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Regulation of cardiovascular calcification by lipids and lipoproteins

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Abstract

Purpose: Lipids and lipoproteins have long been known to contribute to atherosclerosis and cardiovascular calcification. One theme of recent work is the study of lipoprotein (a) [Lp(a)], a lipoprotein particle similar to LDL-cholesterol that carries a long apoprotein tail and most of the circulating oxidized phospholipids.

Recent findings: In vitro studies show that Lp(a) stimulates osteoblastic differentiation and mineralization of vascular smooth muscle cells, while the association of Lp(a) with coronary artery calcification continues to have varying results, possibly due to the widely varying threshold levels of Lp(a) chosen for association analyses. Another emerging area in the field cardiovascular calcification is pathological endothelial to mesenchymal transition (EndMT), the process whereby endothelial cells transition into multipotent mesenchymal cells, some of which differentiate into osteo-chondrogenic cells and mineralize. The effects of lipids and lipoproteins on EndMT suggest they modulate cardiovascular calcification through multiple mechanisms. There are also emerging trends in imaging of calcific vasculopathy, including: intravascular optical coherence tomography for quantifying plaque characteristics, positron emission tomography with a radiolabeled NaF tracer, with either CT or MRI to detect coronary plaque vulnerability.

Summary: Recent work in this field includes studies of Lp(a), EndMT, and new imaging techniques.

Keywords

calcification; lipids; vascular; imaging; cardiovascular

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Conflicts of Interest

None

Introduction

Calcium deposition in cardiovascular tissues is a widespread pathological process. Once considered a degenerative, end-stage, and inevitable condition, it is now recognized as a complex process regulated by key biomolecules and their regulation by metabolic, hormonal, and inflammatory stimuli [1]. In the coronary arteries, calcification progresses substantially with age. Based on optical coherence tomography, it is present in about 5% of people under age 45 and in about 50% of those over age 75 [2]. For decades, the presence and severity of coronary artery calcification (CAC) have been consistently associated with increased risk for major adverse cardiovascular events [3].

Cardiovascular calcification and lipids and lipoproteins

Several lines of evidence link cardiovascular calcification to hyperlipidemia. In a recent study of patients with cerebrovascular symptoms, hypercholesterolemia was associated significantly with carotid artery calcification, especially in male patients [4]. Among patients with heterozygous familial hypercholesterolemia, those with physical signs of tendon xanthomas and corneal arcus had both higher LDL levels and higher CAC score tertiles [5].

Earlier studies have shown an association of Lp(a) with cardiovascular risk, and some recent studies show an association of Lp(a) with cardiovascular calcification. A key unanswered question is whether there is a causal link: Is the risk of Lp(a) dependent or independent of an effect on calcification? The answer appears to depend on the threshold level chosen to define “high Lp(a)” levels. An earlier study by Verweij et al. [6] evaluated subjects with a family history of premature ASCVD and found that elevated Lp(a) levels (> 50 mg/dl) were associated with higher CAC scores (> 100), even after adjusted analyses. Peng and colleagues [7] found that Lp(a) levels > 10 mg/dl were independently correlated with severity of CAC in 2,800 patients. Lp(a) levels remained significantly and positively associated with CAC score even after adjustment for confounders. Similar findings of a clinical relationship between Lp(a) and CAC were reported by Ong et al., who used a slightly higher threshold (30 mg/dl) to define “high Lp(a)” in 6,800 Multi-Ethnic Study of Atherosclerosis (MESA) subjects and found that high Lp(a) was associated with the absolute progression of coronary calcium volume by CT [8].

Clinical studies using higher threshold values for Lp(a), including 50, 60, and 70 mg/dl, have reported a lack of association or interaction between Lp(a) and CAC, suggesting that Lp(a) does not promote risk through an effect on CAC. Using a threshold of ≥ 50 mg/dl for high Lp(a), Mehta and colleagues [9] studied patients with advanced stable coronary artery disease from the MESA study and reported that both CAC and Lp(a) were associated with ASCVD but that they did so “independently” of one another. However, their results suggest a trend toward interaction. For instance, in patients with no CAC, the level of Lp(a) lost its association with ASCVD risk. And, in the highest Quintile (Q5) of Lp(a), the risk was highly dependent on CAC. For instance, in that quintile, absence of calcification carried no significant risk, whereas in moderate calcification, risk was significant and almost doubled, and in those with CAC > 100, risk was even higher with a hazard ratio of 4.71 (3.01 – 7.40).

These results suggest a trend toward interaction of the two factors. A useful analysis would be to test whether CAC affected risk within the lowest quintile of Lp(a) alone, rather than pooled with quintiles 1 – 4. Using an even higher threshold for Lp(a) (< 70 mg/dl), Kaiser et al [10] examined progression of coronary calcific plaque volume and found a 20-fold greater progression of calcific volume in the high Lp(a) group over a 12-month period. However, it did not reach significance ($p = 0.2$). Since these threshold values are within the highest quintiles, a significant correlation may be missed [10].

In vitro evidence suggests a causal link between Lp(a) and calcification. Peng and colleagues tested the direct effects of Lp(a) on human smooth muscle cell cultures and found that Lp(a) also upregulated osteoblastic differentiation genes and induced mineralization, with evidence for a mechanism involving Notch1/BMP-2/Smad signaling [7]. In related mechanistic studies, Rogers and colleagues tested the effects of Lp(a) on calcification of vascular and valvular cell cultures and found that Lp(a), a major carrier of oxidized phospholipids [11], is taken up by the vascular cells and accelerated in vitro calcification [12]. As evidence for a role of oxidized phospholipids, this effect was blocked by administration of the antibody to oxidized phospholipids, E06, developed by the Witztum research group.

Donis et al. [13] fed New Zealand White rabbits with palmitic acid-enriched or control diets and studied the calcification in the aortas. They found Alizarin red staining in the endothelial layer of aortas and aortic valves on the lipid-enriched diet but not in the controls. This positive staining correlated with serum levels of palmitic acid. The pattern and location of Alizarin red staining appear to be unusual for calcification; in most models, calcification occurs as discrete lesions deep in the media [14] or within atherosclerotic lesions. Calcium mineral that usually shows characteristic metachromatic changes on hematoxylin-eosin staining was also apparently not observed in their hematoxylin-eosin staining.

Proprotein convertase subtilisin/kexin type (PCSK9), which internalizes LDL receptors for degradation, is associated with coronary artery calcification, including in patients with renal failure. Lupo et al. [15] provide evidence that PCSK9 contributes causally to arterial medial calcification in both in vitro and in vivo models of chronic kidney disease. The immunohistochemical images show that PCSK9 is expressed highly in the aorta in both control and adenine diet-fed rats, while the medial calcification is only in the latter animals [15]. The authors further assessed whether the effect of PCSK9 on calcification is direct in human smooth muscle cell cultures. The results showed that the calcification was induced by intracellular overexpression of PCSK9 in a calcifying (high phosphate) medium but not by exogenous PCSK9 [15], suggesting that this trafficking protein seems to be necessary but not sufficient to induce calcification.

Endothelial to mesenchymal transition (EndMT) and lipids and lipoproteins

While lipids have long been known to contribute to atherosclerosis and cardiovascular calcification, one mechanism that has recently emerged is their stimulation of ECs to undergo pathological EndMT, the process whereby endothelial cells (ECs) transition into multipotent mesenchymal stem cell-like cells, capable of differentiating into various

cell types. ECs undergoing EndMT have been shown to differentiate into mesenchymal lineages, including chondrogenic and osteogenic cells, which may initiate and propagate atherosclerosis and cardiovascular calcification [16].

The pro-inflammatory lipid, 1-Palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) is found in atherosclerotic lesions. POVPC treatment of human umbilical vein endothelial cells (HUVECs) induced morphologic changes consistent with EndMT [17]. Accordingly, POVPC decreased the expression of the endothelial markers CD31 and endothelial nitric oxide synthase (ENOS) and increased the expression of the mesenchymal markers, alpha-smooth muscle actin, vimentin, Snail-1, Twist-1, transforming growth factor-β (TGF-β), TGF-β receptor II, pSmad2/3 and Smad-2,3 [17]. These effects were mediated by oxidative stress and inhibited by simvastatin, suggesting yet another mechanism for the anti-atherosclerotic effects of statin therapy.

Recent work has identified several candidate-non-coding RNAs that may mediate the effects of lipids on promoting EndMT. The micro-RNA miR-200c-3p is overexpressed in atherosclerotic aortic tissue. In the in vitro experiments using HUVECs, treatment with oxidized LDL (ox-LDL) downregulated endothelial markers CD31 and vWF, while upregulating the mesenchymal markers α-smooth muscle actin and vimentin [18]. Ox-LDL treatment similarly increased the expression of miR-200c-3p, and co-treatment of HUVECs with ox-LDL plus miR-200c-3p potentiated the EndMT phenotype. This effect appeared to be mediated by miR-200c-3p inhibition of the SMAD7/YAP pathway [18].

In HUVECs, ox-LDL treatment resulted in the upregulation of the long non-coding RNA (lncRNA) GAS5, and subsequent increased expression of miR-29a-3p [19]. Pre-treatment with the antioxidant flavonoid myricetin resulted in reduced ox-LDL-induced EndMT, a feature that could be reversed with GAS5 overexpression [19]. Thus, the GAS5/miR-29a-3p pathway appears to help orchestrate ox-LDL-mediated EndMT, which can be targeted with anti-inflammatory therapies such as myricetin. Similarly, another lncRNA, ZFAS1 potentiates the EndMT-inducing effects of ox-LDL in HUVECs via upregulation of Notch3 and downregulation of miR-150–5p signaling [20]. Thus, the identification of non-coding RNAs implicated in lipid and lipoprotein-induced EndMT (Table 1) has helped to provide additional therapeutic targets for EndMT-associated atherosclerosis.

Imaging modalities of calcific atherosclerosis

Calcific atherosclerosis has been imaged for decades, both invasively and non-invasively in humans and in preclinical animal models. Conventional x-ray or CT are widely used to detect calcification in static arteries, and, when the motion of the heart is frozen by gated or ultrafast CT, both valvular and coronary calcification are readily detected and quantified. Clinical echocardiography is widely used to identify valvular calcification and its impact on valve function. Intravascular ultrasound is another modality for clinical detection of coronary calcification.

Two imaging techniques are the focus of recent articles on calcific vasculopathy: intravascular optical coherence tomography (OCT), which characterizes plaque composition

in a quantitative manner suitable for automated analysis, and positron emission tomography imaging with a sodium fluoride tracer, which has been used experimentally with either computed tomography or magnetic resonance imaging to detect coronary plaque features that are associated with vulnerability.

There are several advantages to intravascular OCT as an imaging technique for superficial (close to the lumen) vascular calcification. It has a higher resolution than external imaging techniques, and, unlike IVUS, can penetrate calcium deposits since it is based on interface boundaries. A recent study by Li and colleagues demonstrated use of automated imaging segmentation analysis (see section below on Radiomics) to quantify plaque characteristics [14]. One unusual circumstance where it may mis-categorize a lesion vulnerability is described in a case report of a patient with a calcium deposit that contained a residual necrotic lipid core undetected by OCT [21]. The patient had acute coronary syndrome and a calcium-encased lipid deposit, where rupture appears to have caused the event [21].

With respect to PET scanning, positron-labeled sodium fluoride is being used to identify cardiovascular calcification because the fluoride ion binds to the hydroxyapatite mineral. Bhattaru et al [22] recently found higher 18F-NaF uptake in non-cardiac arteries in both healthy controls and patients at-risk. Although they [22] interpret this as indicating that coronary calcification is a “late manifestation” of atherosclerosis, it is worth noting that they do not necessarily mean to imply that coronary calcification is initiated later than peripheral artery calcification. By “late manifestation,” they may mean that it progresses more slowly and becomes apparent later than peripheral artery calcification due to the threshold for detection. It is also possible that hemodynamic parameters specific to the vascular bed may account for slower progression.

Another recent report utilizes the combination of 18F-NaF PET with MRI. Advantages of this modality over PET-CT and invasive methods are that patients are exposed to less radiation, and they are not exposed to risks of catheter-based interventions needed for intravascular procedures. Wurster et al [23] compared PET/MRI with intravascular coronary OCT in patients with coronary atherosclerosis. Results showed that the PET/MRI scans provided useful information on plaque features associated with vulnerability. In regions of high PET uptake, defined as tissue-to-background ratio > 1.28, the MRI signal levels correlated significantly with the presence of calcified, thin-cap fibroatheroma.

As imaging modalities are widely used, limitations on their interpretation should be noted. Clinical studies using intravascular OCT or ultrasound have shown that areas of “spotty calcification” are associated with higher clinical risk. Such findings have led to the suggestion that microcalcifications pose increased clinical risk. However, the clinical studies demonstrating risk of “spotty calcification” define it as the presence of at least two calcium deposits 1,000 – 4,000 microns in size. Since microcalcifications are defined as 5 – 50 microns in size, two orders of magnitude smaller, “spotty calcification” consists of macrocalcifications.

Another important note is that, although 18F-Na PET has low resolution (~800 – 1,400 microns), it is relatively sensitive to identifying highly porous mineral deposits because

fluoride ions bind with high affinity to the surface of calcium phosphate mineral. As a result, an area or deposit with a high mineral surface area can produce a detectable PET signal even if the deposit is not sufficiently dense to produce > 130 HU of x-ray attenuation on the corresponding CT scanning. Thus, when a PET/CT scan shows an area that is PET-positive and CT-negative, it most likely indicates the presence of a high mineral surface area, which may consist of one or more highly porous (low density) deposits or microcalcifications. It is also important to consider the possibility that such a pair of signals represents a technical artifact, such as a partial-volume effect, spill-over from adjacent slices, or mis-registration between the two types of scans.

Radiomic features of calcific deposits

Clinical findings from the past several years raised the question of whether coronary calcium scores, which are a measure of content, may not be the only, or even the major, determinant of plaque rupture. Several studies have now shown that drugs of the lipid-lowering class, statins, which reduce cardiovascular risk, also promote CAC progression [24]. Similarly, elite athletes, who have, in general, a lower risk of coronary artery disease and higher life expectancy [25], have more rapid progression of atherosclerotic calcification [26]. Thus, other features of calcium deposits that are independent of CT-derived calcium scores, such as volume, shape, and texture may also influence their biomechanical properties and plaque stability. Such features may be quantitatively assessed using radiomics, the extraction of minable imaging features from radiological images. Quantification of such features by radiomics has been valuable in diagnosis and prognosis of tumors in oncology.

Li et al [27] attempted to find radiomic features from coronary CT angiography that would predict flow-limiting stenoses. They found numerous features that were related to significant stenoses, and some of these were used to develop a radiomics model to compare with the conventional model by sensitivity-specificity analysis (area under the receiver operating characteristic curve) [27]. In comparison with conventional analysis, which included a parameter related to spotty calcification, they found better performance of the radiomics model, but determined that it was not statistically significant [27]. One possibility is that inclusion of the highly predictive feature, “spotty calcification,” may have strengthened the conventional model so much that further improvement became difficult.

Radiomic analysis was used in a 9-year follow-up of the Offspring and Third Generation cohorts of the community-based Framingham Heart Study [28]. Features of intensity, shape, and texture were extracted for coronary calcium deposits from CT scans that were positive. Results showed that addition of a quantitative score based on features, textural differences and first-order intensity-statistics, to the conventional calcium scores from cardiac CT scans significantly improved the predictive value (area under the receiver-operating characteristic curve) with respect to major adverse cardiovascular events, and the radiomic score also correlated with the conventional calcium score [28].

In the field of cerebrovascular disease, Le et al. [29] used radiomic analysis of carotid CT angiograms to find features of calcific carotid deposits that predict cerebrovascular events (strokes and TIA’s). They identified robust radiomic features, Grey Level Dependent Matrix:

Dependence Variance, Grey Level Size Zone Matrix: Grey Level NonUniformity, and Grey Level Run Length Matrix: Long run High Grey Level Emphasis, that were more accurate for predicting events than the conventional calcium score, which (as shown in prior studies) failed to predict culprit lesions [29]. Although the biological, structural and/or histological correlates of each radiomics feature may not be apparent, the radiomic algorithms can be useful for prediction in any case, as with biomarkers of unknown function.

Summary

Overall, whether cardiovascular calcification is harmful or beneficial has been controversial. It is associated with dyslipidemia and increased cardiovascular risk, but, at the same time, coronary artery calcification is accelerated in patients otherwise expected to have lower risk, such as elite male endurance athletes and those using statin lipid-lowering therapy. Recent literature may address this conundrum through incorporation of more nuanced considerations such as Lp(a) threshold levels, pathological EndMT, and new imaging modalities and their quantitative analysis using artificial intelligence and radiomics.

- The association of Lp(a) with cardiovascular calcification is still unresolved, possibly because different studies use different thresholds for defining elevated levels.
- Lipid-lowering with statin therapy is associated with reduced cardiovascular risk, but statins are also associated with increased progression of cardiovascular calcification, which is associated with increased cardiovascular risk.
- This paradox suggests that CAC score alone may not be a sufficient determinant for risk assessment, and new imaging and analysis methods are being developed to explore other approaches, including deep learning segmentation and CT radiomics, to assess microarchitectures of cardiovascular calcification.
- Emerging evidence suggests that endothelial-mesenchymal transformation may underlie the relationship of lipids and lipoproteins with cardiovascular calcification.

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Table 1.

Candidate Non-Coding RNAs in Lipid-Induced EndMT

Non-Coding RNA	Experimental Model	Effects on EndMT	Pathways	Reference
Micro-RNA				
miR-150–5p	HUVEC, ox-LDL	Inhibitory	--	[20]
miR-200c-3p	HUVEC, ox-LDL	Stimulatory	Inhibition of SMAD7/YAP pathway	[18]
miR-29a-3p	HUVEC, ox-LDL	Stimulatory	--	[19]
LncRNA				
GAS5	HUVEC, ox-LDL	Stimulatory	Upregulates miR-29a-3p	[19]
ZFAS1	HUVEC, ox-LDL	Stimulatory	Upregulates Notch3, downregulates miR-150–5p	[20]

Abbreviations: EndMT, endothelial-to-mesenchymal transition; HUVEC, human umbilical vein endothelial cells; ox-LDL, oxidized low-density lipoproteins.