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REVIEW ARTICLE

Reactive oxygen species imbalance in atherosclerosis: Biomarker phenotyping, residual risk, and a translational framework for precision prevention

Yasser M. Esmaeil[1]*, Marjona Nurillaeva[1,2], Aamir Khan[1,2], and Matthew Wiesner[1,2]

[1]Department of Internal Medicine, St. John's Episcopal Hospital, New York City, New York, United States of America

[2]Ross University School of Medicine, Bridgetown, Barbados

Abstract

Background: Atherosclerotic cardiovascular disease (ASCVD) leaves substantial residual risk despite intensive low-density lipoprotein-cholesterol lowering and anti-inflammatory therapy. Reactive oxygen species (ROS) have long been implicated, yet neutral broad-antioxidant trials left unclear which dimensions of ROS imbalance are causal, clinically informative, and therapeutically tractable. **Aim:** To critically appraise ROS imbalance in atherosclerosis through a plausibility–association–utility hierarchy and to propose a staged framework for precision cardiovascular prevention. **Methods:** We synthesized mechanistic, biomarker, and translational evidence from targeted PubMed/MEDLINE, Embase, and Cochrane searches (January 2000–February 2026), combining terms for ROS, oxidative stress, atherosclerosis, oxidative biomarkers, and residual cardiovascular risk, with citation chaining. Mass-spectrometry biomarker work, prospective cohorts, and randomized trials received greater interpretive weight. Systematic-review procedures were not applied. **Results:** Compartmentalized, source-specific ROS imbalance contributes to endothelial dysfunction, lipoprotein oxidation, inflammatory amplification, and plaque progression, particularly in high-risk cardiometabolic phenotypes. Human biomarker data are predominantly observational, assay standardization is incomplete, and risk-reclassification evidence is limited. F2-isoprostanes are a relatively mature lipid-peroxidation marker better suited to trial enrichment than treatment selection. **Conclusion:** Oxidative biomarkers are defensibly used for trial enrichment but not individual treatment selection. A staged precision-prevention agenda should test source-selective interventions in biomarker-enriched cohorts using vascular and mechanistic endpoints before large outcome trials. **Relevance for Patients:** Adults whose cardiovascular risk remains high despite cholesterol-lowering and anti-inflammatory treatment—including those with diabetes, kidney disease, smoking, obesity, or recurrent events—may eventually benefit if oxidative stress tests can identify who remains vulnerable and guide targeted prevention.

Keywords: Reactive oxygen species; Oxidative stress; Atherosclerosis; F2-isoprostanes; Residual cardiovascular risk; Precision cardiovascular prevention; Biomarkers

***Corresponding author:**
Yasser M. Esmaeil
(yesmaeil@ehs.org)

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1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) remains a dominant cause of death and disability worldwide despite major advances in preventive cardiology.[1,2] Intensive lipid-lowering, antihypertensive therapy, smoking cessation, antithrombotic strategies, and pathway-specific anti-inflammatory approaches have reduced vascular events, yet recurrent myocardial infarction, stroke, and cardiovascular death still occur in patients receiving guideline-directed care.[3] This persistent burden has renewed interest in biologic pathways that may explain residual risk beyond low-density lipoprotein cholesterol (LDL-C) alone.

The modern era of deep LDL lowering has made this problem especially visible. IMPROVE-IT, FOURIER, and ODYSSEY OUTCOMES confirmed that progressive reduction of atherogenic lipoproteins lowers cardiovascular events, but each trial also showed that very low achieved LDL-C does not eliminate recurrent ischemic events.[4,5] Anti-inflammatory pathway targeting has likewise reduced events: CANTOS demonstrated the principle for interleukin (IL)-1β blockade in patients with residual inflammatory risk, while LoDoCo2 and COLCOT extended the rationale to colchicine.[6,7] These results do not weaken the central role of apolipoprotein B (apoB)-containing lipoproteins in atherogenesis; rather, they show that vascular injury is sustained by interacting processes, including endothelial dysfunction, inflammation, thrombosis, metabolic stress, and tissue-specific maladaptive signaling.[8,9]

Within this multidomain residual-risk landscape, oxidative stress remains biologically compelling but clinically unsettled. Reactive oxygen species (ROS) intersect with nitric oxide (NO) depletion, endothelial activation, lipoprotein modification, inflammatory amplification, plaque remodeling, and platelet dysfunction.[10,11] Decades of disappointing broad antioxidant trials generated skepticism about whether oxidative stress is causal, clinically informative, or therapeutically tractable.[12,13] Contemporary redox biology has moved beyond the older view of diffuse free-radical excess: disease is now understood as arising from sustained, compartmentalized, enzymatically amplified, and inadequately buffered ROS imbalance within specific vascular microenvironments.[14,15]

This review advances three positions: (i) ROS imbalance is a biologically coherent contributor to residual atherosclerotic risk but not yet a clinically validated treatment target; (ii) current oxidative biomarkers are defensible tools for trial enrichment and mechanistic endpoints, not for individual treatment selection; and (iii) progress requires a staged translational framework linking phenotype selection, analytically rigorous biomarker assessment, and source-selective interventions. Particular—but not exclusive—attention is given to F2-isoprostanes (8-iso-prostaglandin $F_{2\alpha}$ [8-iso-$PGF_{2\alpha}$]) as among the more analytically robust markers of *in vivo* lipid peroxidation, considered within a framework that acknowledges assay heterogeneity, incomplete clinical validation, and the multifactorial nature of atherosclerotic disease.[10,16,17]

Framing these positions requires an explicit conceptual stance. Rather than treating oxidative stress as an isolated causal axis, we situate it within a systems- and network-medicine view of atherosclerosis, in which oxidation and inflammation behave as tightly coupled regulatory hubs embedded in a larger disease network rather than as parallel, independent pathways.[18,19] In this framework, redox imbalance is one interacting node among lipoprotein retention, innate-immune activation, metabolic reprogramming, and hemodynamic stress; its clinical meaning depends on how strongly that node is wired to the others in a given phenotype. This perspective reframes the biomarker problem accordingly: single analytes are unlikely to capture a networked disturbance, and the more tractable goal is to position oxidative measures within multidimensional biomarker sets that can be integrated using systems approaches.[20] We return to this integrative view when considering biomarker positioning, distinct high-risk phenotypes, and future multi-omics study design.

2. Methods

We synthesized mechanistic, clinical, and translational evidence on oxidative stress in atherosclerosis through targeted searches in PubMed/MEDLINE, Embase, and the Cochrane Library, supplemented by citation chaining from influential primary studies and prior reviews. Searches covered English-language literature published between January 2000 and February 2026, with seminal earlier publications (e.g., the original F2-isoprostane discovery and oxidative-modification hypothesis papers) added through citation chaining when foundational.

Search strings combined three concept blocks: (i) redox-biology terms ("oxidative stress," "reactive oxygen species," "NADPH oxidase," "mitochondrial ROS," "xanthine oxidase," "eNOS uncoupling," "F2-isoprostanes," "8-iso-$PGF_{2\alpha}$," "oxidized LDL," "oxidized phospholipids," "myeloperoxidase"); (ii) disease terms ("atherosclerosis," "coronary artery disease," "cardiovascular disease," "endothelial dysfunction," "plaque"); and (iii) risk/therapeutic terms ("residual cardiovascular risk," "biomarker," "PCSK9," "statin," "renin–angiotensin," "colchicine," "canakinumab," "precision prevention"). The strategy targeted four evidence categories: mechanistic

vascular ROS biology; human biomarker studies in cardiometabolic and atherosclerotic disease; translational and therapeutic studies of interventions intersecting redox pathways; and contemporary outcome trials framing modern residual risk.

Study selection followed explicit, if non-systematic, prioritization principles. Database and citation-chaining searches returned approximately 1,500 records; after title and abstract screening for relevance to vascular redox biology, oxidative biomarkers, high-risk cardiometabolic phenotypes, or residual-risk therapeutics, approximately 300 full-text sources were examined, of which the 94 most informative articles were retained and cited. Because this is a narrative synthesis rather than a systematic review, selection was interpretive rather than exhaustive, and we did not apply dual-reviewer screening or a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Sources were prioritized by four principles: (i) evidence hierarchy—randomized trials, Mendelian-randomization studies, and large prospective cohorts were weighted above small exploratory or cross-sectional series; (ii) analytic quality—mass-spectrometry biomarker measurements were weighted above immunoassay-only data; (iii) mechanistic informativeness—preclinical studies were included when they clarified source-specific ROS generation not testable in humans; and (iv) recency and representativeness, with deliberate inclusion of null and discordant findings (e.g., neutral broad-antioxidant trials) to counter confirmation bias. Where evidence conflicted (e.g., antioxidant trials versus mechanistic data), we prioritized the higher-evidence study and stated the discordance, distinguishing throughout between mechanistic plausibility, biomarker association, and clinical utility.[21] We also deliberately broadened the literature beyond classical redox biology to incorporate systems- and network-medicine, immunometabolism, inflammasome biology, and proteomic/lipidomic sources, so that oxidative stress is positioned within, rather than abstracted from, the interacting disease network. Methodological limitations of the narrative approach are discussed in **Section 7**.

3. Reactive oxygen species biology in atherosclerosis

3.1. Enzymatic sources of vascular reactive oxygen species

A useful starting point is the distinction between physiologic ROS signaling and pathologic oxidative stress. Under normal conditions, ROS, such as hydrogen peroxide, function as short-range signaling mediators that influence kinase activity, transcriptional responses, vascular tone, and host defense.[14] These effects are constrained by antioxidant systems—superoxide dismutases, catalase, glutathione peroxidases, thioredoxin, and peroxiredoxins—which maintain dynamic redox homeostasis rather than eliminating oxidants.[15]

In atherosclerosis, the relevant abnormality is therefore not the presence of ROS per se, but a sustained shift in redox balance within specific vascular compartments. NADPH oxidase (NOX) enzymes occupy a central role because they are dedicated ROS-generating systems rather than incidental byproducts of metabolism.[22,23] Multiple NOX isoforms are expressed in endothelial cells, vascular smooth muscle cells (VSMCs), macrophages, and adventitial fibroblasts, and are activated by stimuli common in cardiometabolic disease, including angiotensin II, aldosterone, hyperglycemia, advanced glycation end products (AGEs), disturbed flow, and inflammatory signaling.[24,25] NOX activity is therefore particularly relevant in diabetes, hypertension, kidney disease, and obesity.

Mitochondria are a second major vascular ROS source. Low-level electron leakage from the respiratory chain generates superoxide physiologically, but hyperglycemia, ischemia-reperfusion, and mitochondrial dysfunction amplify this leakage, triggering cellular injury.[10,26] Mitochondrial ROS amplify inflammatory signaling, impair endothelial function, and promote further mitochondrial damage, generating a self-reinforcing cycle between redox imbalance and bioenergetic dysfunction.[27]

Xanthine oxidase is a third source of vascular ROS with translational relevance because it is pharmacologically targetable.[28] In metabolic syndrome, ischemia, and heart failure, purine metabolism can become a net source of superoxide and hydrogen peroxide, exemplifying how discrete enzymatic pathways may contribute to disease in specific settings and helping explain why some interventions improve endothelial function without consistently improving outcomes.[29]

These ROS sources do not function independently. ROS-induced ROS release captures how oxidant generation in one compartment promotes further ROS production elsewhere: NOX activation may promote mitochondrial dysfunction, and mitochondrial ROS may uncouple endothelial nitric oxide synthase (eNOS) or activate inflammatory transcription factors, broadening the redox disturbance.[30] This feed-forward architecture strengthens the plausibility that oxidative stress amplifies vascular injury over time, even if the dominant source varies across disease states. Beyond this within-oxidant amplification, oxidation and inflammation behave as two interacting regulatory hubs joined by bidirectional feed-forward loops. Figure 1 integrates this systems-level view, situating

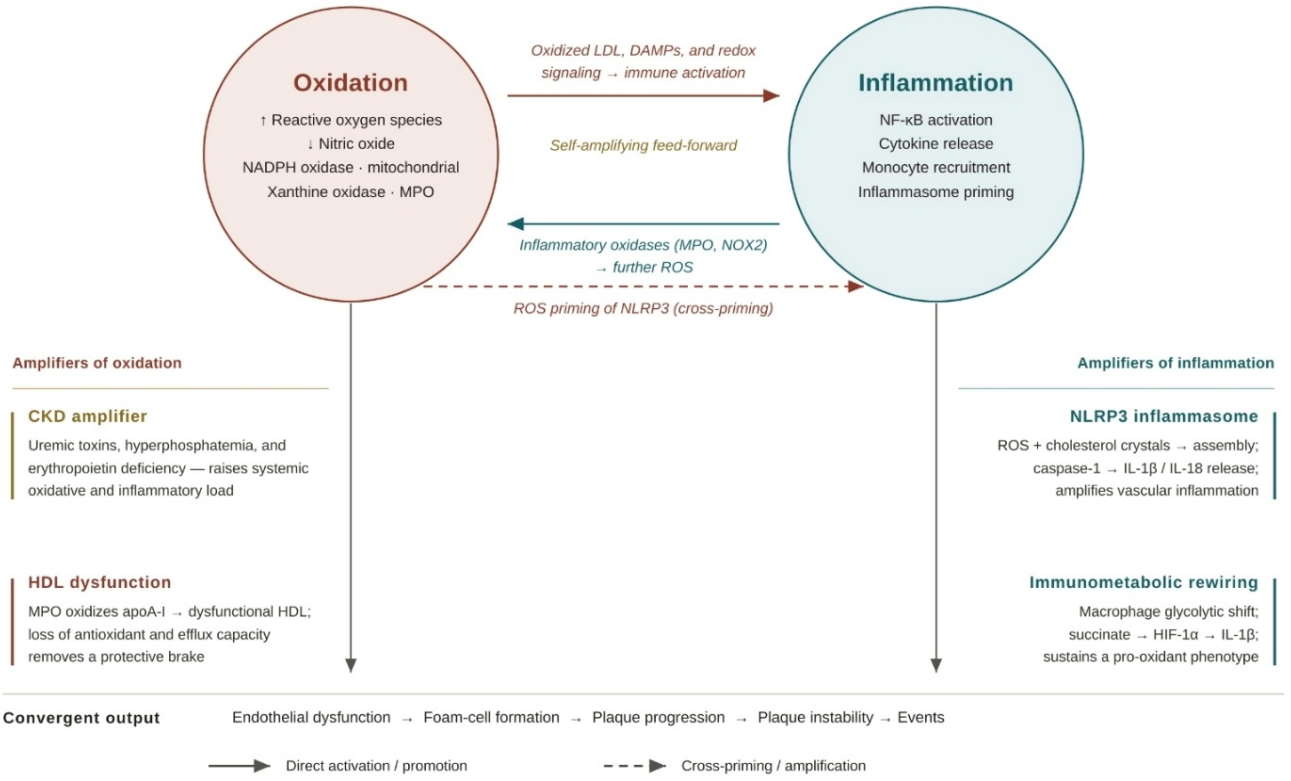

Figure 1. Oxidation and inflammation as interacting regulatory hubs in atherosclerosis. Rather than a strictly linear cascade, oxidative and inflammatory signaling are depicted as two mutually reinforcing hubs. Oxidation (elevated ROS from NOX, mitochondrial electron transport, xanthine oxidase, and MPO, with reduced NO bioavailability) and inflammation (NF-κB activation, cytokine release, monocyte recruitment, and inflammasome priming) are connected by bidirectional feed-forward loops: oxidatively modified LDL, DAMPs, and redox signaling drive immune activation, while inflammation-derived oxidases (MPO, NOX2) generate further ROS. Four modulators feed the network. NLRP3 inflammasome activation (primed by ROS and cholesterol crystals) releases IL-1β and IL-18; immunometabolic reprogramming shifts macrophages toward glycolysis with succinate–HIF-1α–IL-1β signaling that sustains a pro-oxidant phenotype; MPO-mediated oxidation of apoA–I yields dysfunctional HDL, removing a protective antioxidant and cholesterol-efflux brake; and CKD amplifies systemic oxidative and inflammatory load through uremic toxins, hyperphosphatemia, and erythropoietin deficiency. The convergent output is endothelial dysfunction, foam-cell formation, plaque progression, and plaque instability. Solid arrows denote direct activation or promotion; dashed arrows denote cross-priming or amplification. This figure is a conceptual synthesis intended to organize hypotheses, not a quantitative or exhaustive pathway map.
Abbreviations: apoA–I: Apolipoprotein A–I; CKD: Chronic kidney disease; DAMPs: Damage-associated molecular patterns; HDL: High-density lipoprotein; HIF-1α: Hypoxia-inducible factor 1α; IL: Interleukin; LDL: Low-density lipoprotein; MPO: Myeloperoxidase; NF-κB: Nuclear factor kappa B; NLRP3: NLR family pyrin domain containing 3; NO: Nitric oxide; NOX: NADPH oxidase; oxLDL: Oxidized low-density lipoprotein; ROS: Reactive oxygen species.

NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome activation, immunometabolic reprogramming, high-density lipoprotein (HDL) dysfunction, and chronic kidney disease (CKD)-associated amplification within a single self-sustaining network that converges on plaque progression and instability.

3.2. Endothelial dysfunction and nitric oxide biology

Endothelial dysfunction is one of the clearest translational expressions of redox injury. Under physiologic conditions, eNOS generates NO, which promotes vasodilation, suppresses platelet activation, limits leukocyte adhesion, and supports an anti-inflammatory endothelial phenotype.[11] When superoxide levels rise, NO is rapidly scavenged to form peroxynitrite, reducing NO bioavailability precisely where vascular homeostasis depends on it.[31]

The problem is compounded by eNOS uncoupling. Oxidative depletion of tetrahydrobiopterin (BH_4) can shift eNOS from an NO-producing enzyme to a superoxide-producing enzyme, effectively converting a protective pathway into an additional source of oxidative stress.[11] Oxidative stress may also impair dimethylarginine dimethylaminohydrolase (DDAH) activity and increase asymmetric dimethylarginine (ADMA), further weakening

NO signaling and reinforcing endothelial dysfunction; DDAH-deficient mice develop elevated ADMA, reduced NO signaling, hypertension, and impaired vascular function, providing direct mechanistic evidence for this axis.[32]

Reduced NO bioavailability favors vasoconstriction, leukocyte–endothelial interactions, platelet–endothelium crosstalk, and impaired vasomotion, all of which are relevant to early atherogenesis and later plaque activity.[33] Endothelial dysfunction precedes overt plaque complications, bridging redox biology and clinically meaningful vascular disease[34] and helping explain why generic systemic antioxidants—which may neither reach the dominant pathologic source nor preserve beneficial ROS-dependent signaling—have not consistently improved outcomes.[13,14] Figure 2 shows how ROS-mediated endothelial dysfunction operates alongside LDL oxidation with foam-cell formation and VSMC migration and proliferation to drive plaque growth and instability.

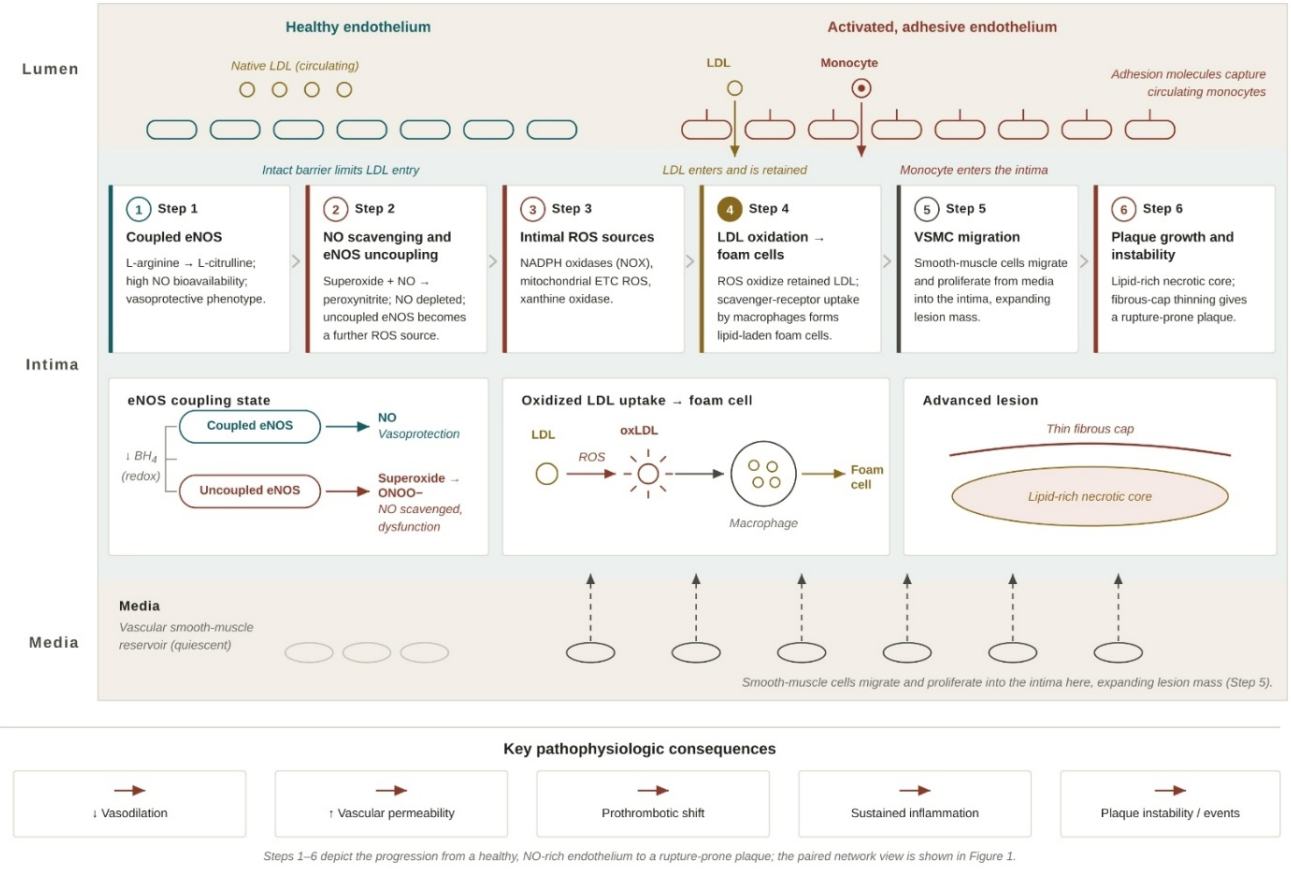

Figure 2. Atherosclerosis: ROS-mediated endothelial dysfunction and plaque development. Schematic depicting ROS-driven progression from healthy endothelium to advanced plaque. Step 1: Coupled eNOS converts *L*-arginine to *L*-citrulline, sustaining high NO bioavailability and a vasoprotective endothelial phenotype. Step 2: Rising $O_2{\cdot}^-$ scavenges NO to form $ONOO^-$, depleting NO bioavailability; eNOS uncoupling further amplifies superoxide generation, converting a protective enzyme into an additional oxidative source. Step 3: Three enzymatic systems—NOX, mitochondrial electron transport, and xanthine oxidase—sustain intimal oxidative stress. Step 4: ROS oxidize retained LDL to oxLDL, which bypasses the regulated LDL-receptor pathway and is internalized via macrophage scavenger receptors, driving foam-cell formation. Step 5: VSMC migration and proliferation from media to intima expand lesion mass. Step 6: Progressive foam-cell accumulation and lipid-rich necrotic core formation, with fibrous cap thinning, produce a rupture-prone plaque. The activated, adhesive endothelium is shown at the right of the luminal surface, with surface adhesion molecules mediating monocyte recruitment. The lower panel summarizes five key pathophysiologic consequences: reduced vasodilation, increased vascular permeability, prothrombotic shift, sustained inflammation, and plaque instability. Notes: Color is used only to distinguish molecular species and cell types: Teal: NO/eNOS; Terracotta: ROS; Ochre: LDL/oxLDL and lipid core; Gray: Foam cells and VSMCs.

Abbreviations: BH4: Tetrahydrobiopterin; eNOS: Endothelial nitric oxide synthase; LDL: Low-density lipoprotein; NO: Nitric oxide; NOX: NADPH oxidase; $O_2{\cdot}^-$: Superoxide anion; $ONOO^-$: Peroxynitrite; oxLDL: Oxidized low-density lipoprotein; ROS: Reactive oxygen species; VSMC: Vascular smooth muscle cell.

3.3. The vascular intima, low-density lipoprotein retention, and lipoprotein modification

The vascular intima is the biologic setting where oxidative stress intersects most directly with the canonical lipoprotein-retention model of atherosclerosis. Native LDL particles cross the endothelium as part of normal lipoprotein trafficking and are not inherently pathogenic when cleared efficiently. The problem begins when apoB-containing particles are retained within the subendothelial matrix through interactions with proteoglycans, prolonging their residence time and increasing their exposure to enzymatic and oxidative modification.[35,36]

Residence time is critical because prolonged retention increases the probability of lipid peroxidation and structural transformation into oxidized LDL. Once modified, LDL is no longer handled primarily through the tightly regulated LDL receptor pathway but is instead taken up via macrophage scavenger receptors, such as CD36 and scavenger receptor class A member 1 (SR-A1), which lack equivalent intracellular feedback constraints and permit progressive cholesterol accumulation and foam-cell formation.[37] In this framework, oxidative modification does not replace the initiating role of lipoprotein retention; it intensifies the biologic consequences of retained lipoproteins.

This distinction matters because it avoids overstating ROS as an alternative to established atherosclerosis biology. Redox imbalance is better understood as an amplifying component within the broader consensus model of disease, especially in high-risk phenotypes with chronic oxidative stress.[16] Framed this way, oxidative modification provides a biologically tractable point of entry for biomarker phenotyping and source-selective intervention within the lipoprotein-retention paradigm, setting the stage for the cell-specific effects discussed next.

3.4. Cell-specific effects within plaque

Atherosclerotic plaques are biologically heterogeneous, and ROS influence multiple cellular compartments. In endothelial cells, oxidative stress reduces NO bioavailability and activates adhesion molecules that promote monocyte recruitment.[8,9] In VSMCs, redox signaling contributes to migration, proliferation, phenotypic switching, and extracellular matrix remodeling, influencing both plaque growth and structural composition.[17] Macrophages integrate lipid handling and inflammatory signaling: ROS promote uptake of oxidized lipoproteins, reinforce cytokine production, and interact with redox-sensitive transcription factors such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), sustaining inflammatory amplification within the lesion.[9,38,39] Oxidative conditions may also favor defective efferocytosis and persistence of necrotic debris, although whether these mechanisms are therapeutically targetable in humans remains uncertain.[40] Collectively, these effects converge on lesion growth, sustained inflammation, matrix turnover, and fibrous-cap weakening,[17] indicating that oxidative stress may influence both plaque initiation and progression while still falling short of proving that ROS-targeted intervention will reduce clinical events.

3.5. From mechanistic plausibility to translational relevance

The biologic literature supports an important role for ROS in endothelial dysfunction, lipoprotein oxidation, inflammatory amplification, and lesion remodeling, particularly in phenotypes such as diabetes, CKD, obesity, smoking exposure, and recurrent events despite guideline-directed therapy.[11] Mechanistic plausibility does not establish ROS as the dominant driver in every patient. Their clinical value depends on whether measurable, source-relevant, phenotype-informed redox disturbance can be demonstrated and modified to improve vascular outcomes.

Viewed through a systems lens, the feed-forward architecture described above—ROS-induced ROS release, eNOS uncoupling, and redox-sensitive inflammatory activation—identifies oxidation and inflammation as reciprocally reinforcing hubs rather than a linear cause-and-effect chain.[18,30] Two consequences follow for how the human literature should be read. First, because these hubs share upstream drivers and downstream effectors, a single oxidative biomarker measured in isolation will inevitably reflect activity distributed across the network rather than a discrete, druggable lesion; this is a structural limitation of reductionist biomarker interpretation, not merely an assay problem.[19] Second, the integration of multidimensional information—oxidative, inflammatory, lipoprotein, and metabolic measures analyzed jointly rather than one marker at a time—is more likely to resolve the phenotype-specific wiring that determines whether redox modulation could plausibly alter outcomes.[20] Network- and multi-omic strategies formalize this integration and are considered in the discussion of future study design.

4. Oxidative stress biomarkers

4.1. The challenge of measuring oxidative stress

Oxidative stress is easier to invoke conceptually than to operationalize clinically. Reactive species are short-lived, biologic compartments differ in redox state, and many measurable products of oxidation reflect downstream injury rather than the upstream source of pathologic

ROS generation. Biomarker studies vary widely in what they measure, how samples are obtained and processed, and how directly the marker maps onto disease-relevant biology.[41]

This heterogeneity has translational consequences. A biomarker may show a biological association with atherosclerosis yet fail to provide incremental clinical value if it lacks analytic reproducibility, standardized thresholds, or a clear relationship to treatment response. Different oxidative markers capture different dimensions of redox injury—lipid peroxidation, protein oxidation, DNA oxidation, myeloperoxidase (MPO)-related inflammatory oxidation, or enzyme-system activation—and no single measure fully characterizes the redox environment of the vessel wall.[41]

A central interpretive distinction follows. Oxidative biomarkers can plausibly support two very different translational uses: (a) *trial enrichment*, in which a biomarker selects participants in whom a candidate pathway is active and trackable, and (b) *treatment selection*, in which a biomarker guides choice of therapy for an individual patient. The evidence supporting trial enrichment is reasonable now—analogous to the role of high-sensitivity C-reactive protein (hsCRP) in CANTOS[6] and to hsCRP-guided enrollment in JUPITER[42]—whereas treatment selection requires demonstration of clinical utility through interventional trials in which biomarker-guided allocation improves outcomes beyond guideline-based care. No current oxidative biomarker meets that second threshold.

4.2. Major classes of oxidative biomarkers

Lipid peroxidation markers—F2-isoprostanes, malondialdehyde (MDA), and oxidized LDL assays—are biologically attractive because lipid oxidation is directly relevant to atherosclerosis, but several of them are limited by preanalytic instability, methodologic inconsistency, or uncertain specificity for vascular oxidative stress.[41] Protein/amino-acid oxidation markers (protein carbonyls, nitrotyrosine-related measures) reflect inflammatory–oxidative crosstalk, and DNA oxidation markers such as 8-oxo-deoxyguanosine (8-oxo-dG) quantify oxidative injury to nucleic acids.[41] Enzyme/pathway markers—MPO, paraoxonase activity, oxidized phospholipids—reflect mixed inflammatory or lipoprotein-related biology rather than a unified measure of oxidative burden.[43,44] Biomarker choice must be guided by the biologic question; association with disease burden does not necessarily predict therapeutic responsiveness.

Table 1 summarizes biomarker classes and their translational status. F2-isoprostanes are used here as a prototype lipid-peroxidation marker for phenotype enrichment and mechanistic trial design (Figure 3).

4.3. Reasons for F2-isoprostanes remaining prominent

F2-isoprostanes have emerged as one of the more attractive candidates for translational work. They are prostaglandin-like compounds formed by free-radical-mediated peroxidation of arachidonic acid, independent of cyclooxygenase activity, and are regarded as relatively robust indicators of *in vivo* lipid peroxidation.[45,46] Their appeal lies partly in analytic maturity, especially when measured using mass spectrometry rather than nonspecific immunoassays.[47]

F2-isoprostanes can be measured in plasma or urine and reflect integrated oxidative injury to lipids rather than the fleeting presence of unstable radical species—important because direct measurement of ROS is often impractical in clinical studies. Elevated F2-isoprostanes in type 2 diabetes were first demonstrated in the mid-1990s and remain a foundational observation; subsequent work has linked them to hypercholesterolemia, endothelial dysfunction, smoking exposure, and vascular disease burden, connecting them to clinical states characterized by chronic redox stress.[48,49]

The emphasis on F2-isoprostanes here is strategic but not exclusive: the framework treats them as one prototype within a broader candidate panel (Table 1) alongside oxidized phospholipids on apoB (OxPL-apoB), MPO, and emerging oxidized lipid measures, rather than as a single-marker strategy.

4.4. Strengths, limitations, and standardization status of F2-isoprostanes

The main strengths of F2-isoprostanes are analytical plausibility, biological relevance, and reproducibility when measured rigorously, particularly 8-iso-$PGF_{2\alpha}$, the most extensively studied species. Mass-spectrometry measurements offer improved specificity over older oxidative assays, and urinary sampling may integrate oxidative burden over time and reduce the technical challenges of transient plasma measurements.[47,50]

Several limitations should be stated explicitly. First, standardization of 8-iso-$PGF_{2\alpha}$ remains incomplete. No widely accepted reference standard or commutable secondary material exists, so results from different mass-spectrometry and immunoassay platforms cannot be assumed to be interchangeable, and immunoassay values frequently overestimate mass-spectrometry values by an order of magnitude.[51] Second, dietary fat composition modulates F2-isoprostane levels: ω-3 polyunsaturated fatty

Table 1. Candidate oxidative stress biomarkers relevant to atherosclerosis

Biomarker	Sample type	Assay maturity	Key strengths	Key limitations	Clinical role	Translational priority
F2-isoprostanes	Plasma, urine	MS preferred; ELISA available	Strong biologic plausibility for lipid peroxidation; relatively robust analytically; measurable in multiple matrices	No standardized clinical thresholds; does not identify ROS source or tissue compartment; variable across platforms	Research tool for phenotype enrichment and mechanistic trials	High
Oxidized LDL	Plasma, serum	Multiple platforms; variable standardization	Direct relevance to foam cell formation and atherogenic biology; conceptually aligned with lipoprotein retention	Substantial assay variability; measures not always comparable across platforms; epitope heterogeneity	Research use; not standardized for clinical decisions	Moderate
MPO	Plasma, serum	Commercial ELISA; MS methods	Links inflammation and oxidative pathways; relevant in ACS; FDA-cleared assays exist	Reflects inflammatory activation more than pure oxidative stress; incremental value over troponin unclear	Investigational risk marker; may complement troponin in ACS	Moderate
MDA (TBARS)	Plasma, urine, tissue	Colorimetry; poor specificity	Historically common; technically simple; low cost	Limited specificity; high susceptibility to artifact; analytic variability; poor correlation with specific pathways	Limited translational value; largely replaced by more specific markers	Low
8-oxo-dG	Urine, plasma	ELISA and LC-MS/MS	Reflects oxidative DNA damage; integrates systemic oxidative burden; stable in urine	Less specific to vascular/atherosclerotic processes; unclear incremental cardiovascular utility	Exploratory research marker; broader oxidative stress assessment	Low–moderate
Protein carbonyls	Plasma, serum	Spectrophotometry and ELISA	General marker of protein oxidation; may reflect inflammatory-oxidative crosstalk	Nonspecific; limited standardization; unclear relationship to atherosclerotic mechanisms	Exploratory research only	Low
Nitrotyrosine	Plasma, tissue	ELISA, IHC, MS	Marker of peroxynitrite formation and nitrosative stress; mechanistic relevance to NO biology	Interpretation complex; preanalytical challenges; standardization incomplete; not cardiovascular-specific	Exploratory research; mechanistic studies of NO dysfunction	Low–moderate

Notes: Translational priority reflects combined assessment of analytic maturity, biologic relevance, feasibility for phenotype-enriched trials, and potential to inform precision prevention strategies. F2-isoprostanes represent the most mature candidate for biomarker-enriched translational investigation based on current evidence.

Abbreviations: 8-oxo-dG: 8-oxo-deoxyguanosine; ACS: Acute coronary syndrome; ELISA: Enzyme-linked immunosorbent assay; FDA: Food and Drug Administration; IHC: Immunohistochemistry; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; LDL: Low-density lipoprotein; MDA: Malondialdehyde; MPO: Myeloperoxidase; MS: Mass spectrometry; NO: Nitric oxide; ROS: Reactive oxygen species; TBARS: Thiobarbituric acid reactive substances.

acids lower urinary and plasma F2-isoprostanes, whereas trans-fatty acids and certain Western dietary patterns raise them, independently of underlying vascular biology.[52] Third, no validated clinical decision cutoffs exist for either plasma or urinary F2-isoprostanes; reported associations rely on within-cohort quartiles or relative thresholds rather than commutable absolute values.[51] Fourth, interpretive specificity is limited: F2-isoprostanes reflect systemic lipid peroxidation but do not identify the dominant enzymatic source, the relevant tissue compartment, or

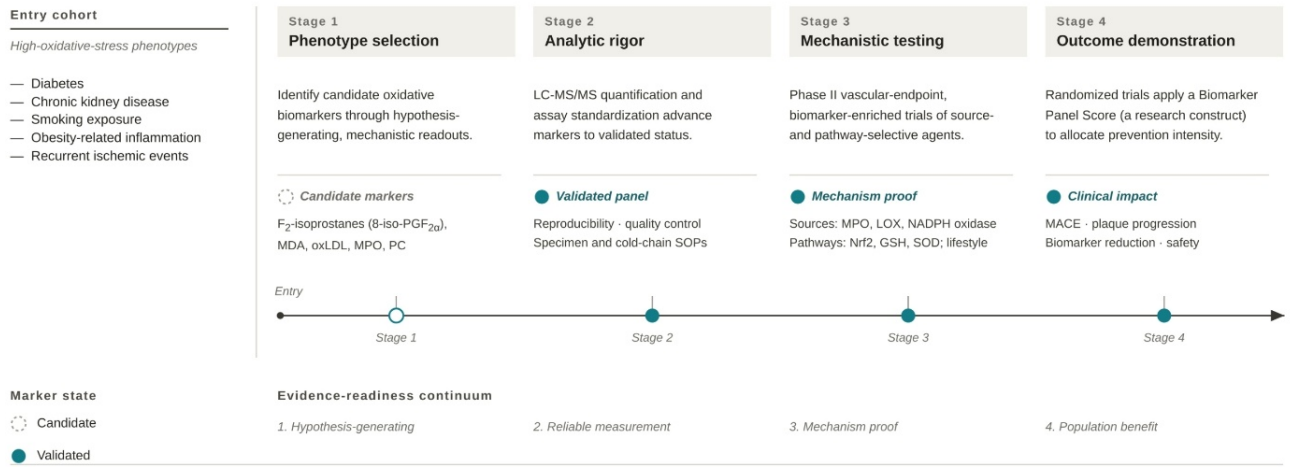

Figure 3. A staged translational framework integrating oxidative biomarkers for prevention in atherosclerosis. Five high-oxidative-stress phenotypes—diabetes, CKD, smoking exposure, obesity-related inflammation, and recurrent ischemic events despite guideline-directed therapy—are carried through four sequential stages aligned with the evidence-readiness continuum. Stage 1 (phenotype selection) identifies candidate oxidative biomarkers (F_2-isoprostanes [8-iso-$PGF_{2\alpha}$], MDA, oxLDL, MPO, PC; dashed circles) through hypothesis-generating mechanistic readouts. Stage 2 (analytic rigor) requires LC–MS/MS quantification and assay standardization before markers advance to validated status (solid circles). Stage 3 (mechanistic testing) employs coordinated biomarker panels in phase II, vascular-endpoint, biomarker-enriched trials targeting enzyme sources (MPO, LOX, NADPH oxidase), antioxidant pathways (Nrf2, GSH, SOD), and lifestyle modification. Stage 4 (outcome demonstration) applies a composite Biomarker Panel Score—a research stratification construct, not a validated clinical instrument—to allocate prevention intensity, with randomized controlled trials measuring MACE, plaque progression, biomarker reduction, and safety against the standard of care. This framework constitutes a precision-prevention research agenda and is not intended to guide individual clinical decisions. For visual clarity, the five phenotypes are represented once at a single entry cohort and then carried through the four stages along one linear progression axis, rather than repeating phenotype and biomarker icons within each stage.
Abbreviations: GSH: Glutathione; LC–MS/MS: Liquid chromatography–tandem mass spectrometry; LOX: Lipoxygenase; MACE: Major adverse cardiovascular events; MDA: Malondialdehyde; MPO: Myeloperoxidase; NADPH: Nicotinamide adenine dinucleotide phosphate; Nrf2: Nuclear factor erythroid 2-related factor 2; oxLDL: Oxidized low-density lipoprotein; PC: Protein carbonyls; SOD: Superoxide dismutase.

whether the oxidative signal is upstream, downstream, or parallel to other active processes. In patients with systemic inflammation, kidney disease, smoking exposure, or metabolic illness, elevated F2-isoprostanes may index a high-oxidative-stress phenotype without pinpointing the causal node most amenable to therapy.

A preanalytic consideration deserves particular emphasis because it directly affects Stage 2 (analytic rigor) of the framework proposed here: the specimen matrix and handling materially influence F2-isoprostane validity. In plasma, the abundance of arachidonic acid substrate makes samples susceptible to ex vivo autooxidation, and platelet cyclooxygenase can generate isoprostane-related species after collection, so that plasma measurements require strict immediate processing—rapid cooling, prompt centrifugation, addition of antioxidant preservative (for example, butylated hydroxytoluene), and storage at −80 °C—to avoid spuriously elevated values.[53] Urinary F2-isoprostanes and their metabolites are comparatively more chemically stable, integrate oxidative burden over time, and are less vulnerable to ex vivo artifacts, which are principal reasons urinary sampling is often preferred for epidemiologic and trial use; urinary specimens nonetheless benefit from antioxidant preservation and controlled frozen storage to maintain stability.[53,54] Consequently, any biomarker-enriched design must prespecify matrix, collection, preservative, and cold-chain protocols, because cross-study discordance in F2-isoprostane values is frequently attributable to preanalytic handling rather than to true biologic difference.[54]

Taken together, these constraints mean that F2-isoprostanes are well-suited for within-study phenotype enrichment, longitudinal mechanistic monitoring, and biomarker-anchored early-phase trials, but are not ready for cross-trial risk stratification, treatment selection, or routine clinical decision-making.

4.5. Comparison with other candidate markers and emerging oxidized-lipid measures

Several oxidative biomarkers beyond F2-isoprostanes have shown cardiovascular relevance, but few have progressed from associative evidence to clinically actionable use. Oxidized LDL assays directly quantify modified lipoproteins implicated in foam-cell formation, yet assay

heterogeneity continues to limit standardization and cross-study comparability.[44] MPO bridges oxidative and inflammatory pathways in neutrophil-rich states and acute coronary syndromes, although it may reflect inflammatory activation at least as much as oxidative burden.[43] MDA and related thiobarbituric-acid–reactive substances retain historical importance but remain constrained by nonspecificity and analytic variability.[41]

A conceptually distinct axis is the functional quality of HDL rather than its concentration. HDL cholesterol-efflux capacity—the ability of HDL to accept cholesterol from lipid-laden macrophages—is inversely associated with incident cardiovascular events independently of HDL-cholesterol level, and captures a protective function that static lipid measures miss.[55,56] Crucially, HDL is itself a target of oxidative modification: MPO-catalyzed oxidation of apolipoprotein A-I generates dysfunctional HDL with impaired efflux and blunted antioxidant activity, and can convert HDL from a protective to a pro-inflammatory particle.[57] Oxidized or dysfunctional HDL therefore sits at the intersection of the oxidative and lipoprotein hubs and is particularly relevant in high-oxidative-stress phenotypes, such as CKD and diabetes, where efflux capacity is reduced; as a biomarker construct, it remains investigational, constrained by assay standardization and the same trial-enrichment-versus-treatment-selection distinction applied throughout this review.[56]

Two conclusions follow. First, no single oxidative biomarker has emerged as a universally accepted clinical standard in ASCVD. Second, the appeal of F2-isoprostanes lies in their greater analytical maturity, biological coherence, and measurement feasibility. Within the staged framework developed here, analytic rigor and independent prognostic association are necessary but not sufficient for clinical adoption; the relevant question is whether a biomarker improves risk classification, alters treatment allocation, or enriches trials for patients most likely to benefit from mechanism-directed interventions.

Table 2 summarizes prognostic and risk-reclassification performance for major oxidative biomarkers. In postmenopausal women, higher urinary 8-iso-$PGF_{2\alpha}$ has been associated with substantially greater risk of fatal cardiovascular disease after multivariable adjustment, supporting independent prognostic relevance.[58] OxPL-apoB improves risk reclassification when added to traditional risk factors, particularly in intermediate-risk populations.[44,59] F2-isoprostanes function as prototype biomarkers for phenotype enrichment and mechanistic trial design rather than as established clinical risk markers; their incremental contribution to risk prediction beyond conventional variables remains uncertain.

The OxPL-apoB complex illustrates how oxidized lipid measures anchored in apoB and lipoprotein(a) biology can be integrated into a staged precision-prevention strategy. Across multiple cardiovascular cohorts, higher OxPL-apoB levels have been associated with increased events independent of traditional risk factors, and incorporation into conventional risk models has improved reclassification, particularly in intermediate-risk groups.[44,60] Even so, improved reclassification alone does not establish clinical utility, and biomarker-guided treatment allocation has not yet been shown to improve hard cardiovascular outcomes beyond guideline-based care.[59] OxPL-apoB assays are best positioned as candidate biomarkers for future application of this framework—for example in deep LDL lowering, residual lipoprotein(a)-related risk, or lipoprotein(a)-enriched trial populations—rather than as established clinical tests.

Taken together, several oxidative biomarkers show independent prognostic associations, few have robust incremental risk-classification evidence, and none meet the evidentiary threshold for routine use in preventive cardiology. Their most defensible role is in phenotype characterization, mechanistic hypothesis testing, and trial enrichment within a staged precision-prevention framework.

4.6. Inflammation–oxidation overlap and biomarker positioning

Inflammation and oxidative stress are mechanistically intertwined and clinically overlapping. A central molecular link is the NLRP3 inflammasome, a cytosolic multiprotein complex that couples redox and metabolic stress to sterile vascular inflammation. Mitochondrial and NOX-derived ROS, cholesterol crystals, and oxidized LDL are among the signals that license NLRP3 assembly, driving caspase-1 activation and maturation of the pro-atherogenic cytokines IL-1β and IL-18.[61] This positions the inflammasome as a redox-sensitive node that converts oxidative and lipid stress into a defined inflammatory output, and provides a mechanistic bridge between the oxidative biology emphasized here and the inflammatory pathway validated clinically by IL-1β blockade in CANTOS.[6] Pharmacologic interest in this axis is expanding, with NLRP3-directed and downstream IL-1 strategies under active cardiovascular investigation.[62] ROS also activate redox-sensitive transcription factors (e.g., NF-κB, AP-1), while inflammatory cells generate ROS through NOX2 and MPO; conversely, hsCRP has been linked to endothelial NO suppression and altered macrophage LDL handling.[9] The clinical literature on residual risk has, to date, advanced furthest on the inflammation side: CANTOS established that on-treatment hsCRP < 2 mg/L identifies patients

Table 2. Prognostic and risk-reclassification performance of oxidative stress biomarkers in cardiovascular disease

Biomarker	Study population and design	*N*	Follow-up	Independent prognostic association	Incremental risk reclassification	Evidence level
Urinary F2-IsoP (8-iso-$PGF_{2\alpha}$)	Postmenopausal women (WHI); prospective cohort	~1,000	18 years	Adjusted HR 1.80 (95% CI 1.16–2.81) for fatal CVD; top vs. bottom quartile	*C*-statistic, NRI, IDI not reported; association established, but incremental value unproven	Moderate
OxPL-apoB	Multiple CVD cohorts; meta-analysis and prospective studies	Variable	5–15 years	OR (1.35–1.58) per SD increase across cohorts; independent of traditional RF	NRI improvement in intermediate-risk groups (reported in EPIC-Norfolk and others)	Moderate–high
Plasma MPO	ACS and stable CAD cohorts; multiple prospective studies	2,000–5,000	1–5 years	HR 1.2–2.5 for MACE, depending on cohort; often independent of troponin and CRP	Modest *C*-statistic improvement (0.01–0.03); NRI data limited	Moderate
Oxidized LDL (multiple assays)	Mixed primary and secondary prevention cohorts	Variable	Variable	Inconsistent; HR 1.1–1.8 across studies; highly assay-dependent	Limited reclassification data; assay heterogeneity limits interpretation	Low–moderate
Urinary 8-oxo-dG	General population and diabetic cohorts	500–3,000	5–10 years	Modest association (HR 1.2–1.4); less cardiovascular-specific	No formal NRI/IDI data available; incremental value unclear	Low
Protein carbonyls	Limited prospective CVD studies	<500	Variable	Association data is sparse and inconsistent	No reclassification studies were identified	Low

Notes: Evidence level reflects quality and consistency of prognostic data, sample size, follow-up duration, and availability of formal risk-reclassification metrics. Independent prognostic association refers to multivariable-adjusted models including traditional cardiovascular risk factors. Incremental risk reclassification assesses whether biomarker addition improves discrimination (*C*-statistic), calibration, or classification (NRI/IDI) beyond established models. Most oxidative biomarkers show independent association but lack robust reclassification evidence, limiting routine clinical implementation. Abbreviations: ACS: Acute coronary syndrome; apoB: Apolipoprotein B; CAD: Coronary artery disease; CI: Confidence interval; CRP: C-reactive protein; CVD: Cardiovascular disease; F2-IsoP: F2-isoprostanes; HR: Hazard ratio; IDI: Integrated discrimination improvement; LDL: Low-density lipoprotein; MACE: Major adverse cardiovascular events; MPO: Myeloperoxidase; NRI: Net reclassification improvement; OR: Odds ratio; OxPL: Oxidized phospholipids; RF: Risk factors; SD: Standard deviation; WHI: Women's Health Initiative.

deriving the greatest absolute IL-1β–blockade benefit,[6] and roughly half of statin-treated atherosclerosis patients carry residual inflammatory risk defined by hsCRP ≥ 2 mg/L despite achieving LDL-C goals.[63] No comparable population-scale quantification yet exists for oxidative biomarkers, and head-to-head studies establishing the incremental value of an oxidative panel beyond hsCRP remain limited.

This has two implications for the framework proposed here. First, oxidative biomarkers should be pursued *alongside* rather than instead of inflammatory markers, because they may capture biology that hsCRP misses—particularly in smoking, diabetes, and CKD where inflammation and oxidative injury are differentially active. Second, biomarker-enriched designs should consider stratifying by hsCRP, oxidative biomarker status, or both to identify subgroups most potentially to benefit from pathway-specific intervention.

A complementary layer is immunometabolism: the recognition that the metabolic state of vascular and

myeloid cells governs their inflammatory and oxidative output. In atherosclerosis, lesional macrophages undergo metabolic reprogramming—shifts in glycolysis, oxidative phosphorylation, and mitochondrial redox handling—that determines cytokine production, efferocytosis, and lipid processing, linking cellular bioenergetics directly to plaque inflammation.[64] Uptake of oxidized LDL and other danger signals can reprogram monocytes toward a durable pro-inflammatory, pro-oxidant phenotype, a process related to "trained immunity" in which epigenetic and metabolic rewiring sustains heightened responsiveness long after the initial stimulus.[65,66] This framework reinforces the systems view: oxidative, metabolic, and inflammatory hubs are not merely correlated but causally coupled through shared metabolic control points, and biomarker strategies that ignore this coupling risk mislabeling a networked disturbance as a single-pathway defect.[20,64]

4.7. Current clinical role of oxidative biomarkers

Oxidative biomarkers remain research tools rather than instruments of routine cardiovascular decision-making. Their defensible current uses are phenotype characterization, biomarker-enriched enrollment for mechanistic and early-phase trials, and exploratory risk stratification. Whether any oxidative biomarker adds sufficient incremental value beyond traditional risk factors, hsCRP, and imaging to justify routine implementation has not been demonstrated. For F2-isoprostanes and OxPL-apoB, the key questions are whether standardized assays identify clinically meaningful high-oxidative-stress phenotypes and whether biomarker-guided interventions improve outcomes beyond guideline-directed care.

5. High-risk phenotypes and residual risk

Several clinical phenotypes provide a biologically plausible setting for oxidative phenotyping because they combine persistent metabolic or inflammatory stress with substantial residual cardiovascular risk despite contemporary preventive therapy. This review emphasizes high-oxidative-stress cardiometabolic phenotypes—diabetes, CKD, smoking exposure, obesity-associated inflammation, and recurrent ischemic events despite guideline-directed therapy—as candidates for biomarker-informed phenotype characterization and trial enrichment.

5.1. Diabetes and metabolic syndrome

Diabetes mellitus and metabolic syndrome are among the most plausible high-oxidative-stress phenotypes. Hyperglycemia, insulin resistance, AGEs, mitochondrial dysfunction, endothelial activation, and low-grade inflammation favor chronic ROS generation and impaired antioxidant buffering. [25-27] Residual cardiovascular risk remains substantial even when LDL-C and glycemic indices are optimized, raising the possibility that oxidative biomarkers capture a dimension of vascular injury not fully reflected by glucose, lipid, or blood-pressure data.[67] This role is stronger at the level of phenotype characterization than as a validated basis for treatment decisions.

5.2. Chronic kidney disease

Chronic kidney disease warrants separate consideration because the associated cardiovascular disease is increasingly understood not as accelerated conventional atherosclerosis, but as a partly distinct vascular phenotype with its own dominant mechanisms.[68,69] Rather than a lipid-driven intimal process alone, CKD-associated cardiovascular disease is characterized by disproportionate medial and intimal vascular calcification, arterial stiffening, and a mineral-bone axis (CKD–mineral and bone disorder) in which dysregulated calcium-phosphate handling, hyperphosphatemia, and altered fibroblast growth factor-23/klotho signaling actively promote vascular calcification.[70] Superimposed on this are retained protein-bound uremic toxins (for example, indoxyl sulfate and *p*-cresyl sulfate) that are poorly cleared by dialysis and directly induce endothelial oxidative stress, NOX activation, and pro-inflammatory signaling.[70] CKD further entails a state of systemic immune dysregulation and persistent low-grade inflammation, together with metabolic reprogramming of vascular and immune cells, that amplifies redox imbalance beyond what traditional risk factors predict.

Lipoprotein biology is also qualitatively altered. Uremia drives remodeling of lipoprotein particles and, in particular, a shift toward dysfunctional, oxidatively modified HDL with impaired cholesterol-efflux and antioxidant capacity, so that HDL may lose its protective role or become pro-inflammatory.[55,71] Proteomic studies of the CKD–atherosclerosis interface reinforce this distinction: label-free and monocyte-focused proteomic analyses from the Łuczak group have shown that the molecular signatures of CKD-related atherosclerosis differ from those of classical coronary artery disease, implicating oxidative-stress–linked and inflammatory protein networks specific to the uremic milieu rather than a simple intensification of the usual atherogenic program.[68,69,71] These observations position CKD as a paradigmatic example of why a network- and multi-omic view is required: the same nominal biomarker (an elevated oxidative marker) can reflect mechanistically different disease depending on phenotype. CKD also illustrates the interpretive hazard directly—altered renal clearance and systemic inflammation can elevate oxidative markers through pathways only partly related to vascular injury—reinforcing that oxidative biomarker inference

in CKD should remain confined to research settings and mechanistically anchored study designs rather than routine clinical stratification.

5.3. Smoking and obesity-related inflammation

Smoking exposure and obesity-associated inflammation are common oxidative phenotypes with clear mechanistic links to vascular injury. Tobacco smoke directly introduces oxidants while promoting endothelial dysfunction, inflammation, platelet activation, and lipoprotein modification.[48,72] Obesity creates a metabolic–inflammatory environment that amplifies vascular oxidative stress through insulin resistance, altered adipokine signaling, and chronic low-grade inflammation.[73] Both states are often associated with persistent residual cardiovascular risk despite standard prevention. Here, oxidative biomarkers are best viewed as tools for phenotype characterization and enrichment of mechanistic or early-phase intervention trials rather than as stand-alone clinical tests.

5.4. Recurrent ischemic events despite guideline-directed therapy

Patients with recurrent myocardial infarction or ischemic stroke despite intensive lipid lowering and contemporary secondary prevention represent a clinically distinct residual-risk phenotype. Very low achieved LDL-C in trials of ezetimibe and PCSK9 inhibition did not abolish events in this group, underscoring the contribution of nonlipid pathways including endothelial dysfunction, inflammation, thrombosis, and oxidative stress.[4,5,74] Within the framework proposed here, these patients are candidates for multidomain residual-risk phenotyping using oxidative biomarkers alongside inflammatory markers, imaging, and metabolic profiling to enrich early mechanistic and vascular endpoint studies.

5.5. Sex- and age-related modifiers of vascular redox biology

Sex and age are biologically meaningful modifiers of vascular redox state and should be treated as prespecified stratification or interaction variables in oxidative biomarker studies and early-phase redox-modulating trials rather than as background adjustment variables. Three lines of evidence support this.

First, sex differences in vascular NOX activity are well documented. NOX activity and expression of NOX1 and NOX4 are approximately two- to three-fold lower in cerebral and systemic arteries of female compared with male animals; ovariectomy abolishes this protection while 17β-estradiol replacement restores it, indicating that endogenous estrogen suppresses vascular NOX activity.[75,76] Accordingly, in vivo biomarkers of oxidative stress, including F2-isoprostanes, tend to be higher in healthy young men than in age-matched premenopausal women,[76] and women appear more likely than men to carry residual inflammatory risk after acute myocardial infarction,[63] suggesting sex-specific patterns in both oxidative and inflammatory residual-risk biology.

Second, aging is associated with rising vascular oxidative stress, increased NOX2 expression, declining endogenous antioxidant reserve, and progressive endothelial dysfunction.[76] In aged vessels, eNOS activity falls, inducible nitric oxide synthase (iNOS) rises, and superoxide-mediated NO scavenging contributes to impaired flow-mediated and acetylcholine-induced dilation.[76] These changes parallel population-level increases in arterial stiffness, pulse-wave velocity, and atherosclerotic burden with age.

Third, the menopausal transition introduces a discrete shift: loss of endogenous estrogen is associated with higher vascular NOX activity, higher systemic oxidative biomarkers, and accelerated endothelial dysfunction, particularly in women with cardiometabolic risk factors.[75]

These observations have direct design implications. Phase II oxidative-biomarker trials should prespecify sex- and age-stratified analyses, consider menopausal status when enrolling women, and use within-sex biomarker thresholds where appropriate. Failure to account for these modifiers risks diluting genuine treatment effects in responsive subgroups and overgeneralizing findings across heterogeneous populations.

5.6. Residual risk in the modern prevention era

The concept of residual risk has become more concrete in the era of contemporary lipid-lowering therapy. As shown in Table 3, FOURIER, ODYSSEY OUTCOMES, and IMPROVE-IT each demonstrated event reduction with progressively deeper LDL-C lowering, yet substantial residual event rates persisted in every trial (9.8% in FOURIER over 2.2 years, 9.5% in ODYSSEY OUTCOMES, and 32.7% in IMPROVE-IT [7-year Kaplan–Meier rate; median follow-up ≈ 6 years]).[4,5,74] Despite these reductions, substantial myocardial infarctions and strokes still occurred, indicating that pathways beyond cholesterol transport—including residual inflammatory risk, thrombosis, and platelet activation—continue to contribute to disease progression and event generation.[77,78]

Anti-inflammatory trials further demonstrate that nonlipid pathways can be clinically actionable. CANTOS established proof of principle for IL-1β blockade in residual inflammatory risk; LoDoCo2 and COLCOT extended the concept to colchicine.[6,7,79] These studies do not establish oxidative stress as the dominant upstream mechanism,

Table 3. Residual cardiovascular event rates in contemporary lipid-lowering trials

Trial (population)	*N*	Follow-up (median)	Therapy and achieved LDL-C	MACE rate (active)	MACE rate (control)	Implication for residual risk
FOURIER (stable ASCVD on statin)	27,564	2.2 years	Evolocumab + statin; median LDL-C 30 mg/dL	9.8%	11.3%	Despite median LDL-C of 30 mg/dL, approximately 1 in 10 patients experienced MACE, highlighting substantial residual risk beyond LDL lowering
ODYSSEY OUTCOMES (post-ACS on high-intensity statin)	18,924	2.8 years	Alirocumab + statin; median LDL-C 40–50 mg/dL	9.5%	11.1%	Even with PCSK9 inhibition after ACS, residual event rate remained ~10%, suggesting non-LDL pathways contribute to recurrent events
IMPROVE-IT (post-ACS)	18,144	6.0 years	Ezetimibe + simvastatin; median LDL-C 54 mg/dL	32.7%	34.7%	Longer follow-up reveals that even modest additional LDL lowering provides benefit, yet one-third still experienced events over 6 years

Note: Data presented demonstrate that contemporary intensive lipid-lowering therapy substantially reduces but does not eliminate cardiovascular events, providing rationale for investigation of complementary pathways including oxidative stress.
Abbreviations: ACS: Acute coronary syndrome; ASCVD: Atherosclerotic cardiovascular disease; LDL-C: Low-density lipoprotein cholesterol; MACE: Major adverse cardiovascular events (composite typically including cardiovascular death, myocardial infarction, and stroke); PCSK9: Proprotein convertase subtilisin/kexin type 9.

because inflammation, thrombosis, clonal hematopoiesis-related biology, metabolic dysregulation, and vascular remodeling may each contribute independently or interactively to residual risk.[80] Oxidative stress represents one plausible component of this multidomain biology, with relevance likely to vary by phenotype and disease stage.

5.7. Lessons from biomarker-enriched anti-inflammatory trials

Biomarker-enriched anti-inflammatory outcome trials provide a precedent for pathway-focused residual-risk strategies. Approximately half of statin-treated atherosclerosis patients carry residual inflammatory risk defined by hsCRP ≥ 2 mg/L despite achieving LDL-C goals, establishing a population-scale rationale for inflammation-targeted enrichment.[63] CANTOS operationalized this principle by enrolling prior-MI patients only if hsCRP remained ≥ 2 mg/L despite statin therapy, enriching for an active IL-1β–IL-6–CRP axis.[6] Event reduction was modest overall, but participants achieving on-treatment hsCRP < 2 mg/L derived greater absolute and relative benefit, with hsCRP functioning as both risk and response marker.[6] CIRT, by contrast, enrolled patients with stable coronary disease and diabetes or metabolic syndrome without requiring elevated hsCRP; methotrexate neither lowered IL-1β, IL-6, or hsCRP nor reduced events.[81] Together, these trials suggest that targeted pathway inhibition is more likely to succeed when enrollment is restricted to patients in whom the relevant biology is demonstrably active and trackable with a reliable biomarker.

CLEAR provides a complementary example in a statin-intolerant population, where bempedoic acid lowered LDL-C by approximately 21%, reduced hsCRP by approximately 22%, and decreased major adverse cardiovascular events (MACE) by 13%.[82] Although hsCRP was not an entry criterion, the parallel changes in lipids and inflammation illustrate how overlapping residual-risk domains can be quantified by distinct biomarker signals. Within the framework summarized in Figure 3, F2-isoprostanes are intended to play a conceptually analogous role in early oxidative-stress research—as an analytically mature biomarker used to enrich early-phase mechanistic and source-selective trials for patients with high oxidative burden—aligning with a broader precision-prevention model in which phenotype selection and biomarker-defined residual risk precede large-scale outcome testing.[83]

6. Therapeutic implications and future directions

6.1. Lessons from failed broad antioxidants

Vitamin-based antioxidant strategies did not produce consistent reductions in cardiovascular events, and this remains the most important historical objection to clinical translation of oxidative stress.[12,13,84,85] Nonspecific systemic scavenging was biologically misaligned with compartmentalized, source-specific, phenotype-dependent redox injury: broad antioxidants may not reach the subendothelial space, mitochondrial membranes, or intracellular microdomains where disease-relevant ROS are generated; may fail to suppress dominant enzymatic sources such as NOX systems and xanthine oxidase; and may interfere with beneficial ROS-dependent signaling. Past trials also did not enroll patients on the basis of measured oxidative burden, potentially diluting benefit within responsive subgroups.[14,15]

6.2. Established therapies that engage redox pathways

Several established cardiovascular therapies engage redox biology in preclinical and translational studies, although the contribution of redox modulation to their clinical outcome benefit has not been isolated in humans. Statins are the clearest mechanistic example: beyond LDL lowering, they reduce isoprenoid intermediates required for prenylation of small GTPases such as Ras-related C3 botulinum toxin substrate 1 (Rac1), attenuating NOX activity, improving endothelial NO bioavailability, and reducing vascular superoxide generation.[86,87] Renin–angiotensin system blockade provides a second example: angiotensin II and aldosterone activate vascular NOX preclinically, and angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers attenuate this axis experimentally, though the contribution to clinical benefit beyond blood-pressure reduction remains undefined.[88] Allopurinol, a xanthine oxidase inhibitor, offers a more direct mechanistic case because xanthine oxidase itself generates superoxide and hydrogen peroxide, although event-level evidence remains mixed.[29]

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) and metformin provide cardiometabolic examples where endothelial and redox effects have been characterized more explicitly. GLP-1 RAs improve endothelial function, reduce vascular oxidative stress, and attenuate inflammatory signaling in experimental models and human studies,[89] in parallel with large-scale outcome trials demonstrating reductions in major adverse cardiovascular events in type 2 diabetes.[90] Metformin similarly prevents hyperglycemia-associated endothelial oxidative stress, improves microvascular and coronary endothelial function,[91] and has been associated with reduced cardiovascular events that appear disproportionate to glycemic lowering alone.[92] These two agents therefore represent widely used, guideline-supported cardiometabolic therapies with relatively well-developed links to vascular oxidative stress pathways.

JUPITER provides a complementary clinical illustration of statin pleiotropy: in apparently healthy adults with LDL-C < 130 mg/dL and hsCRP ≥ 2 mg/L, rosuvastatin reduced both LDL-C and hsCRP and lowered major cardiovascular events by 44%, demonstrating that contemporary statins engage inflammatory—and by extension redox-coupled—pathways in tandem with lipid lowering.[42] Collectively, these therapies show that clinically effective cardiovascular drugs often intersect with oxidative pathways. They should not be interpreted as proof-of-concept ROS-directed therapies; their established clinical benefit derives from LDL lowering, blood-pressure reduction, or glycemic control, with redox engagement remaining a plausible but unquantified contributor.

6.3. Source-selective and tissue-targeted strategies

The major therapeutic challenge is whether redox biology can be targeted more selectively than in earlier antioxidant paradigms. In principle, inhibition of discrete enzymatic ROS sources, modulation of oxygen-sensing pathways, mitochondria-targeted approaches, or lesion-focused delivery systems may offer advantages over broad scavenging.[22] Figure 3 situates these established and emerging therapies within the staged translational trajectory—from exploratory mechanistic readouts to biomarker-enriched trials and, only if successful, outcome-driven integration into preventive care.

Source-selective targeting is biologically plausible but insufficiently validated. Mitochondria-targeted antioxidants (e.g., MitoQ, SS-31) and ROS-responsive or plaque-tropic nanoparticle delivery systems exemplify approaches consistent with broader trends in translational medicine, where context-dependent drug activation and lesion-specific delivery are increasingly valued.[93,94] Major uncertainties remain regarding safety, biodistribution, off-target effects, reproducibility across vascular beds, and whether biomarker-defined enrichment can identify patients most likely to benefit. These approaches should be presented as exploratory rather than near-term solutions.

Beyond source selectivity, the most consequential near-term advance may be analytic rather than pharmacologic. Because oxidation behaves as one hub in an interacting disease network, single-marker strategies are structurally limited, and integration of multidimensional data through

systems- and network-medicine approaches offers a more realistic route to clinically meaningful stratification.[18,20] Multi-omic profiling—coupling oxidative-lipidomic measures with proteomic, metabolomic, and inflammatory data—can, in principle, resolve phenotype-specific wiring (as illustrated by proteomic dissection of CKD-associated atherosclerosis) and identify patients in whom redox modulation is likely to matter.[20,71] Embedding such integrative analytics within the biomarker-enriched designs proposed here, rather than pursuing isolated oxidative endpoints, is the more defensible path from mechanistic plausibility toward precision cardiovascular prevention.[19]

6.4. A four-stage translational framework for biomarker-enriched precision prevention

We propose a four-stage translational framework as the manuscript's central synthesis: it links phenotype selection, analytically rigorous biomarker assessment, mechanistic and source-selective interventional testing, and only then outcome demonstration (Figure 3). The framework is a research agenda, not a clinical algorithm, intended to discipline how oxidative biomarkers and redox-directed therapies enter the cardiovascular evidence base:

(i) Phenotype selection. Identify patient groups in whom persistent oxidative vascular injury is biologically plausible—diabetes, CKD, smoking exposure, obesity-associated inflammation, or recurrent events despite aggressive contemporary therapy—with prespecified attention to sex, age, and menopausal status as effect modifiers.

(ii) Analytic rigor. Measure oxidative biomarkers with standardized methods, ideally emphasizing biomarkers with relatively mature performance characteristics such as mass-spectrometry F2-isoprostanes, alongside complementary measures (OxPL-apoB, MPO) and inflammatory markers (hsCRP) to enable cross-domain stratification.[47]

(iii) Mechanistic and interventional testing. Candidate therapies should be judged not only by biomarker change but also by improvement in endothelial function (flow-mediated dilation, reactive hyperemia), vascular imaging (carotid intima-media thickness, coronary plaque burden,[18] F-fluorodeoxyglucose [FDG] positron emission tomography [PET]–computed tomography vascular inflammation, intravascular ultrasound), plaque-related biology, or other intermediate indicators that plausibly connect to atherosclerotic risk.[34]

(iv) Outcome demonstration. Demonstrate that biomarker-informed or source-informed interventions improve hard cardiovascular outcomes beyond current standard care.

Table 4 illustrates how this framework could be operationalized through biomarker-enriched phase II designs across five high-risk phenotypes, with explicit proposals for sample size, candidate agents, primary endpoints, and feasibility constraints. These designs are illustrative rather than prescriptive: the suggested 100–400-participant cohorts and 12–52-week timelines are design templates that require formal power calculations against pilot biomarker variance, anticipated effect size, and assay coefficients of variation before protocol finalization. Candidate agents range from repurposed drugs (allopurinol, GLP-1 receptor agonists, metformin) to investigational classes (mitochondria-targeted antioxidants, source-selective NOX inhibitors, N-acetylcysteine). Where conventional surrogates such as flow-mediated dilation or carotid intima-media thickness are limited, pulse-wave velocity, vascular FDG-PET, or oxidized-lipid biomarker composites can provide mechanistic confirmation.

The framework makes explicit four assertions often left implicit in the redox-cardiovascular literature: broad antioxidant trials failed largely because of misaligned phenotype, marker, and target; biomarker-enriched trial design—not biomarker-guided treatment selection—is the defensible near-term application; sex, age, and menopausal status are effect modifiers, not nuisance covariates; and progression to outcome testing requires both vascular and mechanistic endpoint success at each prior stage.

6.5. Translational summary for clinicians and trialists

For clinicians, oxidative stress is biologically informative but not validated as a basis for routine therapeutic decisions; no available biomarker is sufficiently standardized or prospectively tested to guide everyday preventive treatment. For trialists, the near-term opportunity lies in biomarker-enriched study designs: selecting high-risk phenotypes, using technically reliable assays (such as mass-spectrometry F2-isoprostanes alongside OxPL-apoB), pairing biomarker assessment with endothelial, imaging, or plaque-related intermediate endpoints, and prespecifying sex-, age-, and inflammation-stratified analyses. Under current evidence, oxidative biomarkers are most defensibly used to clarify mechanisms, refine phenotype characterization, and support early-phase translational testing—not to replace traditional risk markers in practice.

7. Limitations

Several limitations temper any ROS-centered interpretation of atherosclerosis and are summarized here rather than restated across sections:

Table 4. Biomarker-enriched trial design framework for oxidative phenotyping in atherosclerosis

High-risk phenotype	Biomarker enrichment strategy	Proposed intervention example	Primary endpoint (mechanistic phase)	Key implementation challenge	Feasibility
Diabetes with established ASCVD	Plasma F2-IsoP ≥75th percentile for diabetic population	NOX inhibitor or GLP-1RA with documented antioxidant effects vs. placebo	FMD change at 12 weeks; urinary F2-IsoP reduction	F2-IsoP threshold not standardized; assay platform variability	Moderate
CKD stages 3–4 with prior CVD	Elevated urinary F2-IsoP or oxLDL despite statin therapy	XO inhibitor (allopurinol dose-escalation) vs. standard dose	Endothelial function (FMD, RHI); oxidative biomarker panel at 6 months	Altered clearance complicates biomarker interpretation; multifactorial CV risk	Moderate
Recurrent events despite LDL-C <70 mg/dL	F2-IsoP in highest quartile among high-intensity statin users	Source-selective antioxidant (e.g., mitochondrial-targeted) added to GDMT	Vascular inflammation by PET–CT; circulating biomarker panel at 24 weeks	Residual risk is multifactorial; oxidative stress may be epiphenomenal	Low–Moderate
Active smokers with subclinical atherosclerosis	Elevated plasma F2-IsoP and MPO despite smoking cessation counseling	NAC or vitamin C supplementation vs. placebo in smoking cessation program	Endothelial function; arterial stiffness (PWV); F2-IsoP at 12 weeks	Compliance with smoking cessation; direct oxidant exposure from tobacco	Moderate–High
Metabolic syndrome without overt CVD	Oxidative biomarker composite score (F2-IsoP, oxLDL, 8-oxo-dG)	Lifestyle intervention + metformin vs. lifestyle alone	Carotid IMT progression; FMD; biomarker composite at 12 months	Long duration needed for IMT endpoints; high dropout in lifestyle trials	Moderate

Notes: All proposed trials are Phase II mechanistic studies designed to establish proof of concept before outcome trials. Feasibility ratings reflect current assay standardization, recruitment potential, and endpoint accessibility.
Abbreviations: ASCVD: Atherosclerotic cardiovascular disease; CKD: Chronic kidney disease; CV: Cardiovascular; CVD: Cardiovascular disease; F2-IsoP: F2-isoprostanes; FMD: Flow-mediated dilation; GDMT: Guideline-directed medical therapy; GLP-1RA: Glucagon-like peptide-1 receptor agonist; IMT: Intima-media thickness; LDL-C: Low-density lipoprotein cholesterol; MPO: Myeloperoxidase; NAC: N-acetylcysteine; NOX: NADPH oxidase; oxLDL: Oxidized low-density lipoprotein; PET–CT: Positron emission tomography–computed tomography; PWV: Pulse wave velocity; RHI: Reactive hyperemia index; XO: Xanthine oxidase.

(i) Predominantly associative human evidence. Most human data linking oxidative biomarkers to vascular disease are associative rather than decisively causal. Elevated markers may index downstream injury or generalized physiologic stress rather than a modifiable upstream driver, and Mendelian randomization or interventional reversal data of the quality available for LDL-C or lipoprotein(a) do not yet exist for F2-isoprostanes, MPO, oxidized LDL, or OxPL-apoB.

(ii) Assay and analytic heterogeneity. Oxidative biomarkers, including F2-isoprostanes, vary by sample matrix, timing, specimen handling, dietary fat composition, and patient characteristics. 8-iso-$PGF_{2\alpha}$ standardization remains incomplete: no widely accepted reference standard, no commutable secondary material, and no validated clinical decision cutoffs are currently available.[51,52]

(iii) Multifactorial disease network. Oxidative stress operates within an interacting network that includes lipoprotein retention, inflammation, thrombosis, blood-pressure load, metabolic dysfunction, and aging-related vascular change; it may amplify many of these pathways without dominating every phenotype or stage.

(iv) Uneven therapeutic evidence. Although many cardiovascular drugs engage redox biology in preclinical studies, clinical trials have not established ROS modulation as their principal mechanism of

benefit, and direct ROS-targeted interventions have not consistently reduced hard outcomes.

(v) Framework not yet prospectively validated. The four-stage translational framework proposed here is a research agenda derived from current evidence; it has not been tested in prospective biomarker-enriched outcome trials, and its predictive value for trial success remains to be demonstrated.

(vi) Scope and methodology. As a narrative rather than systematic review, this synthesis reflects interpretive study selection without preregistered protocol, dual-reviewer screening, PRISMA flow diagram, or meta-analytic pooling, and was restricted to English-language publications.

(vii) Publication and interpretive bias. Positive biomarker associations and coherent mechanistic narratives may be overrepresented relative to null findings in the cited literature, and the authors' framing—favoring compartmentalized, source-specific ROS biology—necessarily emphasizes evidence consistent with that framework.

8. Conclusion

This review advances three affirmative positions on ROS biology in residual atherosclerotic risk:

(i) ROS imbalance is biologically coherent but compartmentalized. Compartmentalized, source-specific ROS imbalance contributes to residual atherosclerotic risk—not as a competing explanation to lipoprotein retention or inflammation, but as an amplifying component within them. This reframing helps explain why broad antioxidant trials failed and why source-selective approaches deserve renewed investigation.

(ii) Current oxidative biomarkers belong in trials, not in the clinic. F2-isoprostanes and related markers are defensibly used for trial enrichment and mechanistic endpoints, but no oxidative biomarker is ready for individual treatment selection, and no redox-targeted therapy yet improves hard outcomes beyond guideline-directed care. Recognizing this boundary is itself a contribution: it disciplines what oxidative biomarkers can credibly do in the next decade of cardiovascular research.

(iii) A four-stage translational framework offers a disciplined path forward. Phenotype selection with prespecified sex, age, and menopausal stratification; analytically rigorous biomarker measurement alongside hsCRP; mechanistic and vascular-endpoint interventional testing; and only then outcome demonstration—together avoid the missteps of earlier broad-antioxidant trials and provide a research agenda that is testable, falsifiable, and adaptable as evidence accrues.

For clinicians, oxidative biomarkers remain investigational and should not yet alter preventive practice. For trialists, they offer a credible substrate for the next generation of biomarker-enriched, mechanism-anchored studies in high-risk cardiometabolic populations, where residual cardiovascular risk remains the most pressing unmet need in contemporary preventive cardiology.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Yasser M. Esmaeil
Visualization: Yasser M. Esmaeil
Writing–original draft: All authors
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Ethical approval and consent to participate

Not applicable.

Consent for publication

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Not applicable.

Further disclosure

The clinical motivation, central thesis, and scientific positions of this review were developed by the corresponding author based on direct clinical experience caring for patients with residual atherosclerotic cardiovascular risk despite guideline-directed therapy, independent reading of the primary literature, and discussions with mentors. Specifically, the framing of oxidative stress as a compartmentalized, source-specific phenomenon; the distinction between trial enrichment and treatment selection; the application of a plausibility–association–utility hierarchy to oxidative biomarkers; and the staged translational framework with phenotype-enriched trial designs (Table 4) represent the authors' synthesis and intellectual contribution. A large language model assistant was used throughout manuscript preparation under the

authors' direct supervision. The assistant contributed to (i) drafting and refinement of prose based on author-provided outlines, source materials, and instructions; (ii) suggesting candidate citations, all of which were independently retrieved, read in primary form, and verified by the authors against the claims they support; (iii) preparing initial versions of figures and tables, which the authors reviewed, corrected, and finalized; and (iv) language editing and structural organization. The corresponding author critically reviewed and edited all AI-generated content, and the final wording, scientific interpretation, and citation choices reflect the authors' judgment. The AI tool does not meet authorship criteria and is not listed as an author. The authors take full responsibility for the integrity, accuracy, scientific content, and conclusions of the manuscript, including the verification of every cited reference.

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