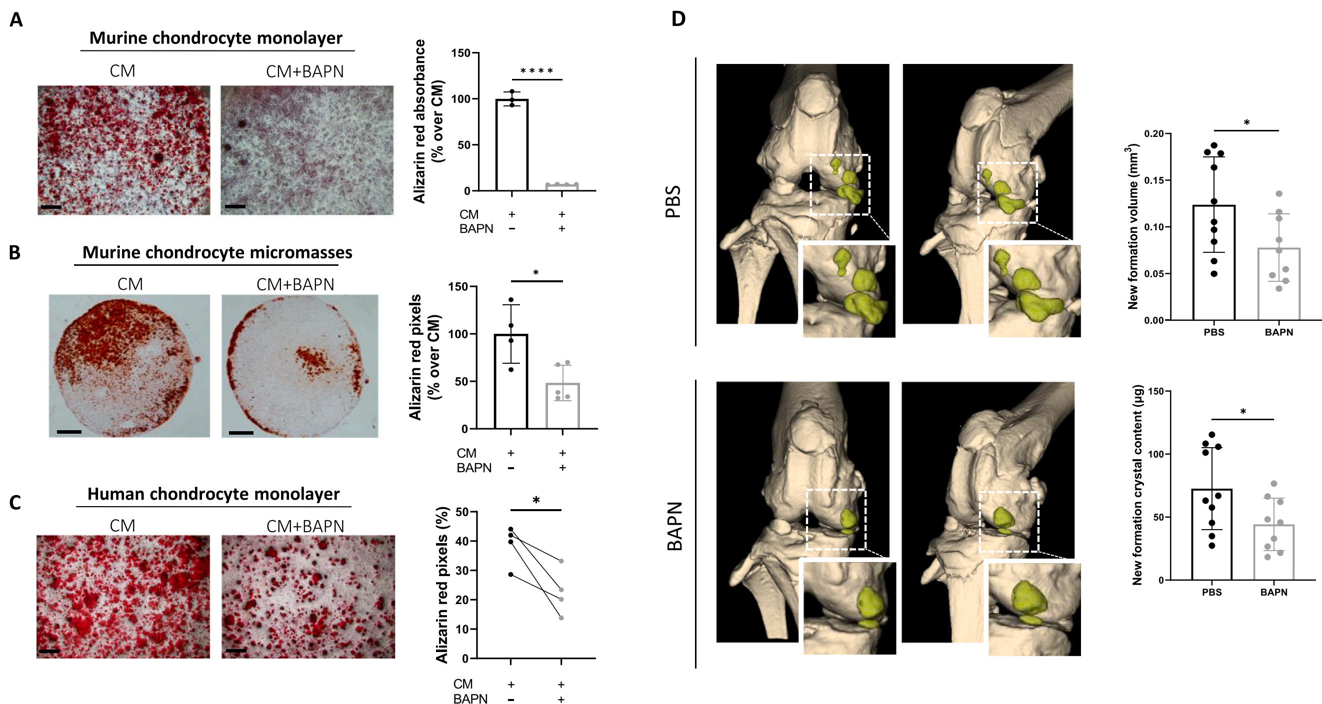

Fig. 5. Inhibition of LOX(L) protects against cartilage calcification in different models. Alizarin Red staining was performed on CM-stimulated murine chondrocytes cultured in (A) monolayer and (B) micromasses, as well as (C) human chondrocytes cultured in monolayer (n=4), with or without BAPN (500 µM) for 14 days. The quantification of Alizarin Red staining is depicted in the corresponding graphs. (D) Representative frontal and lateral 3D microCT scan images of knee joints from OA-induced mice treated with PBS or BAPN for 2 months post-surgery are shown. The right panel presents the quantitative analysis of new formation volumes (mm^3) and new crystal formation (µg). Images modified from [100].

stiffness. Interestingly, matrix stiffness impacted chondrocyte cytoskeleton, resulting in reduced Klotho expression [102]. Klotho is a well-known anti-ageing protein, and its deficiency contributes to cardiovascular PC associated or not with CKD [103–105]. Indeed, a recent meta-analysis showed that Klotho levels were inversely corelated with arterial calcification a well as arterial thickness or stiffness [106]. These data suggest LOX-induced matrix stiffness can regulate Klotho and calcification.

Ligaments can also calcify, such as in ossification of posterior longitudinal ligament (OPLL) in spine. Vascularization of PLL facilitates its calcification. In human and murine tissues with ossification of PLL, LOXL2 regulated the differentiation of ligament cells into endothelial-like cells and osteogenesis. Moreover, a tyrosin kinase inhibitor (sorafenib) suppressed LOXL2-mediated ossification in various rodent models [107].

3.3.2. Protective role of LOX(L) in musculoskeletal calcifications

The roles of LOX(L) have been investigated in mice *in vivo*, revealing their crucial involvement in musculoskeletal tissues. LOX knockout (KO) mice exhibited normal morphology and development of calcified structures but disorganized collagen and elastin fibers [108–111]. Furthermore, LOX$^{-/-}$ osteoblasts cultured *in vitro* demonstrated less mineralization than WT cells [109].

LOXL3 deficiency in mice led to perinatal lethality [112,113]. The surviving mutant mice were smaller, with shorter tibia and femur lengths, craniofacial defects, spinal deformities, and tissue disorganization, contributing to locomotion issues and greater skeletal fragility [112,113]. In addition, LOXL3 KO mice exhibited decreased collagen fibers and collagen cross links [113].

Finally, LOXL1, LOXL2 and LOXL4 have not been implicated in musculoskeletal development [110,114].

In addition to their roles in development, LOX(L) enzymes have also been investigated in musculoskeletal diseases. Notably, osteoporotic features, such as fragile bone structure and abnormal mineral metabolism commonly found in CKD, have been associated with LOX decrease in bones [115,116]. For instance, in rats suffering from renal failure, there is an accumulation of advanced glycation end-products (AGEs) in peri trabecular osteoblasts. This accumulation impairs osteoblast differentiation, evidenced by decreased expression of *Spp1*, and reduces mineralization, as indicated by diminished Alizarin red staining and decreased expression of *Runx2* and *Alpl*. Importantly, AGEs reduced LOX activity and therefore collagen crosslinking [115]. Alternatively, AGEs are also increased in diabetic bones and lead to impaired collagen matrix structure, resulting to improper bone mineralization [117]. Accordingly, mice treated with BAPN displayed a significant decrease of bone volume fraction and trabecular thickness [118].

In the context of bone fracture healing, studies have shown that these enzymes are upregulated in mice following fractures, with expression levels peaking at approximately 7 days post-fracture. This peak coincides with the onset of the endochondral ossification process during healing. *In vitro* experiments using the ATDC5 chondrogenic cell line further elucidated the role of LOXL2 in this process. Knockdown experiments targeting LOXL2 resulted in the abolition of both chondrogenic differentiation and calcification processes [119]. Supplementation of LOXL2 in BMP2-cultured osteoblasts increased collagen extracellular matrix maturity and apatite phosphate formation, thereby reinforcing calcification potential of cells [120].

Calcific tendinopathy is a common disease which can be reproduced in murine models, such as aging. In old mice with calcified tendons, LOX expression and calcification were reduced by uphill treadmill training, suggesting a negative correlation between LOX and tendon calcification [121].

4. Therapeutic inhibition of LOX(L)

Dysregulation of LOX(L) has been implicated in the onset and

progression of various pathologies, leading to the proposal of LOX(L) expression as a potential biomarker for these conditions [3]. In most instances, LOX(L) levels are elevated in these diseases, prompting the development of inhibitors targeting LOX(L). Currently, three classes of LOX(L) inhibitors are under investigation: small molecule inhibitors, monoclonal antibodies, and copper chelators [3,122]. Most of these inhibitors remain in the preclinical phase or are in Phase I-II clinical trials for conditions such as cancer, fibrosis, and neurodegeneration. Notably, they have yet to be tested in clinical trials in calcification conditions illustrated in Fig. 4.

Small molecule inhibitors, such as β-aminopropionitrile (BAPN), are widely utilized for their ability to irreversibly inhibit LOX(L) activity in both *in vitro* and *in vivo* models, as previously discussed. Although there are some conflicting reports regarding its effects on LOX and LOXL2 [3,6,100,122], BAPN is recognized for its role in disrupting the proper formation and assembly of collagen fibrils. This disruption is critical, as collagen fibrils are essential for maintaining the extracellular matrix (ECM) stiffness necessary for optimal tissue biomechanics [6,123]. The administration of this compound has demonstrated significant benefits, including improved cell survival, a reduced inflammatory response, slowed tumor progression, and decreased cardiovascular fibrosis [3]. Additionally, several other small molecules targeting LOX or LOXL2 have been investigated, yielding promising results in combating cancer and fibrosis [3]. Monoclonal antibodies developed to specifically target LOXL2 and LOXL4 isoforms have also proven effective in addressing fibrosis and cancer development, respectively [3]. Interestingly, the monoclonal antibody AB0023 effectively inhibits LOXL2 activity without affecting its catalytic function, suggesting that LOXL2 may play additional roles beyond collagen crosslinking [124]. Furthermore, since LOX(L) enzymes are copper-dependent, the use of copper chelators or inhibitors of copper incorporation has shown notable anti-fibrotic and anti-metastatic effects [3,122].

5. Conclusions

Pathologic calcification represents a significant clinical challenge, contributing to various debilitating conditions across multiple organ systems. The involvement of LOX(L) in the mechanisms underlying PC has garnered increasing attention, as these enzymes play critical roles in collagen cross-linking and extracellular matrix (ECM) remodeling. This review has highlighted the dual role of LOX(L) enzymes in calcification processes, emphasizing both their deleterious and protective roles depending on the specific context and tissue type.

For cardiovascular pathological calcification (PC), the results have been mixed, but a clearer pattern has emerged for musculoskeletal calcification. In musculoskeletal tissues that typically do not calcify, such as cartilage, tendons, and ligaments, LOX(L) enzymes appear to be detrimental because their increased expression leads to greater tissue calcification. In contrast, LOX(L) enzymes are protective in bone tissue, as they support proper calcification and bone health. Notably, pathological calcification in soft tissues is often associated with decreased bone mineral density or abnormal bone turnover, a phenomenon referred to as the 'calcification paradox' [125]. This paradox is particularly evident in conditions such as osteoporosis and CKD, where vascular calcifications occur alongside bone fractures or the development of metabolic bone disease (osteodystrophy) respectively. The association between impaired bone health and vascular calcification raises the question of LOX(L) levels in bone and vascular tissues. Indeed, although definitive answers are still being sought, in osteoporosis or CKD patients we speculate that LOX(L) levels are regulated in bone and vasculature in opposite way. Indeed, in osteoporosis, *in vivo* studies showed that decreased LOX levels caused reduction of collagen cross-links, decreased bone strength, and increased risk of fractures [126]. By contrast, increased LOX(L) in vessels is associated with PC development [127].

While LOX(L) enzymes have been extensively studied in cardiovascular and musculoskeletal tissues, their roles in other soft tissues prone to calcification, such as the brain, skin, and eyes, remain unexplored [128,129]. Furthermore, most investigations into the role of LOX(L) have been conducted on rodent models and require validation in human studies. Lastly, the development of therapeutic tools targeting LOX(L) must carefully consider the calcification paradox to avoid adverse effects on bone health.

Author contributions

E.F prepared the figures and wrote the manuscript. N.B and S.N supervised the work, prepared the figures, and revised the manuscript.

CRediT authorship contribution statement

Elodie Faure: Writing – original draft, Validation, Investigation. **Nathalie Busso:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Sonia Nasi:** Writing – review & editing, Validation, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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References

[1] S. Nasi, M. Romani, N. Busso, Age-associated calcification: insights from murine models, Gout, Urate, Cryst. Depos. Dis. 2 (2024) 236–251.
[2] I. Bernabei, A. So, N. Busso, S. Nasi, Cartilage calcification in osteoarthritis: mechanisms and clinical relevance, Nat. Rev. Rheuma 19 (2023) 10–27, https://doi.org/10.1038/s41584-022-00875-4.
[3] N. Yang, D.F. Cao, X.X. Yin, H.H. Zhou, X.Y. Mao, Lysyl oxidases: emerging biomarkers and therapeutic targets for various diseases, Biomed. Pharm. 131 (2020) 110791, https://doi.org/10.1016/j.biopha.2020.110791.
[4] S. Zaffryar-Eilot, P. Hasson, Lysyl oxidases: orchestrators of cellular behavior and ECM remodeling and homeostasis, Int J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms231911378.
[5] A. Iturbide, A. Garcia de Herreros, S. Peiro, A new role for LOX and LOXL2 proteins in transcription regulation, FEBS J. 282 (2015) 1768–1773, https://doi.org/10.1111/febs.12961.
[6] S.D. Vallet, S. Ricard-Blum, Lysyl oxidases: from enzyme activity to extracellular matrix cross-links, Essays Biochem 63 (2019) 349–364, https://doi.org/10.1042/EBC20180050.
[7] J.L. Grimsby, H.A. Lucero, P.C. Trackman, K. Ravid, H.M. Kagan, Role of lysyl oxidase propeptide in secretion and enzyme activity, J. Cell Biochem 111 (2010) 1231–1243, https://doi.org/10.1002/jcb.22845.
[8] X. Zhang, et al., Crystal structure of human lysyl oxidase-like 2 (hLOXL2) in a precursor state, Proc. Natl. Acad. Sci. USA 115 (2018) 3828–3833, https://doi.org/10.1073/pnas.1720859115.
[9] K. Okada, H.J. Moon, J. Finney, A. Meier, M. Mure, Extracellular processing of lysyl oxidase-like 2 and its effect on amine oxidase activity, Biochemistry 57 (2018) 6973–6983, https://doi.org/10.1021/acs.biochem.8b01008.
[10] A.J. Lopez-Jimenez, T. Basak, R.M. Vanacore, Proteolytic processing of lysyl oxidase-like-2 in the extracellular matrix is required for crosslinking of basement membrane collagen IV, J. Biol. Chem. 292 (2017) 16970–16982, https://doi.org/10.1074/jbc.M117.798603.
[11] L. Knott, A.J. Bailey, Collagen cross-links in mineralizing tissues: a review of their chemistry, function, and clinical relevance, Bone 22 (1998) 181–187, https://doi.org/10.1016/s8756-3282(97)00279-2.
[12] M. Saito, K. Marumo, Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus, Osteoporos. Int 21 (2010) 195–214, https://doi.org/10.1007/s00198-009-1066-z.
[13] P. Garnero, The contribution of collagen crosslinks to bone strength, Bone Rep. 1 (2012) 182, https://doi.org/10.1038/bonekey.2012.182.

[14] M. Saito, K. Marumo, Effects of collagen crosslinking on bone material properties in health and disease, Calcif. Tissue Int 97 (2015) 242–261, https://doi.org/10.1007/s00223-015-9985-5.

[15] M. Yamauchi, M. Sricholpech, Lysine post-translational modifications of collagen, Essays Biochem 52 (2012) 113–133, https://doi.org/10.1042/bse0520113.

[16] X. Grau-Bove, I. Ruiz-Trillo, F. Rodriguez-Pascual, Origin and evolution of lysyl oxidases, Sci. Rep. 5 (2015) 10568, https://doi.org/10.1038/srep10568.

[17] C. Rodriguez, et al., Regulation of lysyl oxidase in vascular cells: lysyl oxidase as a new player in cardiovascular diseases, Cardiovasc Res 79 (2008) 7–13, https://doi.org/10.1093/cvr/cvn102.

[18] X.X. Nguyen, et al., Lysyl oxidase directly contributes to extracellular matrix production and fibrosis in systemic sclerosis, Am. J. Physiol. Lung Cell Mol. Physiol. 320 (2021) L29–L40, https://doi.org/10.1152/ajplung.00173.2020.

[19] R. Oleggini, N. Gastaldo, A Di Donato, Regulation of elastin promoter by lysyl oxidase and growth factors: cross control of lysyl oxidase on TGF-beta1 effects, Matrix Biol. 26 (2007) 494–505, https://doi.org/10.1016/j.matbio.2007.02.003.

[20] S. Martinez-Revelles, et al., Lysyl oxidase induces vascular oxidative stress and contributes to arterial stiffness and abnormal elastin structure in hypertension: role of p38MAPK, Antioxid. Redox Signal 27 (2017) 379–397, https://doi.org/10.1089/ars.2016.6642.

[21] H.M. Lazarus, W.W. Cruikshank, N. Narasimhan, H.M. Kagan, D.M. Center, Induction of human monocyte motility by lysyl oxidase, Matrix Biol. 14 (1995) 727–731, https://doi.org/10.1016/s0945-053x(05)80015-0.

[22] H. Saito, J. Papaconstantinou, H. Sato, S. Goldstein, Regulation of a novel gene encoding a lysyl oxidase-related protein in cellular adhesion and senescence, J. Biol. Chem. 272 (1997) 8157–8160, https://doi.org/10.1074/jbc.272.13.8157.

[23] T. Peng, et al., The interaction of LOXL2 with GATA6 induces VEGFA expression and angiogenesis in cholangiocarcinoma, Int J. Oncol. 55 (2019) 657–670, https://doi.org/10.3892/ijo.2019.4837.

[24] D.M. Csiki, et al., Hypoxia-inducible factor activation promotes osteogenic transition of valve interstitial cells and accelerates aortic valve calcification in a mice model of chronic kidney disease, Front Cardiovasc Med 10 (2023) 1168339, https://doi.org/10.3389/fcvm.2023.1168339.

[25] S. Mokas, et al., Hypoxia-inducible factor-1 plays a role in phosphate-induced vascular smooth muscle cell calcification, Kidney Int 90 (2016) 598–609, https://doi.org/10.1016/j.kint.2016.05.020.

[26] C. Guo, et al., Hypoxia-inducible factor-1alpha regulates high phosphate-induced vascular calcification via type III sodium-dependent phosphate cotransporter 1, Cardiol. Res Pr. 2024 (2024) 6346115, https://doi.org/10.1155/2024/6346115.

[27] V. Wang, D.A. Davis, M. Haque, L.E. Huang, R. Yarchoan, Differential gene up-regulation by hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2alpha in HEK293T cells, Cancer Res 65 (2005) 3299–3306, https://doi.org/10.1158/0008-5472.CAN-04-4130.

[28] V. Wang, D.A. Davis, R. Yarchoan, Identification of functional hypoxia inducible factor response elements in the human lysyl oxidase gene promoter, Biochem Biophys. Res Commun. 490 (2017) 480–485, https://doi.org/10.1016/j.bbrc.2017.06.066.

[29] R. Schietke, et al., The lysyl oxidases LOX and LOXL2 are necessary and sufficient to repress E-cadherin in hypoxia: insights into cellular transformation processes mediated by HIF-1, J. Biol. Chem. 285 (2010) 6658–6669, https://doi.org/10.1074/jbc.M109.042424.

[30] H.Q. He, et al., AGEs-induced calcification and apoptosis in human vascular smooth muscle cells is reversed by inhibition of autophagy, Front Pharm. 12 (2021) 692431, https://doi.org/10.3389/fphar.2021.692431.

[31] T. Tanikawa, Y. Okada, R. Tanikawa, Y. Tanaka, Advanced glycation end products induce calcification of vascular smooth muscle cells through RAGE/p38 MAPK, J. Vasc. Res 46 (2009) 572–580, https://doi.org/10.1159/000226225.

[32] K.K. Papachroni, et al., Lysyl oxidase interacts with AGE signalling to modulate collagen synthesis in polycystic ovarian tissue, 2460-9, J. Cell Mol. Med 14 (2010), https://doi.org/10.1111/j.1582-4934.2009.00841.x.

[33] C. Adamopoulos, et al., Advanced glycation end products upregulate lysyl oxidase and endothelin-1 in human aortic endothelial cells via parallel activation of ERK1/2-NF-kappaB and JNK-AP-1 signaling pathways, Cell Mol. Life Sci. 73 (2016) 1685–1698, https://doi.org/10.1007/s00018-015-2091-z.

[34] J.H. Kim, et al., Matrix cross-linking-mediated mechanotransduction promotes posttraumatic osteoarthritis, Proc. Natl. Acad. Sci. USA 112 (2015) 9424–9429, https://doi.org/10.1073/pnas.1505700112.

[35] Y. Zhu, et al., Advanced glycation end products accelerate calcification in VSMCs through HIF-1alpha/PDK4 activation and suppress glucose metabolism, Sci. Rep. 8 (2018) 13730, https://doi.org/10.1038/s41598-018-31877-6.

[36] D. Zickler, et al., Tumour necrosis factor-alpha in uraemic serum promotes osteoblastic transition and calcification of vascular smooth muscle cells via extracellular signal-regulated kinases and activator protein 1/c-FOS-mediated induction of interleukin 6 expression, Nephrol. Dial. Transpl. 33 (2018) 574–585, https://doi.org/10.1093/ndt/gfx316.

[37] A. Kurozumi, et al., IL-6 and sIL-6R induces STAT3-dependent differentiation of human VSMCs into osteoblast-like cells through JMJD2B-mediated histone demethylation of RUNX2, Bone 124 (2019) 53–61, https://doi.org/10.1016/j.bone.2019.04.006.

[38] S. Nasi, A. So, C. Combes, M. Daudon, N. Busso, Interleukin-6 and chondrocyte mineralisation act in tandem to promote experimental osteoarthritis, Ann. Rheum. Dis. 75 (2016) 1372–1379, https://doi.org/10.1136/annrheumdis-2015-207487.

[39] B. Hegner, et al., Targeting proinflammatory cytokines ameliorates calcifying phenotype conversion of vascular progenitors under uremic conditions in vitro, Sci. Rep. 8 (2018) 12087, https://doi.org/10.1038/s41598-018-30626-z.

[40] J. Shen, M. Zhao, C. Zhang, X. Sun, IL-1beta in atherosclerotic vascular calcification: from bench to bedside, Int J. Biol. Sci. 17 (2021) 4353–4364, https://doi.org/10.7150/ijbs.66537.

[41] R. Laczko, K. Csiszar, Lysyl oxidase (LOX): functional contributions to signaling pathways, Biomolecules 10 (2020), https://doi.org/10.3390/biom10081093.

[42] N. Wang, et al., Role of TGF-beta1 in bone matrix production in vascular smooth muscle cells induced by a high-phosphate environment, Nephron Exp. Nephrol. 115 (2010) e60–e68, https://doi.org/10.1159/000313831.

[43] H. Zhang, M. Ahmad, G. Gronowicz, Effects of transforming growth factor-beta 1 (TGF-beta1) on in vitro mineralization of human osteoblasts on implant materials, Biomaterials 24 (2003) 2013–2020, https://doi.org/10.1016/s0142-9612(02)00616-6.

[44] X. Wang, et al., Inhibition of overactive TGF-beta attenuates progression of heterotopic ossification in mice, Nat. Commun. 9 (2018) 551, https://doi.org/10.1038/s41467-018-02988-5.

[45] M. Chakrabarti, et al., Increased TGFbeta1 and SMAD3 contribute to age-related aortic valve calcification, Front Cardiovasc Med 9 (2022) 770065, https://doi.org/10.3389/fcvm.2022.770065.

[46] J.N. Clark-Greuel, et al., Transforming growth factor-beta1 mechanisms in aortic valve calcification: increased alkaline phosphatase and related events, Ann. Thorac. Surg. 83 (2007) 946–953, https://doi.org/10.1016/j.athoracsur.2006.10.026.

[47] A. Jenke, et al., Transforming growth factor-beta1 promotes fibrosis but attenuates calcification of valvular tissue applied as a three-dimensional calcific aortic valve disease model, H1123-H1141, Am. J. Physiol. Heart Circ. Physiol. 319 (2020), https://doi.org/10.1152/ajpheart.00651.2019.

[48] X. Yang, et al., TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage, J. Cell Biol. 153 (2001) 35–46, https://doi.org/10.1083/jcb.153.1.35.

[49] D.F. Remst, et al., Gene expression analysis of murine and human osteoarthritis synovium reveals elevation of transforming growth factor beta-responsive genes in osteoarthritis-related fibrosis, Arthritis Rheuma 66 (2014) 647–656, https://doi.org/10.1002/art.38266.

[50] J. Xie, et al., TGF-beta1 induces the different expressions of lysyl oxidases and matrix metalloproteinases in anterior cruciate ligament and medial collateral ligament fibroblasts after mechanical injury, J. Biomech. 46 (2013) 890–898, https://doi.org/10.1016/j.jbiomech.2012.12.019.

[51] A. Sethi, W. Mao, R.J. Wordinger, A.F. Clark, Transforming growth factor-beta induces extracellular matrix protein cross-linking lysyl oxidase (LOX) genes in human trabecular meshwork cells, Invest Ophthalmol. Vis. Sci. 52 (2011) 5240–5250, https://doi.org/10.1167/iovs.11-7287.

[52] D. Ngai, M. Lino, M.P. Bendeck, Cell-matrix interactions and matricrine signaling in the pathogenesis of vascular calcification, Front Cardiovasc Med 5 (2018) 174, https://doi.org/10.3389/fcvm.2018.00174.

[53] D. Ngai, et al., DDR1 (Discoidin Domain Receptor-1)-RhoA (Ras Homolog Family Member A) axis senses matrix stiffness to promote vascular calcification, Arterioscler. Thromb. Vasc. Biol. 40 (2020) 1763–1776, https://doi.org/10.1161/ATVBAHA.120.314697.

[54] G. Luo, et al., Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein, Nature 386 (1997) 78–81, https://doi.org/10.1038/386078a0.

[55] K.E. Watson, F. Parhami, V. Shin, L.L. Demer, Fibronectin and collagen I matrixes promote calcification of vascular cells in vitro, whereas collagen IV matrix is inhibitory, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 1964–1971, https://doi.org/10.1161/01.atv.18.12.1964.

[56] H.T. Ding, C.G. Wang, T.L. Zhang, K. Wang, Fibronectin enhances in vitro vascular calcification by promoting osteoblastic differentiation of vascular smooth muscle cells via ERK pathway, J. Cell Biochem 99 (2006) 1343–1352, https://doi.org/10.1002/jcb.20999.

[57] X. Shi, S. Zhang, J. Li, Y. Ke, Y. Bai, Fibronectin/alpha5 integrin contribute to hypertension-associated arterial ageing and calcification through affecting BMP2/MGP imbalance and enhancing vascular smooth muscle cell phenotypic transformation, Gerontology 70 (2024) 1–18, https://doi.org/10.1159/000539399.

[58] K. Gelse, et al., Thrombospondin-1 prevents excessive ossification in cartilage repair tissue induced by osteogenic protein-1, Tissue Eng. Part A 17 (2011) 2101–2112, https://doi.org/10.1089/ten.TEA.2010.0691.

[59] B. Fogelgren, et al., Cellular fibronectin binds to lysyl oxidase with high affinity and is critical for its proteolytic activation, J. Biol. Chem. 280 (2005) 24690–24697, https://doi.org/10.1074/jbc.M412979200.

[60] T. Sasaki, et al., Loss of fibulin-4 results in abnormal collagen fibril assembly in bone, caused by impaired lysyl oxidase processing and collagen cross-linking, Matrix Biol. 50 (2016) 53–66, https://doi.org/10.1016/j.matbio.2015.12.002.

[61] M.V. Panchenko, W.G. Stetler-Stevenson, O.V. Trubetskoy, S.N. Gacheru, H. M. Kagan, Metalloproteinase activity secreted by fibrogenic cells in the processing of prolysyl oxidase. Potential role of procollagen C-proteinase, J. Biol. Chem. 271 (1996) 7113–7119, https://doi.org/10.1074/jbc.271.12.7113.

[62] A. Borel, et al., Lysyl oxidase-like protein from bovine aorta. Isolation and maturation to an active form by bone morphogenetic protein-1, J. Biol. Chem. 276 (2001) 48944–48949, https://doi.org/10.1074/jbc.M109499200.

[63] M.I. Uzel, et al., Multiple bone morphogenetic protein 1-related mammalian metalloproteinases process pro-lysyl oxidase at the correct physiological site and control lysyl oxidase activation in mouse embryo fibroblast cultures, J. Biol. Chem. 276 (2001) 22537–22543, https://doi.org/10.1074/jbc.M102352200.

[64] S. Rosini, et al., Thrombospondin-1 promotes matrix homeostasis by interacting with collagen and lysyl oxidase precursors and collagen cross-linking sites, Sci. Signal 11 (2018), https://doi.org/10.1126/scisignal.aar2566.
[65] N.E. Calabro, et al., Thrombospondin-2 regulates extracellular matrix production, LOX levels, and cross-linking via downregulation of miR-29, Matrix Biol. 82 (2019) 71–85, https://doi.org/10.1016/j.matbio.2019.03.002.
[66] N. Mohammadzadeh, et al., The extracellular matrix proteoglycan lumican improves survival and counteracts cardiac dilatation and failure in mice subjected to pressure overload, Sci. Rep. 9 (2019) 9206, https://doi.org/10.1038/s41598-019-45651-9.
[67] S. Kalamajski, D. Bihan, A. Bonna, K. Rubin, R.W. Farndale, Fibromodulin interacts with collagen cross-linking sites and activates lysyl oxidase, J. Biol. Chem. 291 (2016) 7951–7960, https://doi.org/10.1074/jbc.M115.693408.
[68] K.M. Herum, et al., Syndecan-4 is a key determinant of collagen cross-linking and passive myocardial stiffness in the pressure-overloaded heart, Cardiovasc Res 106 (2015) 217–226, https://doi.org/10.1093/cvr/cvv002.
[69] M. Majora, et al., Functional consequences of mitochondrial DNA deletions in human skin fibroblasts: increased contractile strength in collagen lattices is due to oxidative stress-induced lysyl oxidase activity, Am. J. Pathol. 175 (2009) 1019–1029, https://doi.org/10.2353/ajpath.2009.080832.
[70] C.T. Hu, et al., Oxidative stress in vascular calcification, Clin. Chim. Acta 519 (2021) 101–110, https://doi.org/10.1016/j.cca.2021.04.012.
[71] P. Wang, et al., The role of mitochondria in vascular calcification, J. Transl. Int Med 8 (2020) 80–90, https://doi.org/10.2478/jtim-2020-0013.
[72] K. Morita, et al., Reactive oxygen species induce chondrocyte hypertrophy in endochondral ossification, J. Exp. Med 204 (2007) 1613–1623, https://doi.org/10.1084/jem.20062525.
[73] S. Nasi, H.K. Ea, F. Liote, A. So, N. Busso, Sodium thiosulfate prevents chondrocyte mineralization and reduces the severity of murine osteoarthritis, PLoS One 11 (2016) e0158196, https://doi.org/10.1371/journal.pone.0158196.
[74] S. Jono, A. Shioi, Y. Ikari, Y. Nishizawa, Vascular calcification in chronic kidney disease, J. Bone Min. Metab. 24 (2006) 176–181, https://doi.org/10.1007/s00774-005-0668-6.
[75] M. Wu, C. Rementer, C.M. Giachelli, Vascular calcification: an update on mechanisms and challenges in treatment, Calcif. Tissue Int 93 (2013) 365–373, https://doi.org/10.1007/s00223-013-9712-z.
[76] D.D. Pei, et al., Contribution of mitophagy to cell-mediated mineralization: revisiting a 50-year-old conundrum, Adv. Sci. 5 (2018) 1800873, https://doi.org/10.1002/advs.201800873.
[77] K.M. Kim, Apoptosis and calcification, Scanning Microsc. 9 (1995) 1137–1175, discussion 1175-8.
[78] H.C. Anderson, Matrix vesicles and calcification, Curr. Rheuma Rep. 5 (2003) 222–226, https://doi.org/10.1007/s11926-003-0071-z.
[79] G. Sanchez-Duffhues, C. Hiepen, P. Knaus, P. Ten Dijke, Bone morphogenetic protein signaling in bone homeostasis, Bone 80 (2015) 43–59, https://doi.org/10.1016/j.bone.2015.05.025.
[80] P.P. Lui, L.S. Chan, Y.C. Cheuk, Y.W. Lee, K.M. Chan, Expression of bone morphogenetic protein-2 in the chondrogenic and ossifying sites of calcific tendinopathy and traumatic tendon injury rat models, J. Orthop. Surg. Res 4 (2009) 27, https://doi.org/10.1186/1749-799X-4-27.
[81] E.S. Molloy, G.M. McCarthy, Hydroxyapatite deposition disease of the joint, Curr. Rheuma Rep. 5 (2003) 215–221, https://doi.org/10.1007/s11926-003-0070-0.
[82] M. Ketteler, C. Giachelli, Novel insights into vascular calcification, Kidney Int Suppl. (2006) S5–S9, https://doi.org/10.1038/sj.ki.5001996.
[83] P. Urena-Torres, et al., Valvular heart disease and calcification in CKD: more common than appreciated, Nephrol. Dial. Transpl. 35 (2020) 2046–2053, https://doi.org/10.1093/ndt/gfz133.
[84] C. Ballester-Servera, et al., Lysyl oxidase-dependent extracellular matrix crosslinking modulates calcification in atherosclerosis and aortic valve disease, Biomed. Pharm. 167 (2023) 115469, https://doi.org/10.1016/j.biopha.2023.115469.
[85] E. Jover, et al., Inhibition of enzymes involved in collagen cross-linking reduces vascular smooth muscle cell calcification, FASEB J. 32 (2018) 4459–4469, https://doi.org/10.1096/fj.201700653R.
[86] H.N. Hutson, et al., Calcific aortic valve disease is associated with layer-specific alterations in collagen architecture, PLoS One 11 (2016) e0163858, https://doi.org/10.1371/journal.pone.0163858.
[87] C. Ballester-Servera, et al., Lysyl oxidase in ectopic cardiovascular calcification: role of oxidative stress, Antioxidants 13 (2024), https://doi.org/10.3390/antiox13050523.
[88] K. Uto, S. Yoshizawa, C. Aoki, T. Nishikawa, H. Oda, Inhibition of extracellular matrix integrity attenuates the early phase of aortic medial calcification in a rodent model, Atherosclerosis 319 (2021) 10–20, https://doi.org/10.1016/j.atherosclerosis.2020.12.015.
[89] M.C. McNeill, et al., Substrate stiffness promotes vascular smooth muscle cell calcification by reducing the levels of nuclear actin monomers, J. Mol. Cell Cardiol. 187 (2024) 65–79, https://doi.org/10.1016/j.yjmcc.2023.12.005.
[90] C.R. Kothapalli, C.E. Gacchina, A. Ramamurthi, Utility of hyaluronan oligomers and transforming growth factor-beta1 factors for elastic matrix regeneration by aneurysmal rat aortic smooth muscle cells, Tissue Eng. Part A 15 (2009) 3247–3260, https://doi.org/10.1089/ten.tea.2008.0593.
[91] H. Sugitani, H. Wachi, R.P. Mecham, Y. Seyama, Accelerated calcification represses the expression of elastic fiber components and lysyl oxidase in cultured bovine aortic smooth muscle cells, J. Atheroscler. Thromb. 9 (2002) 292–298, https://doi.org/10.5551/jat.9.292.
[92] B.S. Fleenor, K.D. Marshall, J.R. Durrant, L.A. Lesniewski, D.R. Seals, Arterial stiffening with ageing is associated with transforming growth factor-beta1-related changes in adventitial collagen: reversal by aerobic exercise, J. Physiol. 588 (2010) 3971–3982, https://doi.org/10.1113/jphysiol.2010.194753.
[93] Y. Lei, Y. Xia, Y. Wang, The tropoelastin and lysyl oxidase treatments increased the content of insoluble elastin in bioprosthetic heart valves, J. Biomater. Appl. 33 (2018) 637–646, https://doi.org/10.1177/0885328218807077.
[94] K.L. Hellekson, NIH releases statement on osteoporosis prevention, diagnosis, and therapy, Am. Fam. Physician 66 (2002) 161–162.
[95] T. Sozen, L. Ozisik, N.C. Basaran, An overview and management of osteoporosis, Eur. J. Rheuma 4 (2017) 46–56, https://doi.org/10.5152/eurjrheum.2016.048.
[96] Q. Yao, et al., Osteoarthritis: pathogenic signaling pathways and therapeutic targets, Signal Transduct. Target Ther. 8 (2023) 56, https://doi.org/10.1038/s41392-023-01330-w.
[97] M. Fuerst, et al., Calcification of articular cartilage in human osteoarthritis, Arthritis Rheum. 60 (2009) 2694–2703, https://doi.org/10.1002/art.24774.
[98] J. Herman, et al., Pro-inflammatory effects of human apatite crystals extracted from patients suffering from calcific tendinopathy, Arthritis Res Ther. 23 (2021) 131, https://doi.org/10.1186/s13075-021-02516-9.
[99] S. Matsunaga, T. Sakou, Ossification of the posterior longitudinal ligament of the cervical spine: etiology and natural history, Spine (Philos. Pa 1976) 37 (2012) E309–E314, https://doi.org/10.1097/BRS.0b013e318241ad33.
[100] I. Bernabei, et al., Inhibiting lysyl oxidases prevents pathologic cartilage calcification, Biomed. Pharm. 171 (2024) 116075, https://doi.org/10.1016/j.biopha.2023.116075.
[101] I. Bernabei, et al., CD11b deficiency favors cartilage calcification via increased matrix vesicles, apoptosis, and lysyl oxidase activity, Int J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms24119776.
[102] H. Iijima, et al., Age-related matrix stiffening epigenetically regulates alpha-Klotho expression and compromises chondrocyte integrity, Nat. Commun. 14 (2023) 18, https://doi.org/10.1038/s41467-022-35359-2.
[103] A.P. Singh, et al., AlphaKlotho regulates age-associated vascular calcification and lifespan in zebrafish, Cell Rep. 28 (2019) 2767–2776, https://doi.org/10.1016/j.celrep.2019.08.013.
[104] M.C. Hu, et al., Klotho deficiency causes vascular calcification in chronic kidney disease, J. Am. Soc. Nephrol. 22 (2011) 124–136, https://doi.org/10.1681/ASN.2009121311.
[105] T. Chen, et al., The role and mechanism of alpha-klotho in the calcification of rat aortic vascular smooth muscle cells, Biomed. Res Int 2015 (2015) 194362, https://doi.org/10.1155/2015/194362.
[106] C.D.K. Wungu, et al., Role of klotho and fibroblast growth factor 23 in arterial calcification, thickness, and stiffness: a meta-analysis of observational studies, Sci. Rep. 14 (2024) 5712, https://doi.org/10.1038/s41598-024-56377-8.
[107] L. Wang, et al., Sorafenib inhibits ossification of the posterior longitudinal ligament by blocking LOXL2-mediated vascularization, Bone Res 12 (2024) 24, https://doi.org/10.1038/s41413-024-00327-7.
[108] L. Tjaderhane, et al., The expression and role of Lysyl oxidase (LOX) in dentinogenesis, Int Endod. J. 46 (2013) 581–589, https://doi.org/10.1111/iej.12031.
[109] N. Pischon, et al., Lysyl oxidase (lox) gene deficiency affects osteoblastic phenotype, Calcif. Tissue Int 85 (2009) 119–126, https://doi.org/10.1007/s00223-009-9252-8.
[110] J.M. Maki, et al., Lysyl oxidase is essential for normal development and function of the respiratory system and for the integrity of elastic and collagen fibers in various tissues, Am. J. Pathol. 167 (2005) 927–936, https://doi.org/10.1016/S0002-9440(10)61183-2.
[111] L. Kutchuk, et al., Muscle composition is regulated by a Lox-TGFbeta feedback loop, Development 142 (2015) 983–993, https://doi.org/10.1242/dev.113449.
[112] P.G. Santamaria, et al., Loxl2 and Loxl3 paralogues play redundant roles during mouse development, Int J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms23105730.
[113] J. Zhang, et al., Loss of lysyl oxidase-like 3 causes cleft palate and spinal deformity in mice, Hum. Mol. Genet 24 (2015) 6174–6185, https://doi.org/10.1093/hmg/ddv333.
[114] A. Martin, et al., Lysyl oxidase-like 2 represses Notch1 expression in the skin to promote squamous cell carcinoma progression, EMBO J. 34 (2015) 1090–1109, https://doi.org/10.15252/embj.201489975.
[115] C. Aoki, K. Uto, K. Honda, Y. Kato, H. Oda, Advanced glycation end products suppress lysyl oxidase and induce bone collagen degradation in a rat model of renal osteodystrophy, Lab Invest 93 (2013) 1170–1183, https://doi.org/10.1038/labinvest.2013.105.
[116] S. Moe, et al., Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: improving global outcomes (KDIGO), Kidney Int 69 (2006) 1945–1953, https://doi.org/10.1038/sj.ki.5000414.
[117] Q. Gao, et al., Advanced glycation end products mediate biomineralization disorder in diabetic bone disease, Cell Rep. Med (2024) 101694, https://doi.org/10.1016/j.xcrm.2024.101694.
[118] Y. Shen, et al., The effect of beta-aminopropionitrile on skeletal micromorphology and osteogenesis, Calcif. Tissue Int 103 (2018) 411–421, https://doi.org/10.1007/s00223-018-0430-4.
[119] M. Iftikhar, et al., Lysyl oxidase-like-2 (LOXL2) is a major isoform in chondrocytes and is critically required for differentiation, J. Biol. Chem. 286 (2011) 909–918, https://doi.org/10.1074/jbc.M110.155622.
[120] Y. Shibata, et al., Lysyl oxidase like-2 reinforces unsatisfactory ossification induced by bone morphogenetic protein-2: relating nanomechanical properties

and molecular changes, Nanomedicine 9 (2013) 1036–1047, https://doi.org/10.1016/j.nano.2013.04.008.

[121] L.K. Wood, S.V. Brooks, Ten weeks of treadmill running decreases stiffness and increases collagen turnover in tendons of old mice, J. Orthop. Res 34 (2016) 346–353, https://doi.org/10.1002/jor.22824.

[122] I. Hajdu, et al., Inhibition of the LOX enzyme family members with old and new ligands. Selectivity analysis revisited, Bioorg. Med Chem. Lett. 28 (2018) 3113–3118, https://doi.org/10.1016/j.bmcl.2018.07.001.

[123] A. Herchenhan, et al., Lysyl oxidase activity is required for ordered collagen fibrillogenesis by tendon cells, J. Biol. Chem. 290 (2015) 16440–16450, https://doi.org/10.1074/jbc.M115.641670.

[124] J.A. Bell, et al., Spatial transcriptomic validation of a biomimetic model of fibrosis enables re-evaluation of a therapeutic antibody targeting LOXL2, Cell Rep. Med (2024) 101695, https://doi.org/10.1016/j.xcrm.2024.101695.

[125] V. Persy, P. D'Haese, Vascular calcification and bone disease: the calcification paradox, Trends Mol. Med 15 (2009) 405–416, https://doi.org/10.1016/j.molmed.2009.07.001.

[126] H. Oxlund, M. Barckman, G. Ortoft, T.T. Andreassen, Reduced concentrations of collagen cross-links are associated with reduced strength of bone, 365S-371S, Bone 17 (1995), https://doi.org/10.1016/8756-3282(95)00328-b.

[127] F.F. Stoyell-Conti, et al., Gene inactivation of lysyl oxidase in smooth muscle cells reduces atherosclerosis burden and plaque calcification in hyperlipidemic mice, Atherosclerosis 397 (2024) 118582, https://doi.org/10.1016/j.atherosclerosis.2024.118582.

[128] J.M. Bentin, S. Heegaard, N.R. Jorgensen, L. Grahnemo, S. Hamann, Optic disc drusen: dystrophic calcification, a potential target for treatment, Eye (Lond.) (2024), https://doi.org/10.1038/s41433-024-03138-6.

[129] L.L. de Faria, et al., Soft tissue calcifications: a pictorial essay, Radio. Bras. 53 (2020) 337–344, https://doi.org/10.1590/0100-3984.2019.0100.