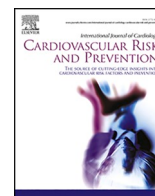




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Lipid oxidation in pathophysiology of atherosclerosis: Current understanding and therapeutic strategies

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ABSTRACT

A marked increase in the global prevalence of ischemic heart disease demands focused research for novel and more effective therapeutic strategies. At present, atherosclerotic cardiovascular disease (ACVD) is the leading cause of the global incidence of heart attacks and a major contributor to many peripheral cardiac diseases. Decades of research have unearthed the complex and multidimensional pathophysiology of ACVD encompassing oxidative stress, redox imbalance, lipid peroxidation, pro-inflammatory signaling, hyperglycemic stress and diabetes mellitus, chronic low-grade inflammation and aging, immune dysregulation, vascular dysfunction, loss of hemostasis, thrombosis, and fluid shear stress. However, the scientific basis of therapeutic interventions using conventional understandings of the disease mechanisms has been subject to renewed scrutiny with novel findings in recent years. This critical review attempts to revise the pathophysiological mechanisms of atherosclerosis using a recent body of literature, with a focus on lipid metabolism and associated cellular and biochemical processes. The comprehensive study encompasses different molecular perspectives in the development and progression of coronary atherosclerosis. The review also summarizes currently prescribed small molecule therapeutics in inflammation and ACVD, and overviews prospective management measures under development including peptides and microRNA therapeutics. The study provides updated insights into the current knowledge of coronary atherosclerosis, and highlights the need for effective prevention, management and development of novel intervention approaches to overcome this chronic epidemic.

1. Introduction

Atherosclerosis is a multifactorial non-communicable coronary disorder, which is characterized by the presence of fibro-fatty lesions or plaque formation in the arterial vasculature. Subsequent complications of the disorder can lead to myocardial infarction and ischemic damage. At present, atherosclerotic cardiovascular disease (ACVD) is the leading cause of the global incidence of heart attacks and is a major contributor to many peripheral vascular diseases [1–4].

The majority of previous studies reported total lipid consumption and extrinsic factors such as smoking as causes of atherosclerotic plaque development. However, decades of research have unearthed the complex and multidimensional pathophysiology of ACVD, and revealed other causative factors including oxidative stress, redox imbalance, lipid peroxidation, pro-inflammatory signaling, hyperglycemic stress and

diabetes mellitus (DM), chronic low-grade inflammation and aging, immune dysregulation, vascular dysfunction, loss of hemostasis, thrombosis, and fluid shear stress [2,3,5–10].

Globally $\geq 75\%$ of deaths due to ischemic heart diseases and strokes occur in low-income and developing countries [3]. Moreover, genetic and epigenetic factors continue to exponentially increase the number of ACVD patients not only in developed countries, but also more rapidly in underdeveloped areas with predisposed populations [7]. In particular, Asian and South-Asian males are now considered the most vulnerable group to acquire obesity, hypercholesterolemia and ACVD [7]. In recent times, CVD and atherogenic complications have transitioned from being a “Western Country Concern” to a worldwide epidemic. However, healthcare, and therapeutic interventions remain a significant financial burden for developing economies with vulnerable populations. Moreover, the scientific basis of therapeutic interventions using conventional

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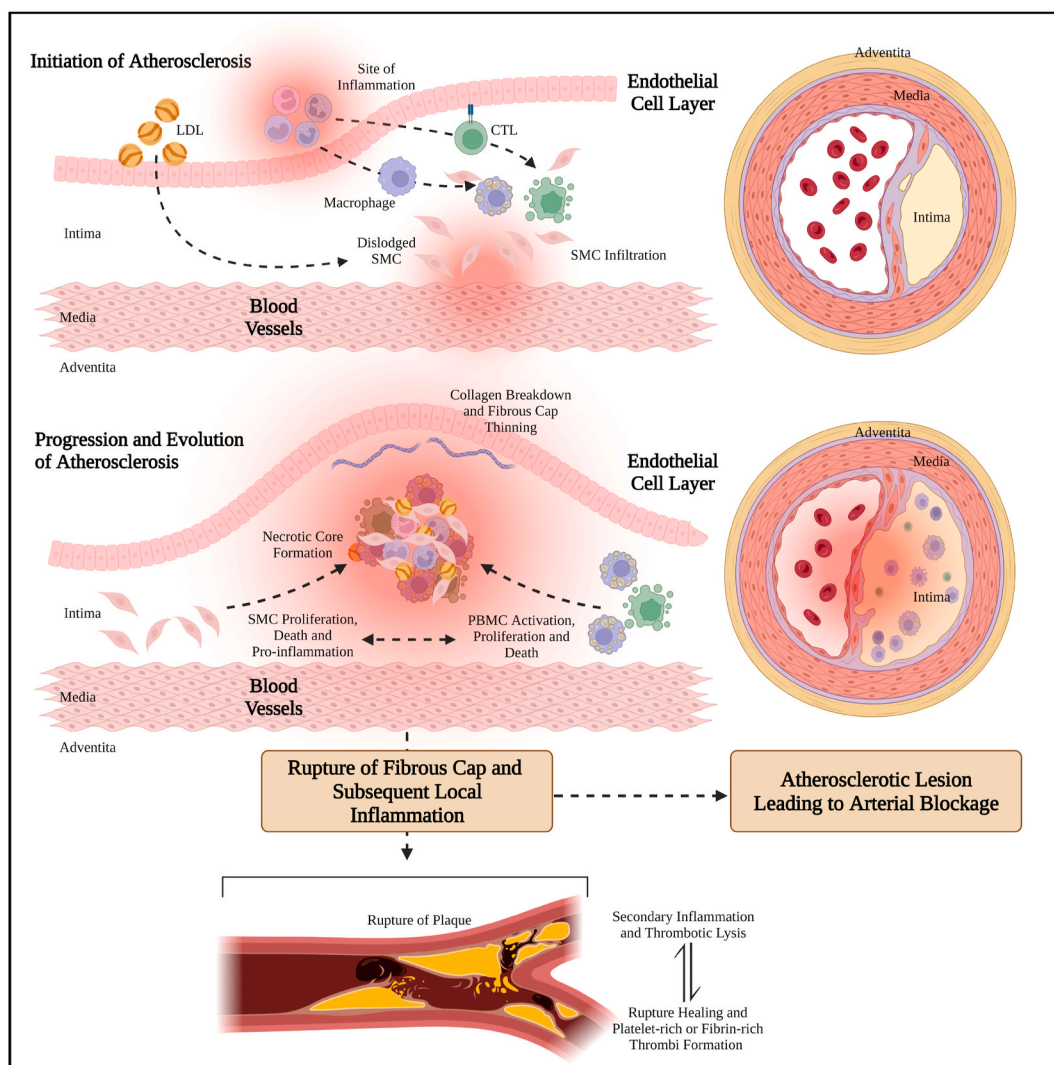


Fig. 1. Genesis, Progression and Evolution of Arterial Atherosclerotic Plaque. Early stages of lesion formation involves LDL infiltration and accumulation on the arterial walls, followed by multiple steps of oxidative modification and inflammatory responses in the arterial microenvironment. Mononuclear cells (PBMC) including macrophages and cytotoxic T-lymphocytes (CTL) circulating in the bloodstream respond to the chemoattractant stimuli by adhering and migrating into the intima. Subsequent mediator release by PBMC in the intima promotes smooth muscle cell (SMC) migration and initiates atheroma growth. Following genesis, the atheroma undergoes complex modifications encompassing proliferation, pro-inflammatory signaling, cell death of SMC and PBMC, and activated platelets in the vicinity. Along with lipid particles, these cellular masses accumulate to form a necrotic fibro-fatty lesion core, which leads to further inflammation and obstruction of arterial flow. Once the lesion core approaches critical mass, the fibrotic cap of the core ruptures and initiates one of a set of possible pathways. The rupture can be healed with fibrinolytic (red) or platelet-rich (white) thrombus formation with the help of tissue growth factors that can further exacerbate arterial blockage and/or the rupture can cause a severe inflammatory response in thrombosis sites leading to ischemic damage and further complications. Figure generated using BioRender. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

understandings of the disease mechanisms has been subject to renewed scrutiny with novel findings over recent years. Parallel to the ever-evolving landscape of atherosclerosis research, the need for new strategies for therapeutic interventions have emerged. However, atheroprotective and by extension, cardioprotective drugs that are conventionally based on antioxidant and anti-inflammatory small molecules have not seen much clinical success in the last few decades [11–13]. This short review attempts to revise the pathophysiological mechanisms of atherosclerosis using a body of recent literature spanning 2012–2022 with a focus on lipid metabolism, and summarizes current and upcoming

therapeutic strategies to address them¹.

2. Pathophysiological mechanisms of atherosclerosis and ACVD

The term atherosclerosis is derived from the Greek word for ‘gruel’ or ‘porridge’, reflecting the yellowish appearance of the lipid material found in the core of a typical atherosclerotic plaque or ‘atheroma’. In the simplest sense, atherosclerotic lesions or plaques require low density lipoprotein (LDL), which carries cholesterol through the blood. Since this is an integral physiological element in almost all living organisms,

¹ Reviews of previous development of experimental basis and current knowledge were interrogated where appropriate. Literature which are written in non-English language, not indexed in PubMed, and that did not include full text through academic licensing were excluded.

the pathophysiological triggering of atherosclerotic plaque formation should logically require a set of stimuli – intrinsic or extrinsic – for the initiation of arterial blockage under disease-related conditions. Indeed, some of these stimuli have been identified as genetic and epigenetic risk factors for atherosclerosis and its thrombotic complications – which include genetic predisposition and congenital defects, hypertension, smoking, alcohol intake, hyperlipidemia, aging, co-occurrence of diabetes mellitus (DM), and physical inactivity [2,3,7,14]. At the cellular and molecular level, these risk factors manifest in the form of immune dysfunction, oxidative stress, and inflammatory surges leading to atheroma development [3].

Atheromata or atherosclerotic plaques formed from fibro-fatty cores in coronary arteries subsequently calcify and develop into more advanced lesions, which impede blood flow in the arterial lumen, often resulting in ischemic damage. In a secondary mechanism, atherosclerotic plaques that do not block blood flow provoke inflammatory responses and accumulate atherothrombotic complexes, which in turn, obstructs arterial function [2,3]. Both of these main routes of arterial blockage present a diverse but fatal set of downstream impacts in different arterial locations, thereby leading to ischemic stroke, myocardial infarction, cardiomyopathy, heart failure, vascular aneurysm, ulceration, cytokine storms, and inflammaging² [3,4].

2.1. Timeline of atherogenesis and atherothrombosis: inflammation driven atheroma development in the arterial microenvironment

Atherosclerotic plaque/lesion formation and maturation in itself is a broad area of active research; nevertheless, a short overview of the initiation, progression and maturation of atheroma is presented herewith.

Libby described the atherosclerosis pathophysiology to occur in three distinct phases: a) Initiation, b) Progression and c) Complications [3]. The initial phase of plaque genesis revolves around cholesterol and spheroidal circulatory LDL particles accumulating in the arterial inner walls or intima; this is followed by a diverse mechanism of oxidative conversion of lipids (including metal ion catalysis), and free radical generation [2,3,7]. Lipid peroxides or oxidized LDL (ox-LDL) products subsequently provoke pro-inflammatory mechanisms involving immune cell mobilization and local cytokine storms. Accordingly, Nf-κB and CrP are actively used as inflammatory biomarkers to assess risk of ACVD and ischemic damage. Another major factor of atherosclerotic initiation can be correlated with glucose abundance, triglycerides (TG) and TG-rich lipoproteins in circulation [7]. Additionally, subsequent glycolytic pathway activation promotes inflammatory chemo-irritant stimulation [7]. Thus, a combination of hyperglycemia, inflammation and adiposity supports the causal links among DM,³ hypertension and ACVD events – the underlying mechanisms at work however, are yet to be fully elucidated.

Interactions of these atherogenic factors with the endothelial lining facilitates endothelial dysfunction and the unregulated migration of peripheral blood mononuclear cells (PBMC) into the vessel core/intima. This in turn disrupts arterial blood flow and initiates smooth muscle cell (SMC) infiltration and early fibrotic lesion formation [7,15,16].

Following the initial lesion formation, SMCs produce a plethora of extracellular matrix (ECM) mediators including elastin, collagen and advanced glycation end products (AGE) which collectively facilitate the

thickening of the intimal layer and disruption of arterial hemodynamic activities. SMCs and macrophages also actively proliferate in the disturbed microenvironment and die off necrotically – leading to further strengthening of the lesion core and creating a pro-inflammatory, mediator-rich, advanced atherosclerotic arterial environment. This in turn, promotes collagen degradation by T-cell and macrophages, which produce IL-1,6, 10, IFN-γ, and other inflammatory mediators, causing fibrous cap thinning, calcification, local degeneration, and eventual rupturing [3,8,15]. Alternatively, tissue growth factors (e.g. TGF-β) that are released to alleviate inflammation consequently result in dysregulated lesion healing and occlusion of ruptured caps – which in turn aggravates arterial blockage and further thrombosis. In either route, the timeline climaxes with the acute thrombosis of coronary arteries, which causes blockages and myocardial infarctions. A simplified adaptation of the process is illustrated in Fig. 1.

For a more elaborate depiction of the multifactorial and ever-evolving field of atherosclerosis research, readers are redirected to more elaborate reviews and updates by experts in the field [2,3,7,8,15,17]. In this study, a more focused outlook on the major biomolecular compartments suggests a set of interconnected metabolic and physiological processes that drive the genesis and evolution of atherosclerosis as a pathology. These compartments can be largely divided into two major areas: a) upstream processes leading to atherosclerotic lesion formation (direct and indirectly associated with lipid oxidation), and b) downstream magnification of atheroma and thrombosis (immune infiltration, cytokine storms, thrombus formation). Of course, other peripheral processes including hypertension, hyperproliferation, and genetic predispositions are involved [3–5,18,19], but these characteristics exceed the scope of this study. Note that these peripheral processes are indirectly modulated by the major processes that will be discussed in the following sections nonetheless.

2.2. Lipid peroxidation and unsaturated fatty acid metabolic products

LDL/LDL-C (LDL-Cholesterol) mediated initiation of atherosclerosis is the fundamental basis of arterial atheroma development. This is majorly driven by the oxidation of LDL-C – particularly unsaturated fatty acids and their subsequent oxidized/hydroxidized products [2,4,20,21]. In context to ACVD – the most important LDL undeniably includes arachidonic acid (ArA), a type of ω-6 polyunsaturated fatty acid (PUFA), which is either present as an endogenous phospholipid in biological membranes or through dietary intake of linoleic acid (LnA) or animal proteins [14,22].

Unsaturated fatty acid oxidation products or free radical lipid peroxides accumulating in LDL particles drive the production of pro-inflammatory aldehydes such as malondialdehyde (MDA) – which activate the migration and foaming of macrophages⁴ [4]. These MDA products are mostly converted from dietary and intrinsic ArA, LnA, and docosahexaenoic acid (DHA) [4,22]. Aldehydes and advanced peroxides promote further oxidative stress and ROS stress, chemotaxis of inflammatory cells, adhesion and migration of cells into blood vessels, cellular proliferation of SMCs and PBMCs, stimulation of IL, IFN, TNF, NF-κB and TGF, and eventual intimal vasoconstriction [3,4,20,21]. Peroxidation products also inhibit anti-inflammatory prostacyclins (PCs) and nitric oxides (NOs), thereby resulting in the aggravation of platelet activation at the site of inflammation [4].

These peroxidation reactions of ArA are initiated by cleavage by phospholipase A2 (iPLA2/sPLA2/cPLA2) and subsequently carried out by the action of oxygenase enzymes – namely cyclooxygenases (COX/PTGS), lipoxygenases (LOX) and cytochrome P450 epoxygenases/

² Cytokine storms refer to hyperactivation of the immune system leading to acute production of inflammatory cytokines, which impacts tissue damage and organ failure. Inflammaging (Inflammation + Aging) refers to chronic production of low-grade inflammatory stress signals that contribute to aging and aging-associated diseases.

³ The current study is focused on the dietary and/or therapeutic management of ACVD-genetic factors are less pronounced in context. Hence, DM mentioned throughout the manuscript primarily includes type II DM (T2DM).

⁴ Foam cells are circulating macrophages that localize to fatty deposits on blood vessel walls that uptake LDLs/ox-LDLs and obtain a foamy appearance. Foam cells are a rich source of proinflammatory stimuli and chemoattractants, and act as a central factor throughout ACVD development and progression.

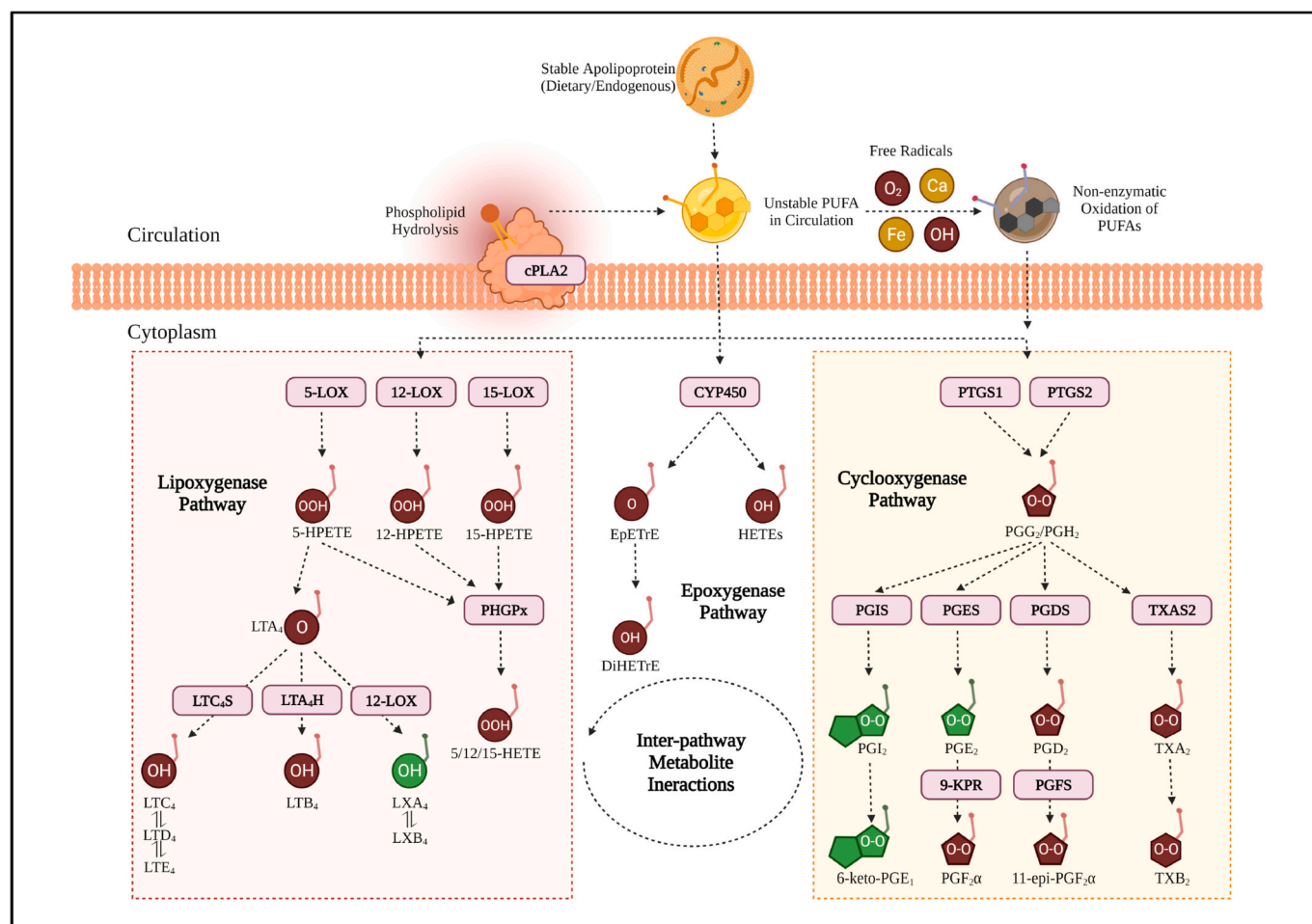


Fig. 2. Summarized Representation of Major Polyunsaturated Fatty Acid (PUFA) Peroxidation Pathways. PUFAs may be cleaved from phospholipids by phospholipase A2 (cPLA2) or imported through apolipoprotein breakdown, which leads to lipid radical (LOO^*) formation. Unstable LOO^* in circulation may undergo further oxidation with the help of transition metals and free radicals to generate oxides such as malonaldehyde and 4-hydroxynonenal. PUFAs trafficked across the cell membrane enter into the cytosol and are subsequently degraded by lipoxygenase (LOX), epoxygenase (CYP450) or cyclooxygenase (PTGS) pathways. Each pathway carries out a set of diverse biochemical conversion of PUFAs to yield mainly pro-inflammatory (red) and some anti-inflammatory (green) lipoxide products. These molecular cascades are intricately intertwined among one another and have multiple checkpoints where they undergo feedback loops and intra-/inter-pathway crosstalk. Figure generated using BioRender. 9-KPR – PGE2 9-ketoreductase; HETE – hydroxyeicosatetraenoic acid; HPETE – hydroperoxyeicosatetraenoic acid; LTX_4 – leukotriene X4; LTX_4H/S – LTX_4 hydrolase/synthase; PGX – prostaglandin X; PGXS – PGX synthase; PHGPx – phospholipid hydroperoxide glutathione peroxidase; TXA_4/B_4 – thromboxane A_4/B_4 ; TXAS2 – TXA_4 synthase 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

monooxygenases (CYP450). Enzymatic degradation of unsaturated fatty acids such as ArA and LnA gives rise to a plethora of bioactive metabolites including eicosanoids and docosanoids. Among these, eicosanoids, particularly eicosapentaenoic acid (EPA) are of much interest within the context of inflammation and ACVD [14].

Cyclooxygenase-1/2 (PTGS-1/2) catalyzes the conversion of eicosanoids to prostaglandins (PGs), thromboxanes (TXs), and PCs (cumulatively known as prostanoids). PTGS-1/2 and subsequent downstream enzymes have been studied extensively as druggable targets in inflammation, immune modulation, nociception, and platelet aggregation [14, 15]. Particularly, downstream induction of $PGF_2\alpha$ and TXs mediates the activation of platelets and sets into motion the progression of atherosclerotic plaques in the arterial lumen [14]. Likewise, lipoxygenase enzymes (15-LOX/5-LOX/12-LOX) oxidize PUFAs to a wide array of unstable hydroperoxides and hydroxy acids including hydroxyeicosatetraenoic acids (HETEs), hydroperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosapentaenoic acids (HEPEs), hydroxyoctadecadienoic acids (HPODEs), 4-hydroxynonenal (4-HNE), LXs, and LTs. These metabolites have been reported to have primary roles in local inflammatory signaling, vascular permeation, epithelial dysfunction,

and vasoconstriction [14,15,21]. CYP proteins, on the other hand, have a diverse set of roles and produce different lipid epoxides and hydrolysates. The direct incorporation of these metabolites into the atherosclerotic disease network is convoluted and contradictory [15]. Regardless, PTGSs and LOXs remain the primary enzymes of interest in anti-inflammatory and atheroprotective therapeutic development.

However, PTGS and arachidonate LOXs themselves are subject to indirect modulation by upstream mechanisms of serum lipid and LDL-C level regulation. One of these enzymes strongly linked to atherogenesis via enzymatic activity in SMCs, PMBCs and ECs is Proprotein Convertase Subtilisin/Kexin 9 (PCSK9); PCSK9 acts as a serine protease that targets and degrades LDL-receptor (LDLR) proteins – modulating circulating LDL-C levels [23,24]. One of the most important LDLRs, Lectin-like oxidized LDLR-1 (LOX1) constructs the Ox-LDL/LOX1/PCSK9 axis, which is another key player in LDL-C level regulation [23,25,26]. However, while important to cholesterol metabolism, both PCSK9 and LOX1 are yet to be fully understood in both endpoint effect and direct underlying molecular mechanisms in atherosclerotic plaque development. Recent studies in both murine models and human cell lines have found ambiguous and conflicting evidence as to whether these are

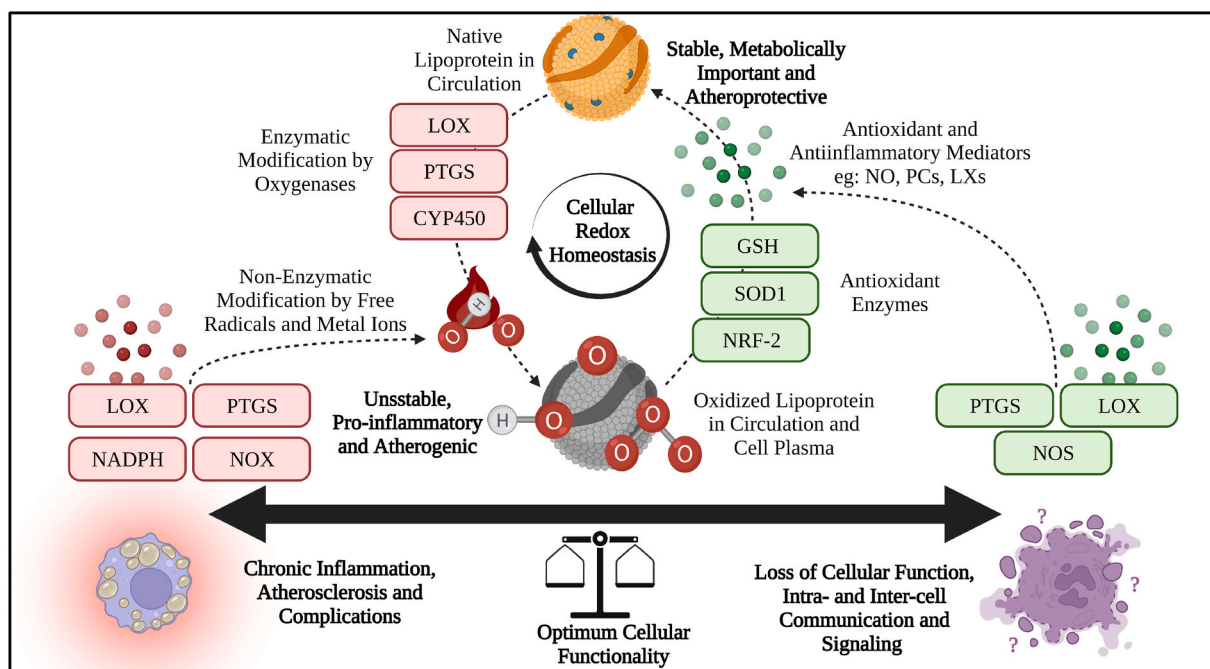


Fig. 3. Oxidative Stress and Cellular Redox Homeostasis in Inflammation and Atherosclerosis [Modified from Ref. [16]]. Intrinsic or dietary phospholipids exist in circulation as stable antioxidant-conjugant lipoproteins which are targeted and oxidized through a complex set of enzymatic and non-enzymatic machinery that propagate and manifest as unstable lipoxides in serum and cellular plasma. These oxidized products have roles in cellular metabolism and signaling but at high concentrations lead to atherogenic complications. Antagonistic mechanisms in the form of antioxidation and stabilization also exists in cells for maintaining redox balance. However, in disease conditions like ACVD, the redox balance is shifted towards pro-inflammatory processes that directly or indirectly suppress antioxidation and manifest more systemic free radicals. Figure generated using BioRender. GSH – reduced glutathione; LXs – lipoxins; NOS – nitric oxide synthase; NOX – NAPDH oxidase; NRF-2 – nuclear factor erythroid-derived 2-like 2; PC – prostacyclin; SOD1 – superoxide dismutase 1.

significantly changed in expression and interaction during atherosclerosis associated cellular developments; this notion is further convoluted by evidence of pro-atheroprotective endpoints of downstream PCSK9 activity [24,26,27]. Ragusa et al. argues that PCSK9 and LOX1 roles in cardiovascular disorders may be beyond LDL-C regulation in serum; however, the extent and degree of these roles are still theoretical if any in context to lipid oxidation mediated atherosclerosis [23]. Nevertheless, the self-looping PCSK9/LOX1 axis ultimately connects to NAPDH oxidase (NOX), Nf- κ B and IL activation – leading to local inflammation and cytokine recruitment, which have been discussed later in this manuscript. For more details on upstream regulation of circulating LDL prior to oxidation mechanisms, we highly recommend some recent review articles on this active area of research [23–27].

In addition to enzymatic peroxidation, PUFAs in circulation can undergo non-enzymatic oxidation with the help of free ROS and metal ions, which results in the production of lipid peroxy free radicals (LOO^{*}). In contrast to stable PUFAs which generally exist as lipoproteins in conjunction with antioxidants (e.g.: Apolipoprotein a/b), LOO^{*}s are highly unstable and cross biological barriers, which leads to further propagation of oxidant radicals into the cellular domain, and consequently, activation of the stress response and inflammation pathways [15,20,21].

LOO^{*}s also interact with non-PUFA esters (e.g.: cholesteryl arachidonate/linoleate etc.) which are subsequently oxidized by LOXs, transition metals, heme, and hemoglobin to produce oxysterols. Oxysterols have a significant role in macrophage foaming, apoptosis, ROS propagation, loss of cellular communication, and atherogenesis. Phospholipids are another source of LOO^{*} generation that undergo LOX-mediated/non-enzymatic oxidation to produce 4-HNE products. 4-HNE produces acute cellular toxicity, DNA damage, proliferation, cell signaling alterations, apoptosis, and protein dysfunction which cumulatively leads to organ damage and atherosclerotic lesion exacerbations [15]. MDA is another important product of the ArA lipoxidation

pathway – it is established as a universal marker of oxidative stress, and is found in damaged and dying cells in SMC and endothelial cell linings [15,20]. A short summary of lipoxidation processes has been illustrated in Fig. 2. To be noted here, PUFAs and peroxidation products such as LnA and eicosanoids are necessary for cellular function and exert beneficial effects in signal transduction and cellular communication at low concentrations [3,15]. However, at excessive levels, the beneficial effects of these lipoxidation products are trumped by hostile reactions. While the specific level of oxidized products for such alterations is yet to be elucidated – a clear incorporation of reactive species balance and oxidative stress response is implied.

2.3. Oxidative stress, redox homeostasis, and ferroptosis

ROS act as signaling molecules in intracellular and intercellular signaling pathways and affect transcriptional factors that are involved in cellular survival and function. At the system level, they actively participate in blood pressure regulation, immune and cognitive regulation, vascular contractility, hemostasis,⁵ angiogenesis, and damage responsive platelet activation [14,15]. Hence, a fine balance is required between a necessary cellular ROS level, and harmful accumulation of free radicals which can lead to dysregulation of redox homeostasis as depicted in Fig. 3.

NOXs are the primary source of ROS in biological systems, thereby forming H₂O₂. NOX enzyme implications in ACVD are well established – with LOO^{*}s and LOXs being major stimulants of NOXs [10,14,15]. NOXs are highly expressed in cardiomyocytes and act as a potent source of H₂O₂ generation as well as PBMC recruitment in atherosclerotic

⁵ Hemostasis refers to a complex intrinsic repair mechanism in response to vascular damage to stop bleeding that involves coagulation, hemolysis, and fibrinolysis, among other processes.

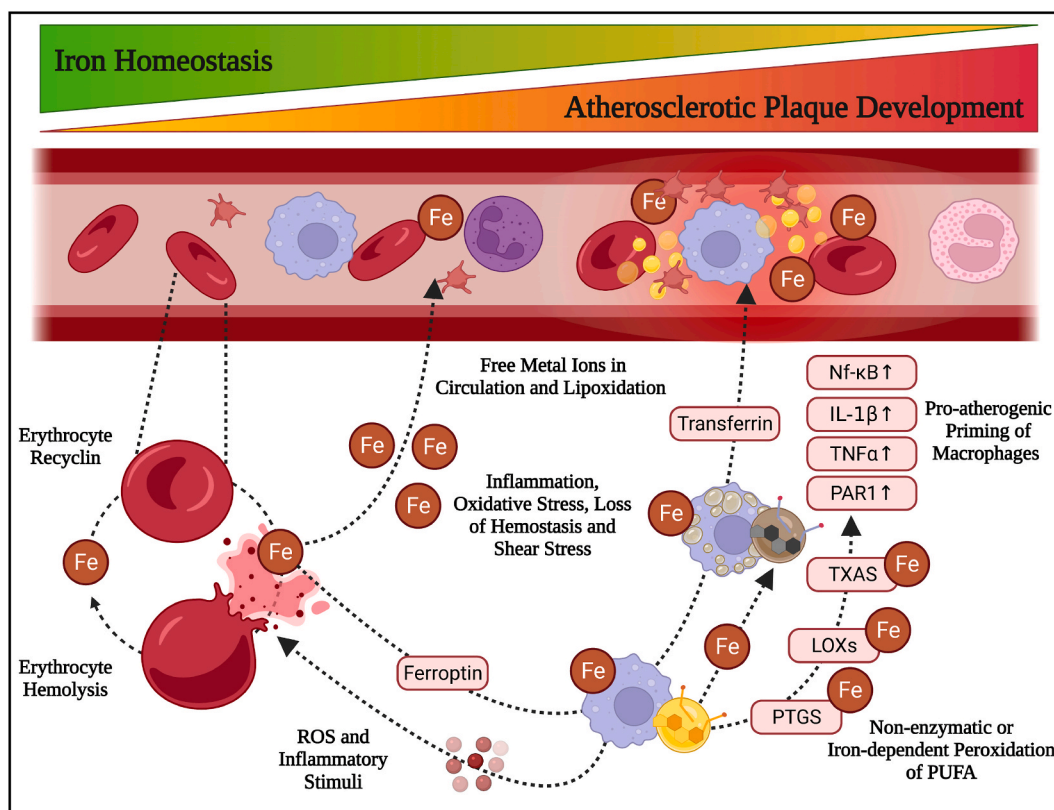


Fig. 4. Role of Iron Metabolism, Ferroptosis and Metal Ion Oxidants in Atheroma Development. Routine recycling of erythrocytes and hemolytic stress induced by ROS and extracellular signaling leads to release of free iron ions into circulation, which are either taken up by PMBCs, or act as non-enzymatic oxidants of LDL-C/PUFAs. Macrophagic iron is also essential in catalytic activation of the iron-dependent peroxidation enzymes, LOXs, PTGSs, and thromboxane A synthase (TXAS), and subsequent downstream enzymes including platelet activation by protease activated receptor 1 (PAR-1), resulting in pro-atherogenic pathway activation and atheroma progression. Figure generated using BioRender.

environments [2,14,15]. Excessive expression of NOX due to an abundance of LOO*s results in redox imbalance in immune cells; NOX products also function to limit antioxidant NO synthesis and promote monocyte adhesion to the vascular lumen [15].

Other sources of cellular ROS consist of LOXs and PTGSs, as discussed in the previous section, along with mitochondrial enzymes involved in energy metabolism, xanthine oxidases, myeloperoxidases, and endothelial nitric oxide synthases [2,10,15]. These enzymes mainly produce O_2^* radicals that are again, stabilized to H_2O_2 . H_2O_2 by itself is not reactive enough to cause notable cellular damage, but it can form extremely active OH^* radicals through the interaction with transition metal ions such as Fe^{3+} [2,15]. H_2O_2 increases the expression of NF- κ B-MAPK-Akt and MAPK-ERK-p38 signaling pathways, thereby contributing to cellular damage and inflammation in SMC and PBMC [2,15]; T[21].

The cellular microenvironment also presents antioxidant components in the form of antioxidant enzymes such as superoxide dismutase 1 (SOD1), nuclear factor erythroid-derived 2-like 2 (NRF-2) and small molecule mediators like PCs, NOs and LXs [2,4,15] that attempt to restore redox balance. However, with exogenous/systemic sources of free radicals, these failsafe mechanisms are easily overrun and/or depressed by LOX/PTGS metabolic products along with transition metal oxidizers [10].

One of these metal ions that are involved in oxidation is iron/Fe. Numerous studies have been conducted to investigate the relationship between iron and ACVD, as reviewed elsewhere [6,20,28]. Iron in circulation is sourced primarily from the inflammatory recycling of erythrocytes by macrophages along with dietary intake. Ferroptin and transferrin regulate the enzymatic transport of Fe^{3+} from macrophages to serum, and then to vascular and endothelial cells [28]. During

erythrocyte hemolysis, free Fe^{3+} in circulation acts as a strong redox agent, which results in the subsequent oxidation of LDL. Ox-LDL produced by non-enzymatic means activates the NF- κ B and IL-1 β pathways, which results in systemic inflammation, followed by macrophage foaming, necrosis, and plaque advancement [20,28]. This alternate mode of non-enzymatic necrotic process initiation by serum iron-lipid interactions or ferroptosis, adds an extra dimension to lipoxidation and ACVD regulation by oxidative stress. Ferroptosis is the concept of accumulation of lipid peroxide mediated damage and inflammatory signaling, which trigger an iron/ferrous dependent programmed cellular death or apoptosis leading to further inflammation in the microenvironment [28,29].

While the direct action of ferroptosis is still underexplored in atherosclerotic research, the erythrocyte inflammation-mediated release of oxidizing iron, macrophagic transportation, and pro-atherogenic pathway activation provide an important avenue for atheroprotective strategies. Furthermore, iron accumulated in cells as heme and non-heme act as essential cofactors for the catalytic reaction of PTGS and LOX-mediated lipoxidation pathways, which leads to activation of protease-activated receptor-1 (PAR1), IL, IFN, and TNF- α [14,28,30]. A simplified scheme of iron metabolism and subsequent mechanisms of ACVD exacerbation is depicted in Fig. 4.

To complete the cycle, excessive production of H_2O_2 and O_2^* coupled with metal ions in arteries and cardiac tissue environments serve as a feedback mechanism to oxidize lipoproteins and LDL-C, which causes further oxidative stress generation cycles and a gradual decline of cellular redox homeostatic balance [20]. These theories of free radical mediated generation of atherosclerosis have prompted the development of antioxidant and metal ion-chelating, small molecule drugs, which are further discussed in section 2.2. While this review focuses on ACVD and

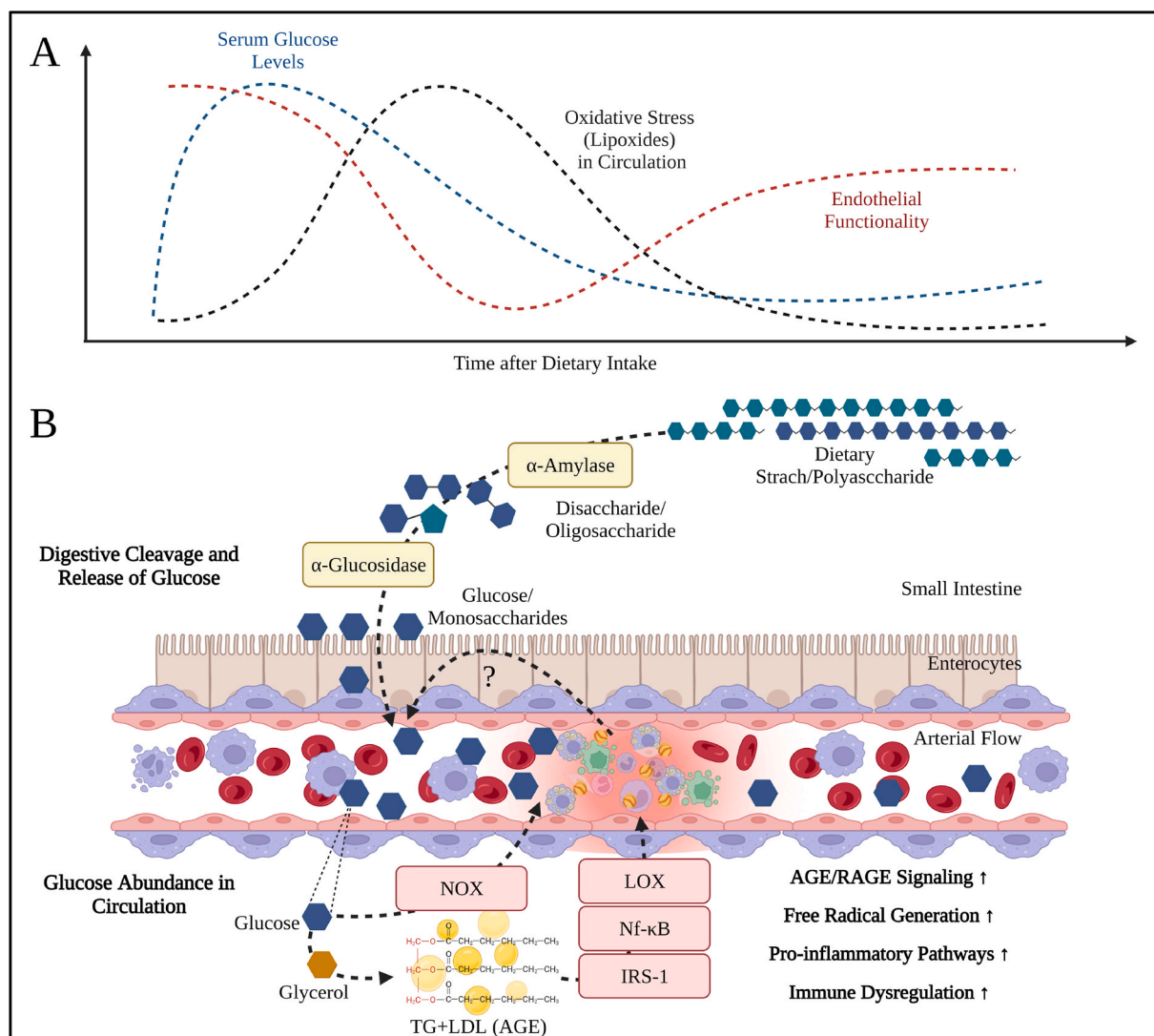


Fig. 5. Role of Glucose Metabolism in ACVD Development. A) Representative time course of changes in plasma glucose levels and its effect on inflammatory trends show effects of glucose uptake on lipoxidation and endothelial dysfunction in ACVD [Adapted [6]]; B) Enzymatic degradation of polysaccharides by digestive enzymes leads to increased glucose abundance and subsequent direct/indirect ROS stress in progressing atheroma. Multiple mechanisms including NADPH oxidase (NOX), insulin receptor substrate-1 (IRS-1), and glycated lipids (AGE) contribute to inflammatory and proliferative pathway activation. However, the causal effect of inflammatory and immune response leading to increased glucose uptake and energy homeostatic dysregulation is yet to be fully elucidated. Figure generated using [BioRender](#).

lipoxidation-relevant modes of oxidative damage, other mechanisms of oxidation have been excellently overviewed elsewhere [10].

Finally, another major source of ROS in biological systems obviously arises from the hyperactivation of mitochondrial energy metabolism, which results in the loss of energy homeostasis and subsequent generation of free radicals [10]. However, from a clinical management perspective, the area is often overlooked regarding the established correlations between ACVD and overnutritional metabolic stress, particularly in the case of obesity and insulin resistance. This presents a paradoxically well-explored and elaborately studied area in molecular biology – yet one that is far from being primed for therapeutic exploitation in the ACVD-DM intersection.

2.4. Hyperglycemia and AGE-RAGE signaling as a gateway to atherogenesis

Sonnweber terms the inflammation-nutrition interaction as a “double-edged sword” where metabolic stress induces inflammation and inflammation disrupts metabolic homeostasis in different tissues [14],

leading to a feedback cycle in ACVD. This interjecting area is as convoluted as the underlying metabolic system itself with contradicting evidence that hinders the proper therapeutic targeting of a multi-pathway approach to management of ACVD, hyperglycemia/DM, obesity, and associated disorders therewith.

Nevertheless, while direct molecular links to hyperglycemia are yet to be fully elucidated – correlating factors including wound healing, TG levels, fat metabolism, and immune function are all similarly affected in DM and ACVD. One possible mechanism as discussed by Sonnweber reflects on the induction of PMBC adhesion to the vasculature, and metabolism-induced ROS generation due to excessive glucose levels [14]. This of course stems from the numerous common pathways that are shared between glucose and lipid metabolism, which lead to common downstream associations. Particularly, the generation of inflammatory glycated lipids or AGE components has garnered attention in ACVD and DM pathogenesis. Interestingly, even in non-DM murine studies – hyperglycemic stress induces PMBC dysregulation and lipoxidation product abundance in blood, as reviewed previously [31]. Furthermore, a recent endeavor by Edgar (reported By Lim), exploring

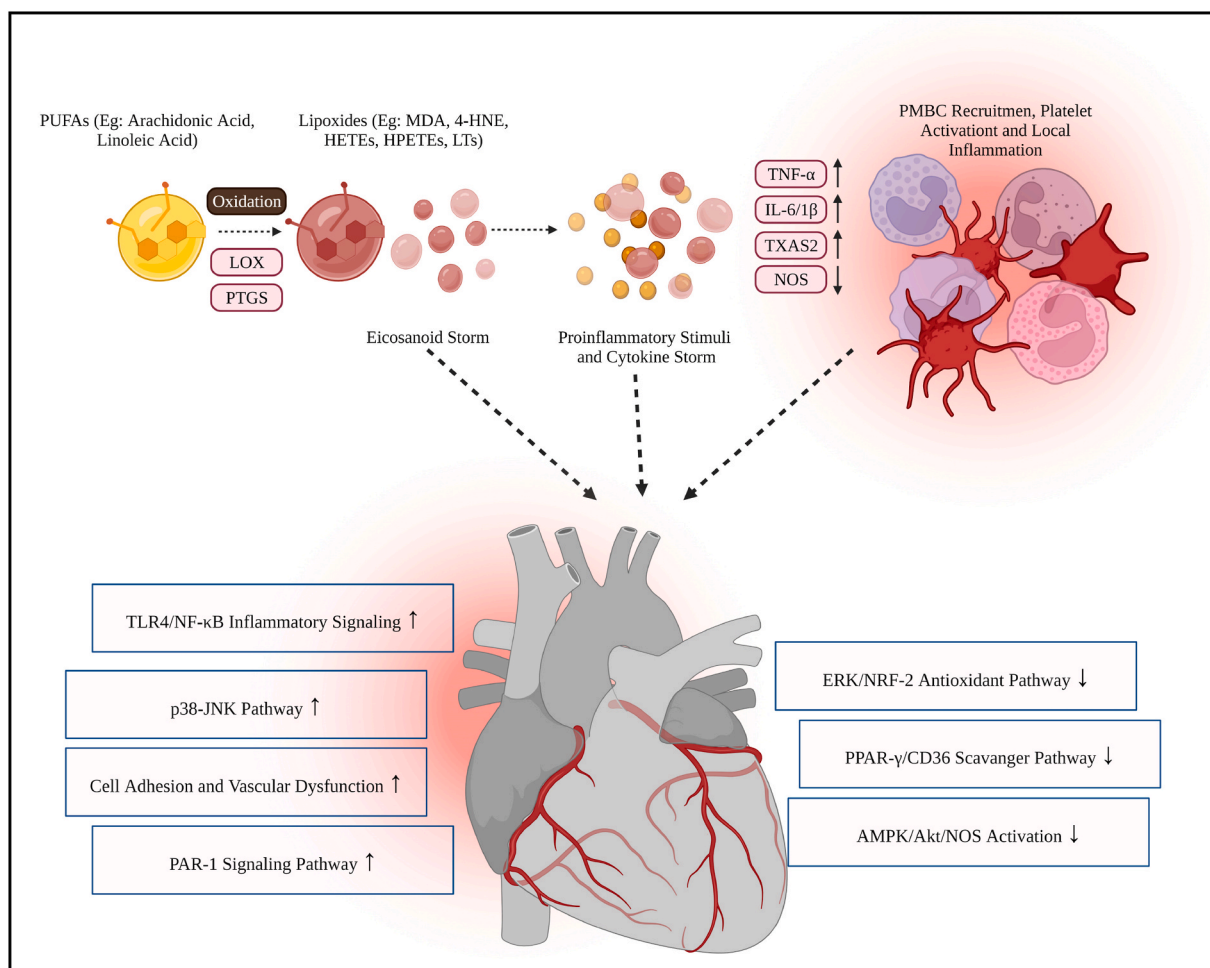


Fig. 6. Interplay Among Peroxidation Products, Inflammation, and Immune Cell Recruitment in ACVD. Oxidized mediators from PUFA metabolic pathways manifest as an accumulation of local eicosanoid outburst or “eicosanoid storms”, which further incorporate chemoattractant molecules. As a result, inflammatory mediators form the signaling basis for recruiting circulating immune cells, which in turn, propagates inflammatory signaling and hyperactivation of stress-responsive pathways in cardiac/arterial SMCs and endothelial cells. The combined action of active monocytes, inflammatory mediators, and signaling molecules exacerbates redox dysbalance and proliferative bias in atheroma microenvironments. Figure generated using BioRender. JNK - jun N-terminal kinase; NRF-2 – nuclear factor erythroid-derived 2-like 2; PAR-1 – protease activated receptor-1; PPAR-γ - peroxisome proliferator activated receptor gamma; TLR4 – toll-like receptor 4.

the glucose-primed immune systems – shows how extracellular glucose abundance not only induces a pro-atherosclerotic phenotype in cardiac patients, but trains macrophages to continue to maintain atherogenic excretions upon lowering blood glucose [32,33]. The study provides new insights into an overlapping realm of overnutritional stress, oxidative stress, lipid peroxidation, and the dysfunctional immune system in ACVD.

At the lipid peroxidation end, ArA itself is a driver of insulin function in β -cells whereas peroxides generated from ArA, particularly LTs, HETEs, HPETEs, and PGs contribute to fostering insulin resistance via induction of TNF- α , IL-6, and ROS-mediated damage to cellular integrity and communication [14,22].

It reviewed the entangled realm of hyperlipidemia, hyperglycemia, ROS, and arterial atherosclerosis, and found that therapies that stabilize glucose levels also reduce oxidative stress in patients with DM. They also posited a causal relationship between glucose uptake in blood, and the subsequent increase in circulating inflammatory lipoxidation products which imparts endothelial dysfunction in ACVD [6]. Hyperglycemia and DM also impact lipid profiles in patients; particularly disrupting LDL/HDL balance, generating AGEs, and activating the AGE-RAGE pathway [6]. Insulin resistance provoked by hyperglycemia and obesity also converges with inflammation and immune modulation in the insulin receptor substrate 1 (IRS-1)/MAPK/Akt/PI3K and

Nf-Kb/IL-1 β /TNF- α /IFN- γ pathways [34]. On the other hand, inhibition of glucose-releasing digestive enzymes α -amylase and α -glucosidase, was significantly correlated with reduced hyperlipidemia and atherogenic peroxides [6,31]. An overview of the current understanding of the glucose abundance impact on ACVD is illustrated in Fig. 5.

2.5. Atheroinflammation, immune dysregulation, and stress response

Exploring the intersection of metabolism, immunity, and ACVD, current ventures into atheroprotective treatment are deeply integrated with immune modulation and systemic inflammation management. While CrPs are a well-established marker of cardiac damage, the role of CrP as a pro-inflammatory mediator of atherogenesis is still largely debated [2,4,7]. Nevertheless, there is a strong correlation among CrP with other inflammatory players like IL and IFN derived from T-cells and macrophages [4].

This links the humoral immune system and inflammation with atherosclerosis and coronary dysfunction. While homeostasis-disrupting traumatic agents (e.g.: ROS, infections, oxidation epitopes, etc.) forming pro-inflammatory phenotypes (PAMPs/DAMPs) initially trigger immune cells – systemic failures and other associated immune-recognition mechanisms regulate the prolongation of chronic inflammation in the atherosclerotic microenvironment [4,16].

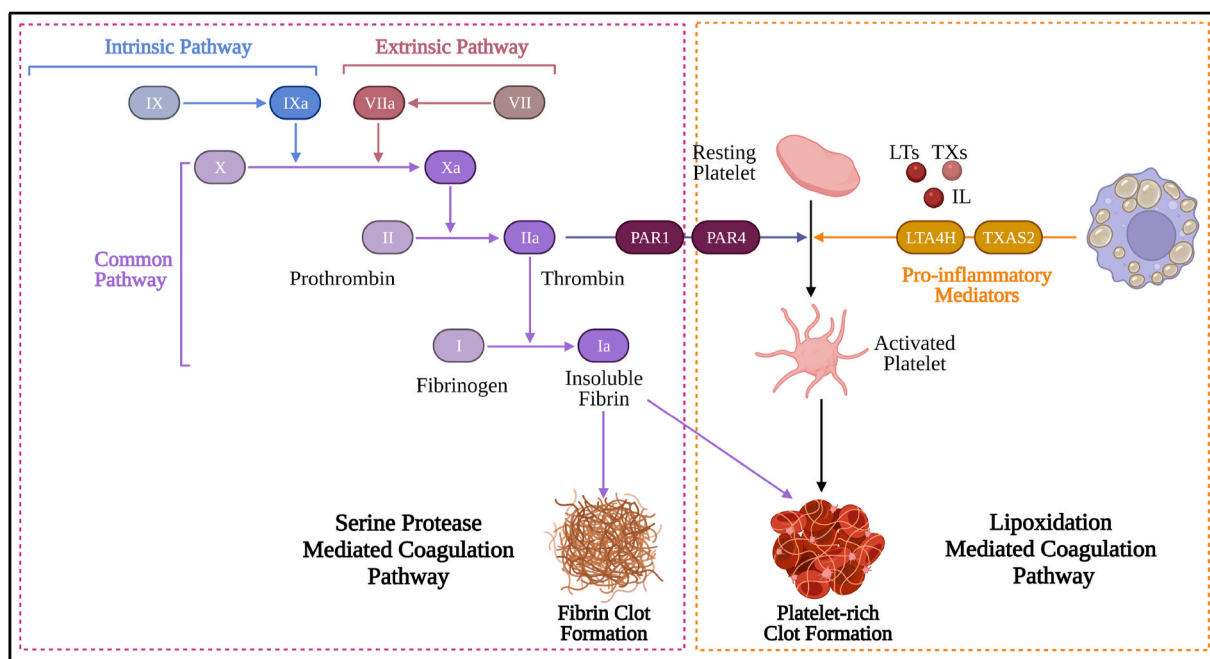


Fig. 7. Thrombotic Pathways Leading to Atheroma Calcification. Classical coagulation pathways converge to a common thrombin-activated fibrinogenic clot formation, which is regulated by a set of serine proteases or coagulation factors. Thrombin also activates the PAR-1/PAR-4 pathway, which in conjunction with lipoxides and inflammatory stimuli converts resting leukocytes to the activated state in platelet-rich-plasma. Both coagulation mechanisms result in thrombotic plaque development, rupture, and ACVD complications. Figure generated using the ‘Coagulation Cascade’ template from BioRender. LT – leukotriene; PAR – protease activated receptor; TX – thromboxane.

Chronic inflammation and prolonged stress response pathway activation has also been dubbed as “Suspect Zero” in atheroma development and progression [35]. A systematic review by Meng explored the role of chronic stress in inflammation, lipid metabolism, endothelial dysfunction, hemodynamics, and blood pressure, plaque stability, autophagy, ferroptosis, and cholesterol efflux – all acting as direct modulators of initial and advanced atherosclerotic lesions [35].

HETEs, HPETEs, MDA, and 4-HNE present important modes in the stimulation of chronic inflammation, thereby resulting in the promotion of TNF α , IL-4, 6, 8, and 10 in hypertension and ACVD patients [2,15,18,22,29]. Additionally, lipoxidation products stimulate scavenger receptors and the toll-like receptor 4 (TLR4)/NF- κ B pathway while suppressing NRF-2/AMPK/Akt/NOS pathways [16]. As a result, MDA and LT levels highly correlate with endothelial dysfunction and inhibition of anti-inflammatory signaling molecules. LDL-C oxidation products were also found to downregulate peroxisome proliferator activated receptor gamma (PPAR- γ) and the scavenger enzyme, platelet glycoprotein 4 (CD36) [29]. As lesions progress, the fibrous cap that is composed of collagen and elastin impregnated with T-cells, macrophages, and dislodged SMC is also driven by immune cell hyperactivation, proliferation, and subsequent apoptosis/necrosis [2]. The downstream interactions of lipoxides with immune cells and resulting pathway modulations are illustrated in Fig. 6.

Another theoretical process of adaptive immune cell pro-atherogenicity resides in the direct antigenicity of circulatory ArA and ox-LDL, which provide for an “auto-antigenic” role of LDL in ACVD [16]. However, Wolf argues that this is insignificant because B cells are rarely found in atherosclerotic plaques [9]. Rather, they posit that innate immune activation of macrophages and platelets, and subsequent thrombosis and dysregulation of hemodynamics have much more substantial impacts in ACVD.

2.6. Role of the thrombotic cascade and hemostasis in atherosclerosis

While ox-LDL and peroxides set into motion a complex set of

chemical reactions to promote inflammation and proliferation, thereby leading to intima thickening and atherosclerotic clot formation – the later stages of atherosclerosis, and by extension atherothrombosis, is driven by the interplay among inflammation, platelet function, and hypercoagulability. Thus, all previously described mechanisms of sclerosis climax with thrombosis as the final common pathway for destabilization of plaques [2]. These thrombotic episodes can be both symptomatic and asymptomatic – but plaque rupture of a thin cap atheroma is the usual lesion responsible for most acute thrombotic events.

The role of inflammation and lipid peroxidation products in thrombosis is to serve as a catalyst for immune recruitment and platelet aggregation, as discussed in the previous section. While the direct mechanisms of inflammatory mediators in thrombosis are unclear, Ambrose et al. speculate that inflammation-mediated hypercoagulability of blood and subsequent thrombus expansion can convert a seemingly innocuous asymptomatic thrombus to a symptomatic coronary syndrome [2].

Ambrose et al. also presents an argument for the bidirectionality of the relationship between inflammation and thrombosis. They posit that platelets activated by oxidized mediators of inflammation in atherosclerosis subsequently release more pro-inflammatory cytokines and chemokines in the environment. Thrombin or prothrombin (Coagulation Factor II) is a major driver of this process, which exerts pro-atherogenic effects to link shear stress, hemostasis, immunity, and inflammation in ACVD [2,20].

Fibrinogen (Coagulation Factor I) levels in circulation initiate a series of serine protease-mediated cleavage leading to the active participation of various clotting factors in coagulation and proliferation promotion in sites of damage or stress. In addition, fibrinolysis end products result in platelet aggregation, endothelial remodeling, cell adhesion to blood vessel linings, and further propagation of inflammation [2,5,13,36]. Platelet-poor and platelet-rich regions of arterial lumen present different mechanisms of thrombotic expressions; although in both scenarios, the underlying mechanisms and the ultimate

Table 1
Currently prescribed therapeutic interventions for atherosclerotic cardiovascular diseases.

Drug Class	Prominent Examples	Primary Mechanism of Action	Reference/ Review
Antioxidant Vitamins	Ascorbic Acid, α -Tocopherol, β -Carotene	Free radical scavenging and antioxidation	[10]
Metal Chelators	Edaravone, Carvedilol, Hydralazine,	Free radical scavenging and reduction of metal ion availability	[15]
Antiplatelets	Aspirin, Warfarin	Inhibition of platelet activation and atherothrombosis	[3]
β -Blockers	Atenolol, Propranolol, Bisoprolol etc.	Blocks effects of the epinephrine and vasodilates arterial walls	[41]
NSAIDs	Celecoxib, Zileuton	Free radical scavenging and inhibition of PTGS	[3]
Natural Antioxidants	Quercetin, Baicalin, Curcumin, Epicatechin Gallate etc.	Underexplored	[4]
PUFAs	Linoleic acid, Fish oils	Underexplored	[6,42]
Monoclonal Antibodies	Canakinumab, Tocilizumab, Eculizumab	Immunosuppressive targeting IL, IFN and TNF	[7,41]
Anti-inflammatory Drugs	Colchicine, Methotrexate, PLA inhibitors	Unclear direct mechanisms; reduction of inflammatory biomarkers; reduces proinflammatory and cytotoxic products	[3,11]
Cholesterol Absorption Regulators	CYP450 modulators, AMPK activators	Regulates glucose metabolism and cholesterol conversion	[11]
PPAR Modulators	Clofibrate, Gemfibrozil, Ciprofibrate etc.	Decreases insulin resistance and suppresses immune hyperactivation; decreases TG-rich lipoproteins	[11]
Statins	Atorvastatin, Fluvastatin, Lovastatin, Pravastatin etc.	Increase NOS expression and lower lipid levels in blood	[3,10]
Anti-hyperlipidemics	Probuocol/succinobucol,	Free radical scavenging and LDL suppression	[43,44]
Anti-hyperglycemics	Metformin, Acarbose	Reduction of digestive increase of blood glucose levels; anti-obesity and anti-hyperlipidemia	[15]
AT1R antagonists	Irbesartan, Valsartan, Candesartan	Blocking ROS production; inhibiting expression of vascular endothelial cells	[10]
ACE Inhibitors	Benazepril, Enalapril, Fosinopril, Lisinopril etc.	Increasing plasma bradykinin and vasodilates arterial lumen	[10]
PCSK9 Inhibitors	Evolocumab	Lower lipid levels in blood and prevent inflammatory symptoms	(Kumar et al., n. d.)

outcome remains similar. The final fate of continuous development and progression of fibrinolytic or platelet-mediated thrombotic plaques results in atheroma vulnerability and eventual rupture leading to acute coronary syndromes and death [2,5,13,36].

Hence, platelet PTGS-1/2 and downstream TXAS2 regulation by NSAID therapeutics further validates this correlation, which inhibits TX synthesis and inflammatory cytokine release, thereby leading to anti-thrombotic benefits [2]. This is one of the major pharmacokinetic bases of antiplatelet drug prescription and administration for coronary and ischemic patients. A schematic representation of the thrombotic pathways associated in atheroma advancement is shown in Fig. 7.

3. Current scenario and trends for therapeutic solutions

The field of cardiac medicine as a whole is greatly beyond the scope of this study and readers should query more generalized reviews and updates on the topic. However, the global epidemic of ACVD [3] is accurately reflected in the niche area of anti-inflammatory and anti-thrombotic therapeutic R&D and production.

3.1. Global market for atheroprotective therapeutics

The global prevalence of ischemic heart disease has increased from about 100 million in 1990 to over 180 million cases in 2019 [7]. According to a recent market study, the global atherosclerosis drugs market was valued at ~ \$45.7 bn in 2018 [37]. Key players in atherosclerosis and anti-inflammatory drug production include Pfizer Inc., AstraZeneca, Merck & Co., Inc., Sanofi US, Amgen Inc., Novartis AG, and GlaxoSmithKline, which are among many industry leaders in a fairly competitive therapeutic landscape dominated by North American companies [38]. Business Wire concurs with these projections and reports an estimated compound annual growth rate (CAGR) > 5% in 2019 with one of the key trends in the field being “Identification of New Drugs and Targets for Therapeutics” [39].

3.2. Available therapeutics: progress and shortcomings

While the field of ACVD and atheroprotective medicine is rich with decades of R&D as well as industrial incentives – Libby reprimands

current therapeutic interventions that have largely been ineffective, unsafe and/or underperforming in significantly affecting atherosclerotic events in clinical trials [3]. Current management seemingly still depends on primordial prevention through lifestyle changes and management of comorbidities. Current medication models mostly rely on three major classes – a) lipid lowering, b) antiplatelet, and c) anti-inflammatory drugs [3].

LDL-C lowering drugs such as statins still remain the most effective mode of controlling CVD and atherosclerosis risk factors. Fibrates are another group of drugs used against atherogenesis that reduce TG-rich lipoproteins in circulation. However, these drugs suffer from under-performance in clinical trials worldwide, in addition to drug-drug interactions and gut dysbiosis resulting in adverse reactions in patients [3]. Another mode of counteracting LDL levels have recently gained momentum using ω -3 fatty acid supplements such as icosapent ethyl. However, several trials using different ω -3 fatty acids have reported contradicting evidence in atherosclerosis and ischemic heart disease management [3,10,12,40].

Another major category of ACVD risk-alleviating drugs includes antiplatelet therapeutics that target the thrombotic cascade and atherothrombosis. In particular, low dose aspirin is the most commonly prescribed medication today for CVD patients. However, there are drastic adversities and side-effects associated with aspirin and anti-coagulant drugs like warfarin – which still presents a lack of suitable and safe therapeutic agents.

Small molecule antioxidants and anti-inflammatory mAb drugs have so far been the most promising interventions in ameliorating cardiac complications associated with atherosclerosis [3]. Notable candidates for immune-modulation and inhibition of inflammatory processes include antioxidant vitamins, probuocol/succinobucol, canakinumab, tocilizumab, colchicine, methotrexate that reduces CrP and IL levels, and NSAIDs such as celecoxib and zileuton that reduce PG synthesis and lipid peroxidation products [3,7,10,18]. Other major metal chelators and antioxidants include Edaravone, Carvedilol and Hydralazine which quench lipoxidation free radicals and prevent formation of atherosclerotic plaques [15].

While a wide class of anti-inflammatory antibodies, steroids, and NSAIDs are currently available in the market – their non-target interactions and low toxic tolerance deem them incompatible with pre-

Table 2

Comparative advantages and disadvantages of peptide therapeutics over small molecule drugs [adapted [45,46]].

Advantages	Disadvantages
Broad range of therapeutic target interactions	Limited oral bioavailability and poor solubility of hydrophobic peptides
Significantly lower toxicity and side-effects	Poor membrane permeability
Higher chemical and biological diversity	Low <i>in-vivo</i> stability
Higher potency and selectivity	May contain immunogenic amino acid sequences
Good efficacy, safety, and tolerability	Short half-life and rapid clearance
Easy excretion and minute accumulation	Elevated development costs
Standard synthetic and possible heterologous expression as cost-effective production mode	

ischemic patients, and often require a risk–benefit analysis before prescription. There are multifactorial dimensions to these shortcomings, but a key area of concern involves the current one molecule-one enzyme approach of inhibiting COX/PTGS proteins as the primary mode of small molecule drug development instead of holistic intervention approaches in ACVD – resulting in undesired physicochemical interactions and lack of synergistic potentials present in pure nutraceuticals.

As an alternative solution, natural products have been explored in the form of fish lipids, plant-derived phenolics, and other antioxidant phytochemicals [4]. However, these compounds fundamentally suffer from the same toxicity of small molecules and extra-target interactions presented in synthetic drugs. As a result, to date, no natural preparations have been proven safe or efficacious enough to be incorporated into atherosclerosis-associated human trials.

Another avenue currently being explored for effective management of atherosclerosis is the glucogenic compartment of the disease and targeting the DM-CVD synapse [7]. This largely targets glucose-lowering mechanisms using anti-hyperglycemic drugs such as metformin and acarbose, and AGE inhibitors [15]. However, an integrated route for the therapeutic treatment of atherosclerosis is yet to be fully elucidated. Combinedly, these challenges further convolute the suitability and safety of conventional small molecule drugs as atheroprotective therapeutic candidates and establish an open area for active research and development of safe and efficacious therapeutics. Some major, currently reported, and prescribed therapeutic compounds are listed in Table 1.

3.3. Prospects of peptide and miRNA therapeutics as alternative intervention strategies

Historically, peptide therapeutic agents were equivocated by the pharmaceutical industry due to low stability, large molecular size, rate of degradation, and poor oral bioavailability. Nevertheless, with improvements in drug delivery and storage technology, peptides have recently again garnered the attention of numerous therapeutic areas. Volpe and Manna summarized the pros and cons of peptides and peptide mimetics over small molecules as adapted in Table 2. Therapeutic peptide research prospects initially settled its roots in atherosclerosis through peptide-mimetics that targeted lipoprotein dysregulation and inflammation through beneficial mimics and agonists [45]. These mimetics and peptide derivatives target a wide variety of atherogenic targets including PCSK9, CD36, IL, and TGF- β [40,47].

Another avenue of peptides in cardioprotection manifests in the form of peptide hormones – particularly natriuretic peptides with substantial anti-inflammatory and anti-proliferative benefits in ACVD [12,46,48]. Several synthetic and recombinant natriuretic peptides (e.g.; Nesiritide) have also been approved by the FDA for heart failure patients. Other synthetic peptides also started budding in the research and development level in inflammatory and atherosclerosis disease mitigation strategies [49–53]. However, major drawbacks of low stability and the need for parenteral administration bridled market propagation of these drugs

beyond high-risk patients [48,54,55].

This is where the recent investigations into stable dietary peptides provide a new promising avenue for ACVD medication. In addition to supplying essential nutrients, some natural food proteins can confer additional health benefits beyond the established nutritional contents in the form of small molecules and peptides. While the small molecule chemistry is a rich and diverse field – the exploitation of these dietary peptides to this day remains inconsequential. Especially in the field of atheroprotective therapeutics – bioactive small peptides, despite numerous interesting preliminary studies, are yet to be incorporated into advanced trials.

Rutherford-Markwick points out that surpassing the generic single drug models – peptides from digestive hydrolysis of foods, particularly plant proteins, provides a complex interaction of multi-peptide interactions with health-modulating benefits [88]. In the succeeding decade, the research niche of bioactive peptides flourished-multiple peptide-rich extracts and candidate peptides from plants and other dietary routes have exhibited significant antihypertensive, antioxidant, anti-inflammatory, anti-thrombotic, and cardioprotective activities. These peptides were derived from digestive or mechanical hydrolysis, fermentation or biochemical modification of plant, microbe, or animal-derived proteins, as elaborately reviewed elsewhere [49,55,67, 88,89]. For narrowing the scope of the current account, only peptides with short amino acid chains (≤ 20 amino acids) from dietary sources are included in Table 3 along with currently reported synthetic and therapeutic peptides.

Another new yet experimental avenue in modern molecular medicine are antisense oligonucleotide microRNAs (miRNAs), which have become somewhat a buzzword in cancer and neuroprotective therapeutics. These miRNA therapeutics are designed or derived from endogenous systems to engineer, mimic or replenish miRNA expression in disease networks. A large body of literature intersecting miRNA and atherosclerosis or inflammation are concerned with downstream regulation of miRNAs by ACVD associated genes or proteins such as TNF, IL-6 or Nf- κ B as biomarkers for diagnostic and surveillance purposes [86,90, 91]. However, in case of lipid oxidation and atherosclerosis management, miRNA based therapeutic interventions have barely gone beyond functional interaction studies in recent years with no candidates currently being considered for human trials [84,86,87].

Nevertheless, specific target regulating miRNAs and functionally effective miRNAs in peripheral pathways prompt a possible new route to administer anti-inflammatory, anti-coagulant and cardioprotective therapeutics. These miRNAs can construct a rich reservoir of potential atheroprotective candidates that can a) target/inhibit ACVD regulating genes/proteins, b) activate/upregulate expression of anti-inflammatory and/or anticoagulant factors in disease networks, and c) replenish or mimic endogenous miRNA expressions. A collection of recent evidence in miRNA therapeutic development in ACVD and relevant diseases has been provided in Table 4.

In cases of both peptides and miRNAs, appropriate delivery vehicle systems, cost efficiency, cell-specific and disease-specific targeting, sever adverse effects (Eg: Mipomersen hepatotoxicity), tranfering *in-vitro* results to complex *in-vivo* models, as well as oral and/or intravenous bioavailability remain major drawbacks and challenges to be addressed in future pre-clinical and clinical investigations.

4. Concluding remarks

The current account revisits conventional understandings of pathophysiological mechanisms in the development, progression and evolution of arterial atherosclerotic complications in coronary diseases. Available literature reflects a core concept of loss of homeostatic balance when it comes to inflammatory and oxidative molecular processes in contrast to previous extremum notions of relevant markers. New insights into the multifaceted roles of unsaturated fatty acids and their oxides in metabolism, nutrition, signal transduction and cellular

Table 3
Recent developments in small peptide therapeutics in ACVD and associated disorders.

Category	Therapeutic Class	Source	Peptide Name/Construct	Highest Evidence Level	Mechanism of Action	Reference/ Review	
Therapeutic Peptides	Antihyperglycemic	Indigenous/Recombinant	Glucagon-like peptide 1	Murine <i>in-vivo</i>	Multifactorial protection in cardiac health	[56]	
	Antihyperlipidemic	Indigenous/Recombinant	Human Neutrophil Peptide 1	<i>in-vitro</i>	Increased hepatic LDL clearance	[57]	
	Anti-inflammatory	Indigenous/Recombinant	Ac-SDKP	<i>in-vitro</i>	Inhibition of cytokine expression	[49]	
	Anti-inflammatory	Indigenous/Recombinant	IIMI	<i>in-vitro</i>	Inhibition of cytokine expression	[49]	
	Anti-inflammatory/ Cardioprotective	Indigenous/Recombinant	Cortistatin	Murine <i>in-vivo</i>	Downregulation of pro-inflammatory pathways and atherosclerotic plaque regression	[58]	
	Anti-inflammatory/ Cardioprotective	Indigenous/Recombinant	Natriuretic peptide (Atrial/B/C)	Human <i>in-vivo</i> / Clinical	Multifactorial protection in cardiac health	[46]	
	Anti-inflammatory/ Immunosuppressive	Indigenous/Recombinant	Vasoactive intestinal peptide	Murine <i>in-vivo</i>	Immune suppression of T-cell activation and macrophage foaming	[59]	
	Antioxidant	Indigenous/Recombinant	Humanin	Murine <i>in-vivo</i>	oxLDL-elicited ROS reduction	[40]	
	Anti-oxidant/Anti-inflammatory	Indigenous/Recombinant	Carnosine	Murine <i>in-vivo</i>	Selectively lowered LOX product levels in serum	[15]	
	Synthetic Peptides	Immunosuppressive	Indigenous/Recombinant	Neuropeptide Y (NPY)	<i>in-vitro</i>	Macrophage regulation	[49]
Antihyperglycemic		Apolipoprotein A-I Mimetics	ELK-2A2K2 E	Murine <i>in-vivo</i>	Increased blood HDL; augmented ROS and cholesterol efflux	[54,60]	
Anti-inflammatory		Antimicrobials peptide hybrid	LL-37-Ta1	<i>in-silico</i> / murine <i>in-vivo</i>	TNF- α , IFN- γ , IL-6 and IL-1 β reduction	[21]	
Anti-inflammatory		Designer Chensinin-1 Mutants	MC1-1/2/3	<i>in-vitro</i> / <i>in-silico</i>	Inhibition of TNF- α and IL-6	[61]	
Anti-inflammatory		Solid phase peptide synthesizer	FWY, FYS, YWG	<i>in-vitro</i>	Inhibition of soy bean 15-LOX	[62]	
Anti-inflammatory		Solid phase peptide synthesizer	FWY, YWCS, FYS, FWCS	<i>in-vitro</i>	Inhibition of 12-LOX	[62,63]	
Anti-inflammatory		Solid phase peptide synthesizer	MHP1	<i>in-vitro</i>	Inhibition of LPS-induced cytokine storm	[45]	
Anti-inflammatory		Solid phase peptide synthesizer	Trp-His	Murine <i>in-vivo</i>	Reduced atherosclerotic lesion	[64]	
Anti-oxidant/Anti-inflammatory		Solid phase peptide synthesizer	9Pbw2/9Pbw4/AIP6	<i>in-vitro</i>	Inhibition of NO production; suppressed immune activation	[49]	
Anti-oxidant/Anti-inflammatory		Solid phase peptide synthesizer	Oxpholipin 11D	<i>in-vitro</i>	Antagonists of lipoxidation products	[49]	
Anti-thrombotic		Arg-gly-asp mimetic	Eptifibatide	Human <i>in-vivo</i> / Clinical	Prothrombin inhibition	[65]	
Anti-thrombotic		Hirudin mimetic	Bivalirudin	Human <i>in-vivo</i> / Clinical	Prothrombin inhibition	[65]	
Immunosuppressive		GHRP anlaogue	EP 80317/Hexarelin	Murine <i>in-vivo</i>	CD36 antagonist	[40]	
Immunosuppressive		Solid phase peptide synthesizer	Chemerin 15	<i>in-vitro</i>	Suppression of chemotaxis and adhesion of PMBC	[49]	
Immunosuppressive		Solid phase peptide synthesizer	IDR-1018	<i>in-vitro</i>	Macrophage regulation	[49]	
Natural/ Dietary Peptides		Antihyperglycemic	<i>Glycine max</i>	Soymorphin-5	Murine <i>in-vivo</i>	Activation of PPAR α ; lowered glucose/TG levels	[66]
		Antihyperlipidemic	Chicken bone collagen hydrolysates	Complex mixture	Murine <i>in-vivo</i>	Unexplored	[67]
		Anti-inflammatory	<i>Amaranthus hypochondriacus</i>	Seed glutenin fragment 75	<i>in-vitro</i>	Unexplored	[68]
	Anti-inflammatory	<i>Annona montana</i>	Cyclomontanin A/B/C/D; corytuberine; annomuricatin C	<i>in-vitro</i>	Unexplored	[49,68]	
	Anti-inflammatory	<i>Annona reticulata</i>	Fanlizhicyclopeptide A/B	<i>in-vitro</i>	Unexplored	[49]	
	Anti-inflammatory	<i>Apis mellifera</i>	Melittin	Murine <i>in-vivo</i>	Multifactorial protection against inflammation	[49]	
	Anti-inflammatory	<i>Dianthus superbus</i>	Dianthin A/B	<i>in-vitro</i>	Unexplored	[68]	
	Anti-inflammatory	<i>Gluten</i>	Pyro-EL	<i>in-vitro</i>	Inhibition of NOS and IL-1 β	[49]	
	Anti-inflammatory	<i>Glycine max</i>	Lunasin	<i>in-vitro</i>	Reduced ROS production; Inhibition of PTGS, iNOS, PGE2,	[68]	
	Anti-inflammatory	<i>Glycine max</i>	PepT1	<i>in-vitro</i>	Reduced TNF- α , IL-6, IL-1 β , IFN- γ and IL-17 level	[68]	
	Anti-inflammatory	<i>Heterometrus laoticus</i>	Hetlaxin	Murine <i>in-vivo</i>	Unexplored	[49]	
	Anti-inflammatory	<i>Lateolabrax maculatus</i>	DAPAPPSQLEHIRAA, AADGPMKILGY	<i>in-vitro</i>	Unexplored	[69]	

(continued on next page)

Table 3 (continued)

Category	Therapeutic Class	Source	Peptide Name/Construct	Highest Evidence Level	Mechanism of Action	Reference/ Review
	Anti-inflammatory	<i>Marine Streptomyces</i> sp.	Cyclomarin C	Murine <i>in-vivo</i>	Reduced TNF- α , IFN- γ , IL-6 and IL-1 β	[49]
	Anti-inflammatory	<i>Pennisetum glaucum</i>	Complex Mixture	<i>in-vitro</i>	Reduced TNF- α , IL-1 β and PGE2 level	[70]
	Anti-inflammatory	<i>Pyropia yezoensis</i>	PPY1	<i>in-vitro</i>	Reduced ROS production; Inhibits PTGS, TNF- α , iNOS and IL-1 β	[71]
	Anti-inflammatory	Sea snake extracts	Hydrostatin-SN1	<i>in-vitro</i>	Inhibition of TNF/TNFR1 downstream targets	[49]
	Anti-inflammatory	<i>Tabanus</i> sp.	Cecropin-TY1	<i>in-vitro</i>	Inhibition of MAPK and NF- κ B activation	[72]
	Anti-inflammatory	<i>Theonella swinhoei</i>	Perthamides C/D	Murine <i>in-vivo</i>	Reduced TNF- α , IFN- γ , IL-6 and IL-1 β	[49]
	Anti-inflammatory/ Cardioprotective	<i>Oryza sativa</i>	GEQQQQPGM	Murine <i>in-vivo</i>	Reduced ROS production; Inhibition of VCAM, iNOS; Increased PHGSx and SOD expression	[73]
	Anti-oxidant/Anti-inflammatory/Anti-coagulant/Cardioprotective	<i>Citrus hystrix</i> , <i>Citrus reticulata</i> , <i>Citrus aurantiifolia</i> , <i>Citrus limon</i> , <i>Citrus limetoides</i>	Complex Mixture	<i>in-vitro</i>	Free radical scavenging; Inhibition of α -amylase, glucosidase, PTGS-1/2, 5/12/15-LOX, and serine proteases	[74]
	Antioxidant	<i>Brassica napus</i>	Complex Mixture	<i>in-vitro</i>	Free radical scavenging; Inhibition of lipoxide production	[75]
	Antioxidant	Egg albumin	IRW, IQW	<i>in-vitro</i>	Free radical scavenging; Inhibition of lipoxide production	[75]
	Antioxidant	<i>Oryza sativa</i>	FRDEHKK	<i>in-vitro</i>	Free radical scavenging; Inhibition of lipoxide production	[75]
	Anti-oxidant/Anti-inflammatory	Kefir	Complex Mixture	<i>in-vitro</i>	Reduced lipid deposition, ROS, macrophage accumulation, IL-1 β and TNF- α levels	[76]
	Anti-oxidant/Anti-inflammatory	<i>Lupin mutabilis</i>	Complex Mixture	<i>in-vitro</i>	Reduced ROS production; inhibits TNF- α , IL-6 and IL-1 β	[77]
	Anti-oxidant/Anti-inflammatory	<i>Setaria italica</i>	PFLF, IALLIPF	<i>in-vitro</i>	Reduced ROS production; inhibits TNF- α , IL-6 and IL-1 β	[78]
	Antioxidant/Anti-thrombotic	Milk Protein	Casein/ κ -casein/ Lactoglobulin hydrolysates	<i>in-vitro</i>	Free radical scavenging; inhibition of thrombin-induced platelet aggregation	[79,80]
	Anti-thrombotic	<i>Amaranthus hypochondriacus</i>	Seed glutenin fragment 3/59	<i>in-vitro</i>	Unexplored	[68]
	Anti-thrombotic	<i>Arachis hypogaea</i>	Unnamed	<i>in-vitro</i>	Unexplored	[81]
	Anti-thrombotic	Yogurt	Complex mixture	<i>in-vitro</i>	Unexplored	[67]
	Cardioprotective	<i>Citrus natsudaidai</i>	Cyclonatsudamine A	Murine <i>in-vivo</i>	Vasorelaxation	[68]
	Cardioprotective	<i>Leonurus japonicus</i>	Cycloleonoripeptide D/E/F	Murine <i>in-vivo</i>	Inhibition of PTGS-2; vasorelaxation	[82]
	Cardioprotective	<i>Stellaria dichotoma</i>	Dichotomin D/F/G	Murine <i>in-vivo</i>	Inhibition of PTGS-2; vasorelaxation	[82]
	Cardioprotective	<i>Undaria pinnatifida</i>	ACEi WH	Murine <i>in-vivo</i>	Vasorelaxation	[68]
	Cardioprotective	<i>Vaccaria hispanica</i>	Segetalin F	Murine <i>in-vivo</i>	Vasorelaxation	[68]
	Immunosuppressive	<i>Jatropha curcas</i>	Curcacycline A/B	<i>in-vitro</i>	Unexplored	[68]
	Immunosuppressive	<i>Leonurus japonicus</i>	Cycloleonorinin	<i>in-vitro</i>	Unexplored	[68]
	Immunosuppressive	<i>Linum usitatissimum</i>	Cyclolinopeptide A-I	<i>in-vitro</i>	Unexplored	[68]
	Anti-inflammatory/ Immunosuppressive	<i>Annona squamosa</i>	Cyclosquamosin A-F/1	<i>in-vitro</i>	Unexplored	[68]

maintenance adds a previously unaccounted for dimension in atherosclerosis and associated complications with ACVD. Amalgamating these ideas of homeostatic balances along with interconnected cascades and a complex network of intertwined molecular processes spanning redox product clearance, stress and damage response, glycemic stress, atheroinflammation, inflammaging and thrombotic complications expose the lack of holistic approaches in currently available therapeutic strategies. As a result, one drug-one enzyme based small molecule regulators and treatments beg to be newly evaluated for their applicability and extent in atheroprotection compared to a holistic approach to a multifactorial disorder. Although prospective strategies such as peptide therapeutics and anti-sense miRNAs are slowly but steadily being introduced to the pipeline, more detailed investigations and revisions of our understanding of the pathophysiology of ACVD are still required for

effective prevention, management and developing novel interventions for this chronic epidemic.

Author contributions

RS prepared the data and visuals, and wrote the original draft; FA, ANH and MMB reviewed and edited the manuscript; MEI and KMDI conceptualized and supervised the work. All authors discussed the results and agreed on the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 4
Recent Developments in miRNA Therapeutics in ACVD and Associated Disorders.

Category	Target Gene/ Transcript	Mechanism of Action	Interacting miRNA	Highest Evidence Level	Reference/ Review	
Endogenous Therapeutic miRNAs	LOX12	Functional target interactions	miR-185-5p	<i>in-vitro</i>	[83]	
	LOX5		miR-19a-3p, miR-125b-5p	<i>in-vitro</i>		
	IL-6		miR-26a-5p, miR-365a-3p, miR-98-5p, miR-107, miR-223-3p, miR-149-5p, miR-146b-5p, miR-9-5p, miR-146a-5p, miR-125a-3p, miR-136-5p, miR-451a	murine <i>in-vivo</i>		
		IL-6 Receptor		miR-23a-3p, miR-124-3p, miR-124-3p, miR-125b-5p, miR-125b-5p, miR-451a, miR-451a, miR-34c-5p, miR-34b-3p, miR-34a-5p, miR-34a-5p, miR-34a-5p, miR-221-5p	<i>in-vitro</i>	
		IL-10		miR-106a-5p, miR-194-5p, miR-19a-3p	<i>in-vitro</i>	
		IL-11		miR-204-5p, miR-211-5p, miR-211-5p, miR-379-5p, miR-1-3p, miR-30c-5p	<i>in-vitro</i>	
		PTGS-1		miR-1-3p	<i>in-vitro</i>	
		PTGS-2		miR-16-5p, miR-101-3p, miR-101-3p, miR-101-3p, miR-101-3p, miR-26b-5p, miR-26b-5p, miR-26b-5p, miR-137, miR-143-3p, miR-143-3p, miR-199a-3p, miR-199a-5p, miR-146a-5p, miR-589-5p, miR-558, miR-26a-5p, miR-26a-5p, miR-144-3p, mmu-miR-101a-3p, miR-101-3p, miR-143-5p, miR-137, miR-146a-5p, miR-146a-5p, miR-26b-5p, miR-26b-3p, miR-21-5p	murine <i>in-vivo</i>	
		TNF- α		miR-19a-3p, miR-19a-3p, miR-19a-3p, miR-203a-3p, miR-187-3p, miR-130a-3p, miR-130a-3p, miR-143-3p, miR-125b-5p, miR-17-5p	murine <i>in-vivo</i>	
		PPAR- γ		miR-27b	<i>in-vitro</i>	[84]
	Importins	Disruption of Nf- κ B signaling	miR-181b	murine <i>in-vivo</i>		
	IRAK1/2, TRAF6	Disruption of Nf-Kb and MAPK signaling	miR-146a	murine <i>in-vivo</i>		
	CaMKII α	Inhibition of pro-inflammatory cytokine production	miR-148, miR-152	<i>in-vitro</i>		
Anti-sense miRNAs	Apo-B	Sequence-specific inhibition of transcripts	Mipomersenn	human <i>in-vivo</i>	[85]	
	miR-92		MRG110	human <i>in-vivo</i>	[86]	
Atheroprotective Gene Regulators	miR-128-1		Locked nucleic acid antisense oligonucleotides	<i>in-vitro</i>	[84]	
	VCAM-1	Endothelial repair signaling and differentiation	miR-126, miR-143, miR-145, miR-155	murine <i>in-vivo</i>	[87]	
	ABCA1	Regulation of cholesterol efflux	miR-302a, miR-125a-5p, miR-146a	murine <i>in-vivo</i>	[84,87]	

the work reported in this paper.

Data availability

PlantPepDB, THPdb, and MirTarBase are freely accessible online repositories and latest editions of the databases are available at www.nipgr.ac.in/PlantPepDB, webs.iitd.edu.in/raghava/thpdb, and https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022.

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