Review

Vascular Aging and Atherosclerosis: The Modulatory Impact of Selenium—A Comprehensive Review

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Vascular Aging and Atherosclerosis: The Modulatory Impact of Selenium—A Comprehensive Review

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Abstract

Selenium (Se), a vital trace element, plays a significant role in maintaining vascular health and may offer protective effects against atherosclerosis. Its actions are mediated through Se-dependent selenoenzymes and selenoproteins, which enhance antioxidant defense, modulate inflammatory responses, and promote autophagy. These processes collectively help prevent cellular senescence - a state associated with age-related vascular decline characterized by oxidative stress, DNA damage, pro-inflammatory activity, and endothelial dysfunction. Epidemiological evidence consistently shows that low Se status is associated with increased risk of atherosclerotic cardiovascular disease within a narrow concentration range. However, clinical trials have not demonstrated clear reductions in cardiovascular events or mortality with Se supplementation alone. Overall, current evidence indicates that Se modulates key mechanisms involved in vascular aging and atherosclerosis, particularly redox balance, immune activation, and vascular cell homeostasis. This comprehensive review summarizes current epidemiological, clinical, and experimental research on the role of Se in cardiovascular health. It underscores Se's potential as a promising strategy for the prevention and treatment of atherosclerosis, while also acknowledging the complexities and nuances of its effects on vascular health. A deeper understanding of the cellular and molecular mechanisms involved could pave the way for targeted interventions aimed at reducing the burden of atherosclerotic cardiovascular disease.

Keywords: selenium; selenoproteins; atherosclerosis; coronary heart disease; cardiovascular disease; cellular senescence; oxidative stress; inflammation; endothelial dysfunction; DNA damage

1. Introduction

Atherosclerosis, a chronic and progressive inflammatory condition affecting the intima of medium- and large-sized arteries, characterized by endothelial dysfunction, lipid infiltration, and foam-cell formation, ultimately leading to plaque development, represents the leading cause of coronary heart disease (CHD), myocardial infarction (MI), ischemic stroke, and peripheral arterial disease [1–3]. Despite a global reduction in mortality from IHD and ischemic stroke between 1990 and 2019 across all adult age groups - attributable to advances in public health strategies and early treatment - recent estimates indicate an increasing incidence of atherosclerosis over the same period [2]. This rise is largely driven by global population aging, lifestyle patterns characterized by high-fat dietary habits and insufficient physical activity, and a growing prevalence of chronic diseases such as hypertension and diabetes, which constitute major risk factors for atherosclerosis [2]. While atherosclerosis was historically viewed as a mere consequence of dyslipidaemia - driven by the accumulation of cholesterol-rich lipoproteins, primarily low-density lipoprotein (LDL), within the arterial intima - by the end of the last century its inflammatory nature had been proposed, supported by the observation of circulating monocytes within the fatty streak [4,5]. Furthermore, accumulating evidence indicates that aging, defined as a progressive decline in cellular function caused by multiple factors, including genomic instability, telomere dysfunction, stem-cell exhaustion, and oxidative stress, contributes to the senescence of vascular and immune cells, thereby representing a pivotal

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driver in the promotion of atherosclerosis [3,6]. Indeed, although cellular senescence initially prevents the replication of damaged cells and thus protects against tumorigenesis, in the long term the secretion of inflammatory mediators and proteolytic enzymes by senescent cells - known as the senescence-associated secretory phenotype (SASP) - promotes vascular inflammation and remodelling [3,4].

Selenium (Se), an essential trace element involved in a wide range of physiological functions through its incorporation into selenoproteins as selenocysteine (Sec), is recognized as one of the most potent antioxidant systems in humans [7–9]. As a component of selenoproteins, Se regulates the antioxidant defence system, primarily through glutathione peroxidase (GPx), thioredoxin reductase (TrxR), and selenoprotein P (SELENOP), attenuates inflammatory processes triggered by excess reactive species [10], enhances immune function [11], modulates thyroid activity [12], exerts anti-cancer effects [13], and supports male fertility [14] as well as female reproductive health [15]. An expanding body of research indicates that Se may also help reduce the risk of CVD, primarily by mitigating oxidative-stress–induced damage, thereby reducing inflammation and endothelial dysfunction and inhibiting vascular cell apoptosis and vascular calcification [16–18]. Meanwhile, the antioxidant activity of Se, which reduces DNA damage and preserves telomere length, plays a crucial role in counteracting aging and preventing age-related diseases [19].

In this comprehensive literature review, we examine the current body of research from experimental and clinical studies on the role of Se in reducing the risk of atherosclerosis by acting at multiple levels, including the mitigation of cellular senescence, and discuss how Se supplementation may represent a valuable strategy to curb atherosclerosis and associated CVD.

2. Atherosclerosis and Cellular Senescence

2.1. Pathophysiology of Atherosclerosis

Atherosclerosis is an immunoinflammatory and fibroproliferative disease that originates in early childhood and progresses silently through adulthood [1]. It is defined by the progressive accumulation of lipids and fibrous tissue within the tunica intima of medium- and large-sized arteries [20,21]. The process is initiated by endothelial activation, which triggers a cascade of cellular and molecular events that ultimately promote atheroma formation and, over time, give rise to clinically overt cardiovascular complications [20,21] (Figure 1). The vascular endothelium, a monolayer of endothelial cells (ECs) lining the vessel lumen and positioned between circulating blood and the underlying tissues, functions as a selectively permeable barrier and as a transducer of both mechanical signals (e.g., wall shear stress, WSS) and metabolic cues to the other layers of the vascular wall [20,21]. Turbulent blood flow at arterial curvatures and bifurcation points, exacerbated by pathological conditions including hypercholesterolemia, hypertension, and type 2 diabetes, can lead to a reduction in WSS [1,20]. Low or oscillatory WSS induces mechanical stress that promotes endothelial dysfunction and facilitates LDL infiltration into the tunica intima through transcytosis, a process mediated by endothelium scavenger receptor B1 and activin A receptor-like type 1 [20,21]. Once retained, LDL particles undergo oxidative modification (oxLDL), amplifying oxidative stress, and activating pro-inflammatory pathways such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [20,21]. This, in turn, increases the secretion of cytokines (interleukins (IL-1, IL-6, tumor necrosis factor alpha - TNF-α), chemokines (monocyte chemoattractant protein 1 - MCP-1), and adhesion molecules (VCAM-1, ICAM-1), ultimately fosteringmonocyte recruitment and their differentiation into M1 macrophages [20,21]. The release of these pro-inflammatory molecules drives the migration of T- and B-lymphocytes, dendritic cells (DCs), and vascular smooth muscle cells (VSMCs) - the predominant cell type located in the arterial media - into the intima, thereby intensifying the inflammatory response [1,21]. Within this inflammatory milieu, endothelial nitric oxide synthase (eNOS) becomes uncoupled, generating the harmful peroxynitrite radical, which diminishes the glutathione' scavenging capacity and reduces the nitric oxide (NO) availability - an atheroprotective molecule that physiologically restrains platelet aggregation and supports

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endothelial function, vasorelaxation, angiogenesis, and metabolic homeostasis - thus further aggravating endothelial dysfunction [20,21].

Once formed, oxLDL undergo lysosomal processing and are also taken up by activated macrophages, converting an initially protective mechanism into the accumulation of lipid-laden macrophages, or foam cells. Foam cells, together with DCs, activate the cytosolic multiprotein NOD-like receptor protein-3 (NLRP3) inflammasome within macrophages, leading to caspase-1 activation and the subsequent secretion of IL-1β and IL-18, thereby creating a highly pro-inflammatory microenvironment at the macrophage surface [20,21]. The migration of VSMCs from the media into the intima contributes to at least 50% of the total foam-cell population [20]. Once within the intimal microenvironment, VSMCs - similarly to macrophages - upregulate scavenger receptors, enabling the uptake of modified lipoproteins and the formation of smooth-muscle–derived foam cells enriched in cholesteryl esters [22]. This process markedly expands the foam-cell pool and facilitates the transition toward fatty streak development [21]. Early atherosclerotic lesions evolve into plaques characterized by a lipid-rich necrotic core covered by a fibrous cap composed of VSMCs and extracellular matrix (EM) [21]. As disease progresses, plaques undergo calcification as VSMCs transdifferentiate into osteoblast-like phenotypes, acquiring the capacity to secrete a mineralized EM that nucleates and enlarges calcium deposits [20]. In advanced stages of atherosclerosis, the fibrous cap becomes progressively thinner due to increased VSMC apoptosis and heightened metalloproteinase activity, and is infiltrated by macrophages, T-lymphocytes, and B-lymphocytes [23]. As a result, the cap loses its structural integrity and becomes susceptible to rupture [23]. Plaque rupture releases necrotic core material into the bloodstream, activating platelets and the coagulation cascade, and potentially leading to ischemic cardiomyopathies, such as cardiac insufficiency or angina pectoris when coronary blood flow is reduced, and to MI and stroke when the obstruction becomes complete [20,24]. Alternatively, thrombi may remain small clinically silent [21]. Moreover, lipid-lowering therapy can attenuate inflammation and stabilize the plaque, thereby preserving adequate blood flow [20].

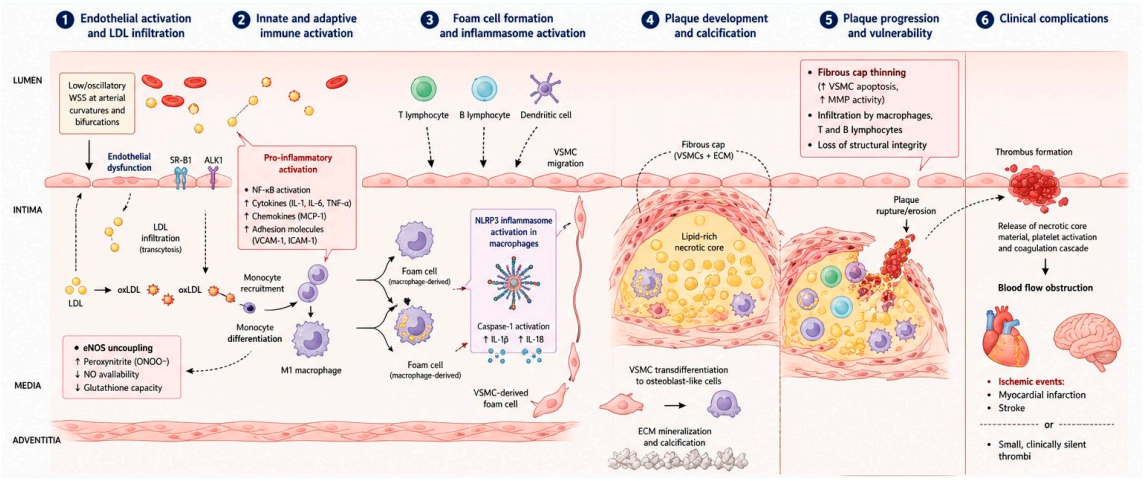

Figure 1. Pathophysiological progression of atherosclerosis from endothelial dysfunction to plaque rupture and clinical complications (see text for details). Image generated with AI ChatGPT 5.0. Abbreviations: ALK1: activin A receptor-like type 1; ECM: extracellular matrix; eNOS: endothelial nitric oxide synthase; ICAM-1: intercellular adhesion molecular-1; IL: interleukin; LDL: low-density lipoprotein; MCP-1: monocyte chemoattractant protein-1, MMP: matrix metalloproteinase; NF-κB: nuclear factor kappa B; NLRP3: NOD-like receptor protein 3; NO: nitric oxide; oxLDL: oxidized LDL; SR-B1: scavenger receptor B1; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion molecule-1; VSMC: vascular smooth muscle cell; WSS: wall shear stress.

2.2. Cellular Senescence

Cellular senescence, first described more than fifty years ago as a process limiting cell proliferation in in vitro models, has progressively gained attention due to its central role in tumor

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suppression [25]. It is currently defined as a state of irreversible cell-cycle arrest affecting most division-competent cells, with the notable exception of embryonic stem cells, and has historically been viewed as a mechanism that prevents the propagation of damaged DNA, thereby reducing cancer risk [25,26]. Telomere shortening and DNA damage are recognized as major inducers of cellular senescence and ageing [27]. Telomeres consist of simple tandem DNA repeats and associated proteins that, under physiological conditions, protect chromosome ends from degradation by suppressing DNA damage responses (DDR) [26]. Telomerase - an RNA-dependent DNA polymerase that synthesizes telomeric sequences at chromosome ends - maintains telomere length and prevents sequence loss during repeated DNA replication [28]. Nonetheless, when telomerase components are impaired, nucleolytic processing causes telomeres to shorten by 50–200 nucleotides per cell division, eventually reducing their length below the threshold required to fully repress the DDR, thereby triggering senescence [27–29]. Activation of the DDR pathway induces p21, a cyclin-dependent kinase inhibitor that halts the cell cycle to allow damage repair, and p16, which reinforces p21-mediated arrest and contributes to the long-term maintenance of the senescent state [26]. Prolonged oxidative stress, characterized by excessive accumulation of reactive oxygen species (ROS) following UV radiation exposure or mitochondrial dysfunction, together with a concurrent decline in cellular antioxidant capacity, exacerbates the loss of redox homeostasis [26]. A key contributor to this impaired defense is the reduced activity of nuclear factor erythroid 2–related factor 2 (Nrf2), the master antioxidant transcription factor responsible for inducing multiple downstream cytoprotective genes, including GPx, superoxide dismutase, and catalase [30,31]. This combined failure to counteract ROS accumulation markedly increases cellular vulnerability and promotes the onset of senescence [26]. Senescent cells exhibit profound mitochondrial alterations, including increased size and volume [32]. Concurrently, the mitochondrial membrane potential declines, leading to reduced respiratory capacity and impaired efficiency of the electron transport chain [33]. As a result, ATP production through oxidative phosphorylation (OXPHOS) decreases, whereas the generation of ROS rises [33]. The ensuing oxidative damage to mitochondrial DNA, proteins, and lipids activates DDR signaling, thereby reinforcing and propagating the senescent phenotype [33]. In dysfunctional mitochondria, reduced cytosolic NAD^+/NADH ratios activate AMP-activated protein kinase (AMPK), which phosphorylates p53 and stabilizes p16 mRNA [32]. Importantly, telomere integrity and mitochondrial biology are reciprocally linked in a self-perpetuating cycle [34]. Telomere dysfunction, through repression of peroxisome proliferator–activated receptor gamma coactivator 1α/β (PGC-1α/β), impairs mitochondrial biogenesis and function, leading to increased ROS production [34]. In turn, excess ROS damages mitochondrial DNA (mtDNA) and exacerbates mitochondrial injury, while also accelerating telomere shortening and dysfunction, thereby perpetuating cellular damage and aging-related decline [34].

Senescent cells exhibit the SASP as a defining hallmark, characterized by increased β-galactosidase activity at pH 6.0, reflecting expanded lysosomal content [26,35]. This altered functional state is accompanied by the secretion of pro-inflammatory cytokines, including IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), which collectively sustain a chronic low-grade inflammatory milieu commonly referred to as inflammaging [26,36,37]. Senescent cells also release matrix metalloproteinases (MMPs), such as MMP-1 and MMP-3, as well as transforming growth factor-beta (TGF-β), all of which contribute to tissue remodeling and organ dysfunction through EM degradation [26,36]. In addition, ROS generated by senescent cells further exacerbates local oxidative stress, amplifying cellular damage and reinforcing pro-inflammatory signaling [37]. Multiple interconnected pathways, including mammalian target of rapamycin (mTOR) protein, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and p38/mitogen-activated protein kinase (p38/MAPK), sustain the SASP phenotype and support the long-term survival and immune resistance of senescent cells [3,37].

Senescent cells also undergo marked morphological changes, displaying an enlarged and flattened morphology together with an increased number of cytoplasmic vacuoles, reflecting the

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accumulation of β-galactosidase and other catabolic by-products and conferring the characteristic granular appearance of the senescent cytoplasm [26].

Another defining feature of senescent cells is their resistance to programmed cell death, or apoptosis [26]. Apoptosis is typically an anti-inflammatory form of cell death and is followed by efferocytosis, a process in which apoptotic cells are rapidly engulfed and cleared by phagocytes [38]. This prevents the release of intracellular components that would otherwise activate damage-associated molecular patterns and trigger inflammation [38]. During efferocytosis, phagocytes also secrete anti-inflammatory cytokines such as TGF-β and IL-10, contributing to the resolution of tissue inflammation [38]. In contrast, senescent cells develop a pro-inflammatory and immunogenic phenotype and can persist for prolonged periods, often indefinitely, unless they are actively cleared by the immune system or eventually undergoing necrotic death [38].

Importantly, although cellular senescence was initially described as a phenomenon exclusively driven by excessive telomere shortening resulting from repeated cell divisions - now referred to as telomere-dependent replicative senescence - subsequent research has identified multiple additional forms of senescence [38,39]. Oncogene-induced senescence represents a particularly complex senescence program: while it activates potent tumor-suppressive responses, the associated SASP also acquires tumor-promoting features through the secretion of pro-inflammatory cytokines, C-X-C motif chemokine ligand 1 and vascular endothelial growth factor (VEGF) [40]. These mediators collectively enhance angiogenesis and support the growth and survival of neighboring cells, thereby creating a microenvironment that can paradoxically favor tumor progression [26,40]. Furthermore, emerging evidence highlights the multifunctional nature of cellular senescence, underscoring its physiological relevance across multiple biological processes [41]. Thus, although senescence provides an essential short-term barrier against malignant transformation, its long-term persistence, sustained by chronic inflammation, becomes a central contributor to tumor progression and to a broad spectrum of age-related diseases, including cardiovascular and cerebrovascular diseases, neurodegenerative disorders, fibrotic pulmonary diseases, hepatic steatosis, and metabolic dysfunction [4,41,42] (Figure 2).

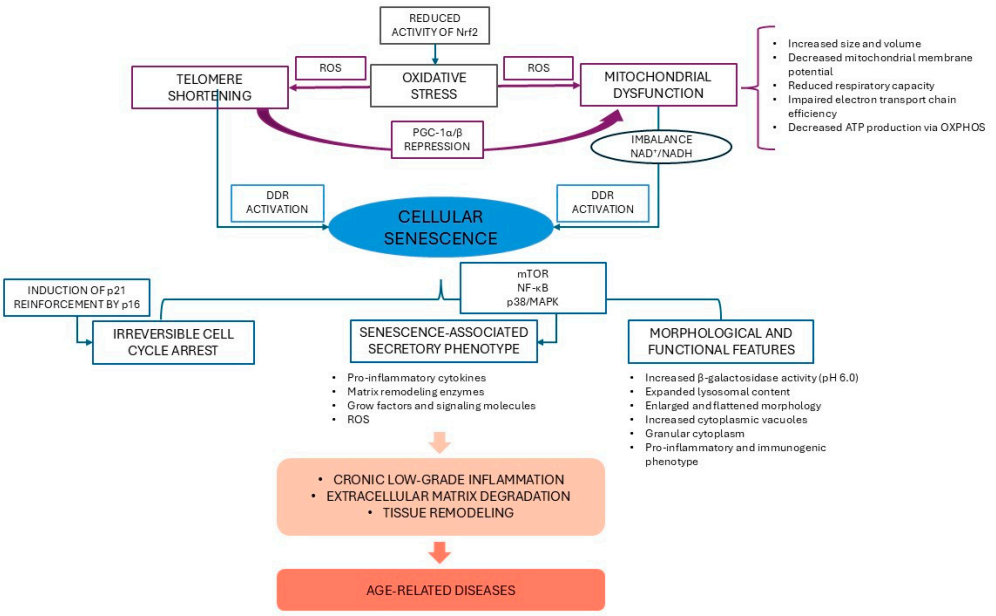

Figure 2. Molecular mechanisms linking telomere dysfunction, oxidative stress, and mitochondrial impairment to cellular senescence and age-related diseases. Abbreviations: DDR: DNA damage response; mTOR: mammalian target of rapamycin; Nrf2: nuclear factor erythroid 2–related factor; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; OXPHOS: oxidative phosphorylation; p38/MAPK: p38/mitogen-activated protein kinase; PGC-1α/β: proliferator–activated receptor gamma coactivator 1α/β; ROS: reactive oxygen species.

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2.3. The Role of Cellular Senescence in Atherosclerosis

Accumulating evidence indicates that cellular senescence is a major driver of atherosclerosis [3,4]. In addition to traditional stressors - such as mechanical forces (shear stress and elevated or pulsatile pressure), oxidative stress (ROS), and metabolic stimuli (glucose, lipids, and amino-acid metabolites) that induce DNA damage and mitochondrial dysfunction - various cardiovascular risk factors, including hypertension, hyperlipidemia, hypercholesterolemia, obesity, diabetes, and smoking, also contribute to the development of cellular senescence [3,43]. Notably, several of these risk factors are closely linked to ageing, underscoring the interdependence among cellular senescence, ageing, and atherosclerosis [3]. In the following sections, we describe how senescent vascular and immune cells participate in atherosclerosis (Figure 3).

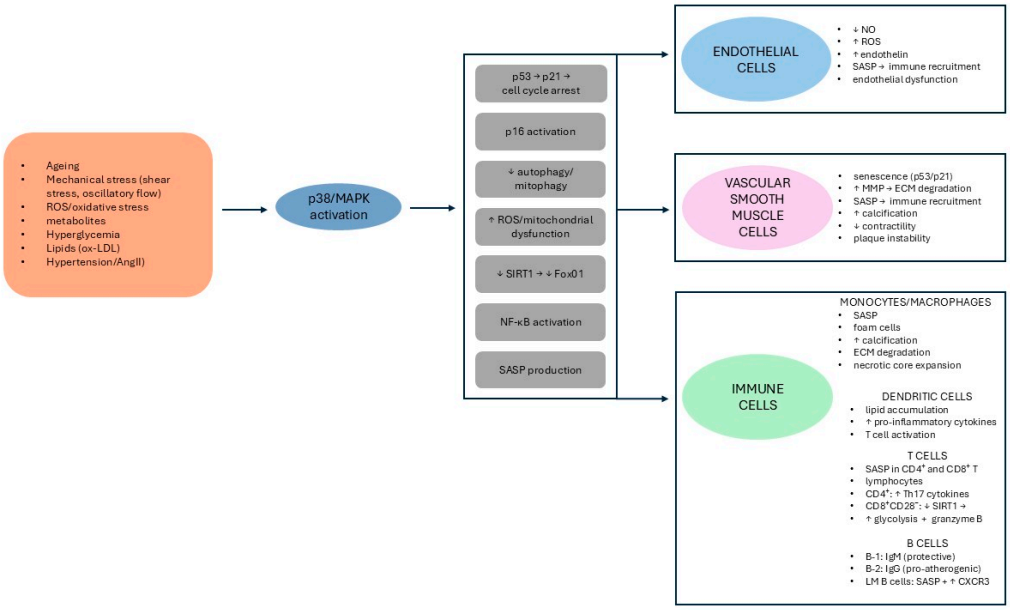

Figure 3. Integrated role of cellular senescence in vascular and immune dysfunction during atherosclerosis (see text for details). Abbreviations: AngII: angiotensin II; ECM: extracellular matrix; LM: late memory; MMP: metalloproteinase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NO: nitric oxide; oxLDL: oxidized low-density lipoprotein; p38/MAPK: p38/mitogen-activated protein kinase; ROS: reactive oxygen species; SASP: senescence-associated secretory phenotype; SIRT: sirtuin.

2.3.1. Vascular Endothelial Cells

Vascular ECs are among the first cell types to exhibit signs of ageing due to their constant exposure to circulating pathogenic stimuli, as well as to low and oscillatory shear stress at arterial branches and curvatures [44]. These disturbed-flow regions experience higher mechanical stress than sites exposed to laminar flow and show an increased rate of endothelial turnover, ultimately leading to telomere attrition, activation of DDR, engagement of the p53–p21 pathway, and progression toward replicative senescence [3]. Under conditions of excess pro-inflammatory and pro-atherogenic factors (e.g., TNF-α, hydrogen peroxide, oxLDL), deactivation of protein kinase B (Akt) – a key regulator of endothelial cell growth, migration, survival, and vascular tone - reduces telomerase activity, thereby promoting EC ageing [45,46]. Obesity, hyperlipidemia and diabetes further promote EC senescence [3]. In vivo and in vitro model systems have shown that hyperglycemia-induced downregulation of sirtuin-1 (SIRT1), the major nicotinamide adenine dinucleotide-dependent enzyme in the SIRT family exhibiting protective roles in stress resistance and cell survival in various disease, accelerates ageing in ECs [47]. SIRT1 plays a central role both as histone deacetylase at DNA damage sites and as a regulator of proteins involved in DNA repair and DDR [48]. SIRTs also modulate oxidative stress and inflammation by regulating transcriptional factors associated with

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redox homeostasis, such as Nrf2 and Forkhead box protein O3 (FoxO3a), as well as antioxidant enzymes, through epigenetic and post-translational modifications [49]. Consistently, glucose-induced oxidative stress and accelerated aging in ECs are mediated by depletion of mitochondrial SIRTs [50]. Senescent ECs display increased ROS production, which promotes peroxynitrite formation and reduces NO production in response to shear stress [51]. Reduced NO availability, together with enhanced p53 activity - which suppresses expression of VEGF, fibroblast growth factor, and hypoxia-inducible factor - and increased synthesis of endothelin, the most potent EC-derived vasoconstrictor, ultimately impairs endothelium-dependent vasodilation and ischemia-induced angiogenesis [44,52,53]. In parallel, senescent ECs promote a prothrombotic and proatherogenic phenotype by upregulating plasminogen activator inhibitor-1 and thromboxane A2, thereby increasing thrombogenicity and susceptibility to atherosclerosis [44,51]. Furthermore, the production of SASP components, including inflammatory cytokines (IL-1, IL-6, IL-8, TNF-α), chemokines (MCP-1, chemokine ligand 11, and growth factors (ICAM-1), facilitates immune cell infiltration into vascular tissues [3,44] (Figure 3).

2.3.2. Vascular Smooth Muscle Cells

As highlighted in Section 2.1, vascular smooth muscle cells (VSMCs) play a central role in the pathogenesis of atherosclerosis, owing to their predominant presence throughout all stages of disease progression [54,55]. Senescent VSMCs - characterized by diminished proliferative capacity and prolonged cellular survival - are primarily observed in aged vessels and advanced atherosclerotic plaques [56]. In addition to replicative senescence driven by telomere shortening, VSMCs may undergo senescence through proliferation-independent mechanisms, collectively referred to as stress-induced premature senescence. This process can be triggered by multiple stimuli, including angiotensin II (Ang II), chronic inflammatory signaling, and sustained oxidative stress [44,57]. Ang II, an octapeptide generated from angiotensinogen through sequential proteolytic cleavage by renin and angiotensin-converting enzyme, is the principal effector molecule of the renin–angiotensin–aldosterone system and is a key mediator in the pathophysiology of cardiovascular disease [58,59]. Through its pleiotropic vascular actions - including the promotion of endothelial dysfunction, pathological neovascularization, VSMC proliferation, and inflammatory activation - Ang II orchestrates a cascade of molecular and cellular events that drive the initiation and progression of atherosclerotic lesion formation [59]. At the cellular level, Ang II induces premature VSMC senescence and a pro-inflammatory phenotype through activation of the p53/p21 signaling axis [60]. Conversely, suppression of p21 expression markedly attenuates these Ang II–mediated effects, underscoring the central role of this pathway in regulating VSMC senescence [60]. Elevated Ang II levels further contribute to vascular inflammation and fibrotic remodelling by upregulating TGF-β signaling, activating NF-κB and MMP pathways, increasing ROS generation, and reducing NO bioavailability [61]. Collectively, these mechanisms promote maladaptive arterial remodeling and structural destabilization of the vascular wall [61]. Enhanced expression and activity of MMP-2 and MMP-9 in VSMCs play a pivotal role in vascular calcification and inflammation, respectively and, along with increased elastase activity, contribute to ECM degradation and increase the susceptibility of atherosclerotic plaques to rupture [44,57]. In parallel, TGF-β signaling promotes the selective upregulation of collagen types I and III, which together constitute up to 60% of the extracellular matrix protein content within atherosclerotic plaques [62,63]. In advanced lesions, dysregulation of the balance between collagen synthesis and degradation results in progressive thinning of the fibrous cap, thereby markedly increasing plaque vulnerability and the risk of rupture [63]. Furthermore, TGF-β–mediated inflammatory signaling stimulates the release of cytokines that amplify MMP activity, promoting degradation of collagen IV, an essential structural component of the basement membrane that supports endothelial cell integrity and maintains vascular barrier function [63]. Consistently, senescent VSMCs develop a SASP characterized by the secretion of numerous pro-inflammatory mediators, including IL-1α, IL-1β, IL-6, IL-8, IL-18, and TNF-α, which reinforce this cytokine-driven inflammatory cascade [55]. These factors suppress anti-inflammatory pathways,

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while promoting the recruitment and chemotaxis of immune cells within the vascular wall [44]. The resulting inflammatory milieu is further exacerbated by increased ROS production, driven by transcriptional reprogramming and a concomitant decline in cellular antioxidant capacity [55,57]. Aging VSMCs also exhibit reduced contractile capacity due to decreased expression of proteins involved in muscle contraction and acquire a pro-calcific phenotype, marked by increased production of osteogenic mediators such as osteopontin, osteoprotegerin, runt-related transcription factor 2, bone morphogenetic protein 2, and alkaline phosphatase in response to oxidative stress and inflammation, thereby contributing to arterial stiffness [44,55]. Importantly, while oxidative stress and inflammation are key trigger of VSMC senescence, VSMCs may promote survival through autophagy, a lysosome-regulated process that degrades and recycles unnecessary or dysfunctional components to maintain intracellular homeostasis and energy balance [54,55,57,64]. The mammalian target of rapamycin (mTOR) pathway plays a crucial role in regulating cell growth and ageing [65]. Increased mTOR activity is associated with VSMC senescence and reduced levels of autophagy-related proteins, whereas mTOR inhibition exerts the opposite effects [65]. Autophagy is also induced by modest amounts of oxLDL; however, high concentrations impair autophagic flux and accelerate the development of stress-induced premature senescence, indicating that autophagy and senescence are profoundly interconnected and inversely correlated in atherosclerosis [54,55] (Figure 3).

2.3.3. Immune Cells

Immunosenescence, defined as the gradual deterioration of immune function that accompanies ageing, further contributes to the onset of inflammaging and the development of atheroma and progression of atherosclerotic plaques [66].

Monocytes, a heterogeneous cell population distinguished by differential surface expression of the CD14 and CD16 antigens, are critically involved in all stages of atherosclerosis and play a pivotal role in early plaque formation and maturation [67,68]. Notably, all three monocyte subsets display hallmarks of senescence, which amplify their inflammatory and pro-atherogenic activities [69]. Among them, non-classical monocytes ($CD14^{+}CD16^{++}$) represent the least proliferative subset but exhibit the shortest telomere length, indicating that they have undergone more replication cycles than the other subsets [70]. These cells also show pronounced SASP-like activation, likely driven by high expression of membrane-bound IL-1α and upregulated NF-κB signaling, and release substantial amounts of cytokines and chemokines such as TNF-α, CCL3, and CCL4 [70]. Together with intermediate monocytes ($CD14^{++}CD16^{+}$), they also produce high levels of IL-6, IL-8, IL-1β, and CCL5, generating a stongly proinflammatory milieu in the elderly population [69,70]. In addition, senescent non-classical monocytes display increased expression of expression of proatherogenic chemokine receptors (e.g., CCR2, CCR5, CCR7, and CX3CR1) and adhesion molecules involved in endothelial binding (e.g. VCAM and ICAM-1), along with enhanced monocyte–endothelial adhesion capacity [71].

Senescent macrophages play a central role in atherosclerosis, by producing inflammatory cytokines, lipid accumulation, foam-cell formation, and expansion of the necrotic core due to their reduced ability to clear apoptotic cells [3,37]. Their lineage heterogeneity is well established, with macrophage polarization varying according to tissue localization and the surrounding inflammatory environment [72]. While macrophages have traditionally been categorized into M1 (pro-inflammatory, releasing factors such as IL-6, IL-12, and TNF-α) and M2 (anti-inflammatory, involved in tissue repair) subtypes [73], single-cell RNA sequencing has revealed three macrophage populations in atherosclerotic aortas [74]. These include resident-like macrophages with a gene expression profile similar to aortic resident macrophages, which can proliferate and resemble an M2-like phenotype; inflammatory macrophages, the predominant type within the plaque intima and the main drivers of lesional inflammation, characterized by elevated expression of IL-1β; and a novel subset of aortic $TREM2^{hi}$ macrophages [74,75]. These foamy, lipid-laden $TREM2^{hi}$ macrophages exhibit an M2-like phenotype, contribute to plaque rupture through the release of MMP-9, and are enriched in numerous genes involved in osteoclastogenesis, suggesting a key role in atherosclerotic

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lesion calcification [69,74]. Macrophages are among the cells with the highest levels of p16, senescence-associated β-galactosidase activity, and increased mRNA expression of a subset of transcripts encoding factors involved in the SASP such as MMP3 and MMP13, which promote ECM degradation, plaque instability, and eventual rupture, as well as the inflammatory cytokines IL-1α and TNF-α [69,75–77]. Since activated p38/ MAPK increases p16 expression and promotes cellular senescence, and oxLDL induces lipid deposition in macrophages p38/MAPK activation, it is plausible that the p38/MAPK/p16 pathway contributes to oxLDL-induced senescence in plaque macrophages [77]. Notably, CD9 - a tetraspanin membrane protein that regulates cellular senescence through the phosphoinositide 3-kinase–Akt–mTOR–p53 signaling pathway and whose levels increase in arterial tissues with ageing - is also upregulated in macrophages within atherosclerotic lesions [77]. Conversely, CD9 ablation is associated with reduced expression of p53 and p16 in macrophages [78].

Although the role of DC senescence in atherosclerosis remains poorly explored, DCs - antigen-presenting cells responsible for processing and presenting specific antigens to T cells via major histocompatibility complexes (MHCs) - show a reduced capacity to interact with T cells during ageing [69,79]. Residing in the intimal and adventitial layers of arteries, where they act as sentinel cells probing for antigens, intimal DCs accumulate during both the initiation and progression of atherosclerosis, as well as during ageing [80,81]. Based on the expression of specific markers, DCs can be further classified into conventional type 1 and type 2 dendritic cells, plasmacytoid dendritic cells, and monocyte-derived dendritic cells [79]. These subsets exert heterogeneous roles in atherogenesis; nevertheless, plasmacytoid dendritic cells, which colocalize with T cells in atherosclerotic plaques, appear to contribute to disease progression through the production of IFN-γ [79]. Despite this heterogeneity, all DC subsets express varying levels of MHCII and CD11c, and ageing has been associated with the accumulation of $CD11c^+$ cells in mouse aortas [80]. Following activation by autoantigens such as oxLDL, DCs may accumulate intracellular lipids and transform into $CD11c^+$ foam cells, thereby acquiring the ability to induce T-cell activation and proliferation, as well as the release of IFN-γ and TNF-α, ultimately promoting atherosclerosis progression [80,81]. Furthermore, $CD11c^+$ DC produces several metabolites relevant to atherogenesis, including indoleamine 2,3-dioxygenase, one of the rate-limiting enzymes in the kynurenine pathway whose metabolites have been associated with prevalent atherosclerosis [82]; cyclooxygenase, the rate-limiting enzyme in prostaglandin synthesis that is upregulated in atherosclerotic lesions [83]; and inducible nitric oxide synthase [83]. NO production, by promoting glycolytic reprogramming, supports prolonged DC survival, sustained secretion of pro-inflammatory cytokines, and an enhanced capacity to stimulate T cells, thereby contributing to plaque development. [84].

T lymphocytes, together with other immune cell populations, play a pivotal role in both the initiation and progression of atherosclerosis [85]. Within atherosclerotic lesions, three major T-cell groups can be distinguished, each exerting pro- or anti-atherogenic effects depending on the specific subset involved [3,85]. Among $CD4^+$ T cells, multiple T helper (Th) subsets have been characterized: Th1 cells, which predominantly produce IFN-γ and are strongly pro-atherogenic; Th2 cells, which secrete IL-4 and IL-13, although their contribution to disease modulation remains controversial; and Th17 cells, defined by IL-17 production and implicated in plaque development and destabilization [85]. Additional $CD4^+$ subsets include T follicular helper cells, producers of IL-21 and key regulators of B-cell activation and differentiation, as well as $CD28^-$ T cells, both associated with pro-atherogenic activity. Conversely, regulatory T (Treg) cells exert protective effects during early disease phases but may convert into pro-inflammatory ex-Tregs during advanced atherosclerosis, thereby contributing to disease progression [85]. $CD8^+$ T cells are also abundant within lesions and display a dual role, with evidence supporting both protective and pathogenic functions depending on their activation state and microenvironmental context [85]. Non-conventional T-cell subsets further contribute to disease pathogenesis. Invariant natural killer T (iNKT) cells, capable of producing a broad spectrum of Th-like cytokines, have been implicated in accelerating plaque progression and promoting structural destabilization, including necrotic core expansion [85]. Similarly, γδ T cells, which secrete IL-17, exhibit functional properties overlapping with Th17 cells and are considered pro-atherogenic

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[85]. As outlined in the previous sections, activation of p38/MAPK plays a cruciall role in establishing the senescent phenotype by activating p53, inducing the expression of p16 and p21 to enforce cell cycle arrest, inhibiting autophagy, disrupting redox balance and promoting mitochondrial dysfunction, and regulating SASP release [86]. Consistently, within the immune system, p38/MAPK signaling drives cellular senescence and regulates the SASP phenotype in both $CD4^+$ and $CD8^+$ T lymphocytes [86,87]. $CD8^+$ T cells, in addition to secreting high levels of IL-18, a pro-inflammatory cytokine that induces IFN-γ release, also produce large amounts of metalloproteinases, particularly ADAM28, a member of the ADAM family involved in cell adhesion, migration, proteolysis, and signaling [87,88]. In senescent $CD4^+$ T lymphocytes, p38/MAPK activation disrupts mitophagy, leading to the accumulation of dysfunctional mitochondria and increased production of Th17-type cytokines, including IL-6, IL-17A, IL-17F, IL-21, IL-23, and GM-CSF [86]. Importantly, these effects can be reversed by treatment with BIRB-796, a specific p38/MAPK inhibitor, which markedly reduces mitochondrial mass, ROS levels, and secretion of Th17-type mediators [86]. Furthermore, SIRT1 expression, whose levels decline with age in multiple organs and tissues, is significantly downregulated in $CD8^+CD28^-$ T cells, a population of terminally differentiated memory T cells that expands during ageing [89]. Despite their impaired proliferative response to antigen-specific stimulation, $CD8^+CD28^-$ T cells retain high cytotoxic potential and produce elevated levels of effector molecules such as IFN-γ, granzyme B, and perforin [89]. Reduced SIRT1 levels compromise the expression and function of FoxO1, a transcription factor involved in proliferation, differentiation, cell survival, glucose metabolism, longevity, and resistance to oxidative stress [89,90]. Consequently, $CD8^+CD28^-$ T cells exhibit enhanced glycolytic capacity and increased granzyme B production [89]. Conversely, when deacetylated, FoxO1 translocates to the nucleus promoting endothelial dysfunction, therefore SIRT-1 decline may exert an atheroprotective action against plaque progression [69,91].

B cells have been identified in both healthy and atherosclerotic aortas, where they are mainly localized in the adventitial layer surrounding atherosclerotic regions, whereas they are only rarely detected within plaques [92,93]. Mature B cells can be broadly divided into two major subpopulations based on activation requirements, anatomical localization, and surface markers: the less prevalent B-1 cells ($CD20^+CD27^+CD43^+CD70^+$), which can be further subdivided into B-1a ($CD11b^+$) and B-1b ($CD11b^-$) subsets, and the conventional B-2 cells, which include follicular B cells, marginal zone B cells, and regulatory B cells (Bregs) [93,94]. B-cell subsets exert distinct effects in atherosclerosis primarily through antibody production [93]. While B-1 cells confer protection by producing natural IgM antibodies that bind oxLDL and neutralize its pro-inflammatory effects, B-2 cells promote disease progression through the production of atherogenic IgG, which can bind oxLDL and modulate macrophage activation [93,95]. Beyond their antigen-presenting capacity and immunoglobulin secretion, B cells also release a broad array of cytokines that can either promote or attenuate atherosclerosis: B-1 cells secrete IL-12, IFN-γ, and TNF-α; B-2 cells produce IL-2, IL-4, TNF-α, and IL-6; and Bregs release IL-10 and TGF-β [92]. The prevalence of late/exhausted memory (LM) B cells, one of the four peripheral B cells subsets, increases with age [96]. Although LM B cells do not proliferate in vitro in response to mitogenic stimulation, they are transcriptionally active and express multiple SASP markers, such as pro-inflammatory cytokines (TNF-α, IL-6, IL-8), cell cycle regulators (p16), inflammatory micro-RNAs (miRs, miR-155, miR16, miR93) [96,97]. Expression of these SASP components is associated with activation of NF-κB and p38/MAPK signaling following spontaneous AMPK activation, a key regulator of metabolic homeostasis [97,98]. Notably, aged LM B cells show increased expression of the chemokine receptor CXCR3, enabling their preferential migration toward vascular sites where its ligands are released by activated endothelial and epithelial cells [3,97] (Figure 3).

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3. Selenium, Cellular Senescence and Atherosclerosis

3.1. Selenium: An Overview

Selenium, a metalloid belonging to the same group as oxygen and sulfur, is a well-established essential trace element in humans, where it serves as a key structural component of enzymes with antioxidant, anti-inflammatory and immunomodulatory properties [9,99]. The total amount of Se in humans ranges between 3-20 mg, with intake primarily derived from food sources and, to a much lesser extent, from water and atmospheric inputs [9,100]. Although cereals represent the main dietary source of Se in terms of bioavailability, plant-based foods generally contain lower Se levels (0.01–0.55 μg/g) compared with animal-based foods (0.08–0.7 μg/g) [100]. This difference largely reflects the variability of Se concentrations in soils, which range from 0.005 to 3.5 mg/kg depending on the country and region, even within the same country, with a global average soil Se concentration of approximately 0.32 mg/kg [101,102]. Soil Se bioavailability is influenced not only by physicochemical properties (e.g., pH, redox status, soil moisture, organic matter content, microbial activity) but also by its chemical speciation [103]. In soil solution, the oxyanions (Se(VI)), predominant in well-aerated alkaline soils, and selenite (Se(IV)), more abundant in neutral to acidic soils, represent the most mobile and bioavailable forms for plant uptake [101,103]. Their interconversion largely depends on soil pH and redox potential, with selenite frequently bound to iron hydroxides in acidic soils [99,101,103]. Organic Se species - including Se-containing proteins, metabolites, and Se covalently bound to soil organic matter - constitute an additional plant-available pool through direct uptake (e.g., selenomethionine - SeMet, selenocysteine - SeCys, methylselenocysteine), oxidation to inorganic oxyanions, or mobilization during organic-matter turnover [101]. In contrast, insoluble elemental Se (Se^0) and selenide (Se^{2-}), which predominate under reducing conditions, are poorly available to plants [103]. In foods, selenoamino acids exhibit higher bioavailability than inorganic Se species [100]. SeMet, synthesized exclusively by plants and fungi, reaches 95–98% bioavailability in plant-derived matrices, whereas SeCys is the predominant chemical form in animal-based foods, reflecting its direct incorporation into selenoproteins [9,100].

Following absorption in the duodenum, Se is distributed to tissues with different affinities, with the liver and skeletal muscle each accounting for approximately 30% of total body stores [9,104]. In enterocytes, dietary Se is incorporated into selenoproteins in the form of SeCys, which is essential for their biological activity [9,100]. SeCys, the 21st naturally occurring amino acid, lacks a cognate aminoacyl-tRNA synthetase in humans and therefore requires a specialized biosynthetic pathway for its incorporation into selenoproteins [100]. SeCys is inserted into SELENOP, a glycoprotein containing 10 SeCys residues and accounting for up to 40% of total circulating Se, thus serving as the main Se transport protein to deliver Se to other cells [100]. To date, 25 selenoproteins have been identified in humans [100]. Distributed across different organs, most selenoproteins, including GPx and TrxR, play essential roles in antioxidant defense [100]. Their antioxidant activity has also been related to chemopreventive mechanisms and a reduced cancer risk [105]. Se supplementation enhances both innate and adaptive immune responses, with immunostimulatory effects detectable also in individuals with adequate Se status [10,100]. SELENOP, SELENOW, and GPx4 are among the most abundantly expressed selenoproteins in the brain, underscoring their importance in preserving neural function; notably, the brain contains one of the largest Se reservoirs in the body - second only to the liver - and this concentration is maintained under conditions of dietary Se depletion [100,105]. Beyond its transport function, SELENOP exhibits redox properties that protect endothelial cells from oxidative injury, while other selenoproteins such as GPx3, SELENOS, and SeAlb have been associated with cardiovascular health through their roles in regulating oxidative stress and inflammation [105].

The World Health Organization and the Food and Agriculture Organization recommend a daily selenium intake of 55–60 μg for adults; however, global dietary guidelines are not standardized, and recommendations vary across countries and organizations [7,9,106]. The European Food Safety Authority has set an adequate intake of 70 μg/day and a tolerable upper intake level of 255 μg/day for adults [107,108].

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Se exerts both beneficial and adverse effects depending on its concentration [109]. Chronic high-level Se exposure - resulting from over supplementation, frequent consumption of Se-rich foods, or occupational exposure (e.g., Se processing plants) - can lead to selenosis, a condition characterized by nail brittleness, hair loss, fatigue, and skin lesions [109,110]. As the condition progresses, neurological impairment may develop, including cognitive decline and paralysis, and in severe cases it can be fatal [110]. Moreover, high Se intake has been associated with renal dysfunction, increased cardiovascular risk, MI, and heart failure, highlighting that the beneficial effects of Se occur within a narrow range of plasma concentrations [9,111–113].

3.2. Selenium and Cardiovascular Disease in Human Studies: Focus on Atherosclerotic Cardiovascular Diseases

Despite inconsistent findings from early observational studies and Se supplementation trials, an increasing body of evidence supports a role for Se in the optimal functioning of the cardiovascular system [114]. In this context, a recent meta-analysis of 13 observational studies and randomized controlled trials (RCTs) reported a 15% reduction in cardiovascular disease (CVD) incidence per 10 μg/L increase in blood Se concentration, together with a statistically significant nonlinear dose–response relationship between blood Se levels and CVD mortality [115]. The lowest risk was observed at Se concentrations between 30 and 35 μg/L, whereas CVD risk increased at concentrations above 300 μg/L, indicating that Se may exert protective effects only within a physiologically optimal range for both CVD incidence and mortality [115].

In one of the earliest comprehensive assessments, Flores-Mateo and colleagues investigated the association between Se and CHD by conducting a meta-analysis of observational studies and RCTs [116]. In the analysis of observational studies (14 prospective cohort and 11 case–control studies), the pooled relative risk (RR) comparing the highest with the lowest category of blood Se concentration was 0.85 (95% Confidence Interval - CI: 0.74–0.99) in cohort studies and 0.43 (95% CI: 0.29–0.66) in case–control studies. Moreover, dose–response analyses indicated a 24% reduction in CHD risk associated with a 50% increase in Se concentration [116]. By contrast, the meta-analysis of RCTs (six trials: two using Se alone and four using Se in combination with vitamins or minerals; Se doses of 75, 100, or 200 μg/day) showed a lower, but not statistically significant, risk of CHD with Se supplementation compared with placebo (pooled RR= 0.89; 95% CI: 0.68–1.17) [116]. It should be noted, however, that the inverse associations observed in observational studies require confirmation after adequate adjustment for potential confounders, including other dietary antioxidants (such as vitamin E, folate, and β-carotene), overall dietary patterns and Se intake from foods or supplements, as well as residual confounding related to socioeconomic status, educational level, and other cardiovascular risk factors [116]. By contrast, the limited evidence from randomized trials may, at least in part, be explained by relatively small number of participants and by substantial heterogeneity across studies, related to differences in baseline Se status, treatment duration, and intervention regimens, including combined supplementation strategies [116]. Notably, Zhang et al. confirmed these findings when the outcome was expanded to include total CVD [117]. Specifically, the meta-analysis of 16 prospective studies (N=35,607 participants and 4,421 incident CVD cases) showed a significant inverse association with CVD risk within a relatively narrow range of blood Se concentrations (55–145 μg/L), which became null at concentrations exceeding 145 μg/L [117]. In contrast, the meta-analysis of 16 RCTs (37,572 participants) found no significant effect of oral Se supplementation (median dose: 100 μg/day) on CVD risk over follow-up periods ranging from 6 to 114 months [117]. Indeed, although beneficial effects of Se may be more likely in deficient populations, adequate or high Se status may be associated with neutral or even adverse effects [117]. Moreover, the authors could not exclude potential changes in compliance among trial participants or heterogeneity in Se concentration responses following supplementation [117,118].

In a subsequent meta-analysis including 16 RCTs and a total of 43,998 participants, Ju et al. observed a non-significant trend toward reduced CHD mortality among individuals receiving Se supplementation compared with controls (Odds Ratio - OR = 0.88; 95% confidence interval 0.76–1.02;

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$p = 0.087$) [119]. Consistently, Se supplementation was not significantly associated with total cholesterol, LDL or high-density lipoprotein cholesterol levels [119]. By contrast, the intervention was associated with significantly lower serum levels of C-reactive protein, a major inflammatory marker and a well-established risk factor for CHD that is directly correlated with both coronary and peripheral atherosclerosis [120,121], as well as with increased GPx activity [119]. Accordingly, Se supplementation appears to attenuate inflammation and oxidative stress, despite the absence of significant effects on lipid profiles or CHD mortality [119]. The lack of association with CHD mortality, in particular, may reflect the chronic and progressive nature of the disease, characterized by a high burden of complications and fatal events; thus, short-term Se supplementation may be insufficient to reduce mortality, while still being effective in improving inflammatory and pro-oxidant status [119]. Additionally, given that in two trials participants receiving placebo exhibited higher mortality than those receiving combined Se and coenzyme Q10 supplementation, the combination of Se with other supplements may have a greater impact on reducing CHD mortality than Se alone [119]. This notion is further supported by a meta-analysis of 43 RCTs by Jenkins et al., which showed that Se supplementation was associated with reduced cardiovascular and all-cause mortality only when combined with antioxidant mixtures (e.g., vitamin A, vitamin C, vitamin E, β-carotene, zinc, copper) (RR = 0.77, 95% CI: 0.62–0.97; RR = 0.90, 95% CI: 0.82–0.98) [122]. In contrast, neither Se alone nor antioxidants alone had significant effects, suggesting that Se-related effects are finely balanced and may depend on synergistic antioxidant interactions [122]. A recent systematic review and meta-analysis of 884 RCTs evaluating the effects of 27 different micronutrients (median duration intervention: 3 years) on CVD outcomes in a total of 883,627 participants found a moderate beneficial effect of Se on dyslipidemia [123]. However, no significant associations were observed with the incidence of CHD (RR= 0.89, 95% CI: 0.64–1.23), MI (RR=0.87, 95% CI: 0.59–1.30), or CVD mortality (RR=0.97, 95% CI: 0.83–1.14) [123]. Importantly, several the intervention trials had very short durations (<1 month), and the effects of antioxidant mixtures were not evaluated, raising the possibility that the overall cardioprotective effects of Se supplementation may have been underestimated [123].

A meta-analysis of 12 observational studies (10 cohort and 2 case–control studies), including a total of 25,667 individuals, confirmed that low Se levels were associated with an increased risk of both all-cause and CVD mortality [124]. When comparing the lowest with the highest category of circulating Se levels, the pooled RRs were 1.36 (95% CI: 1.18–1.58) for all-cause mortality and 1.35 (95% CI: 1.13–1.62) for CVD mortality [124]. In contrast, the association with CHD mortality, assessed in four studies, was not statistically significant (pooled RR=1.43, 95%CI: 0.93-2.19) [123]. This lack of significance may be partly explained by the reliance on a single baseline measurement of Se, which could have led to exposure misclassification and dilution of risk estimates across Se level categories [124]. On the other hand, because high-normal Se concentrations (>150 μg/L) may be associated with an increased risk of mortality [125], the inclusion of the highest Se category as the reference group may have led to an underestimation of the association between low Se levels and CVD mortality [124]. Consistently, the protective effects of low Se status on CVD and all-cause mortality were more evident in Europe and Asia, regions characterized by populations with low Se intake (<50 μg/day) or low Se status (serum concentrations <100 μg/L) [115,123]. More recently, Yang et al. examined differences in Se levels between patients with CHD or MI and healthy controls within a meta-analysis including 38 studies and 25 cohort populations [126]. Both MI and CHD patients exhibited significantly lower blood Se levels (Standard Mean Difference – SMD= -3.64, 95%CI: -4.43, -2.85; SMD= -0.47, 95%CI: -0.67, -0.28) compared to healthy controls, supporting the hypothesis that low Se status may be associated with an increased risk of atherosclerotic cardiovascular disease (ASCVD) [126]. Notably, circulating Se levels also demonstrated a favorable diagnostic performance for MI, with relatively high sensitivity (77.27%) and specificity (72.73%) [126]. Based on data from the U.S. National Health and Nutrition Examination Survey (NHANES) 2011–2016, a cross-sectional study involving 5,101 participants reported a strong L-shaped association - rather than a U-shaped relationship as previously described by Kuria et al. [115] - between blood Se concentrations and the

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prevalence of CHD [127]. In addition, participants in the second, third, and fourth quartiles of blood Se concentration exhibited significantly lower odds of CVD morbidity compared with those in the lowest quartile, consistent with a dose–response pattern (OR=0.71, 95% CI: 0.53–0.96, $p = 0.024$; OR=0.73, 95% CI: 0.55–0.99, $p = 0.041$; and OR 0.74, 95% CI: 0.55–0.98, $p = 0.038$, respectively), indicating that even the highest Se quartile was associated with a protective effect [127]. However, these findings should be interpreted with caution, as the cross-sectional study design does not allow causal inferences regarding the relationship between Se status and CVD. Cui et al. were the first to assess the relationship between Se status, selenium-related biomarkers, and CVD mortality in a meta-analysis of population-based studies (n=9; 41,548 participants) [128]. In pooled analyses, each standard deviation increase in Se/SELENOP concentration was associated with an 11% reduction in CVD mortality across nine studies including 41,548 participants (RR=0.89, 95% CI: 0.84–0.94) [128]. This finding contrasts with the results reported by Kuria et al. [115], who observed no significant association between a 10 μg/L increase in blood Se concentration and CVD mortality (RR=0.93, 95%CI: 0.83–1.05); notably, however, their meta-analysis was not restricted to population-based studies [128].

Of interest, Liang et al. recently explored the relationship between dietary Se intake and the risk of CVD in U.S. adults using data of 39,372 participants from the NHANES 2003–2018 [17]. The overall prevalence of CVD was 8.57% and progressively decreased across increasing tertiles of dietary Se intake (11.10% in the lowest tertile vs. 6.75% in the highest tertile) [17]. Furthermore, after adjustment for potential confounders, a significant inverse association between dietary Se intake and CVD risk was observed when comparing the highest tertile with the reference category (OR=0.73, 95% CI: 0.63–0.86, $p < 0.0001$) [17]. Similarly, higher dietary Se intake was associated with a significantly reduced risk of CHD (OR=0.78, 95% CI: 0.64–0.95, $p = 0.01$; third vs. first tertile) and ASCVD (OR = 0.85, 95%CI: 0.74–0.98, p = 0.02; second vs. first tertile) [17]. Notably, the relationship between dietary Se intake and both CVD and ASCVD followed a significant non-linear pattern ($p = 0.002$), with risk decreasing beyond an inflection point at 135.28 μg/day and subsequently increasing at higher intakes, suggesting a potential increased risk of adverse outcomes at excessive Se intakes [17]. Subgroup analyses further showed that the inverse association between higher dietary Se intake and ASCVD risk was particularly evident among females, individuals younger than 60 years, and those with obesity or hypertension [17]. Moreover, hypertension status significantly modified the association between dietary Se intake and ASCVD risk (P for interaction = 0.034) [17]. Despite these relevant findings, it is important to note that the cross-sectional study design cannot capture potential variations in dietary patterns over time, and such changes may influence the observed relationship between Se intake and CVD [17]. In addition, reliance on self-reported CVD outcomes may have introduced recall bias [17].

Taken together, human studies provide divergent evidence depending on study design. While multiple observational studies meta-analyses consistently suggest an inverse or non-linear association between Se status and ASCVD risk, RCTs largely fail to demonstrate significant benefits on reduced CHD/CVD events or mortality with Se supplementation alone. These discrepancies highlight the importance of baseline Se status, the identification of an optimal dose - possibly in combination with other beneficial antioxidants - and adequate study duration when designing and implementing RCTs. Furthermore, the chemical form of Se supplementation may influence circulating Se concentrations, with SeMet being among the most effective organic selenocompounds for improving Se status; this aspect may contribute to an underestimation of the beneficial effects of Se supplementation in some trials.

On the other hand, observational studies are inherently subject to potential bias. In particular, reliance on a single baseline Se measurement may lead to exposure misclassification, while the observational nature of these studies cannot fully rule out residual confounding (diet quality, antioxidant intake, socioeconomic status). Causality remains uncertain, as observational associations may be influenced by residual and reverse causation. In addition, substantial heterogeneity in the cut-off values used to define Se status across studies limits the ability to identify precise optimal circulating Se levels in the clinical setting (Figure 1).

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Future trials should be stratified by baseline Se status, focusing on deficient or low-status individuals, to clarify whether supplementation benefits are restricted to specific subgroups. There is a need for long-term RCTs with adequate duration and statistical power, given the chronic and progressive nature of atherosclerosis and CHD.

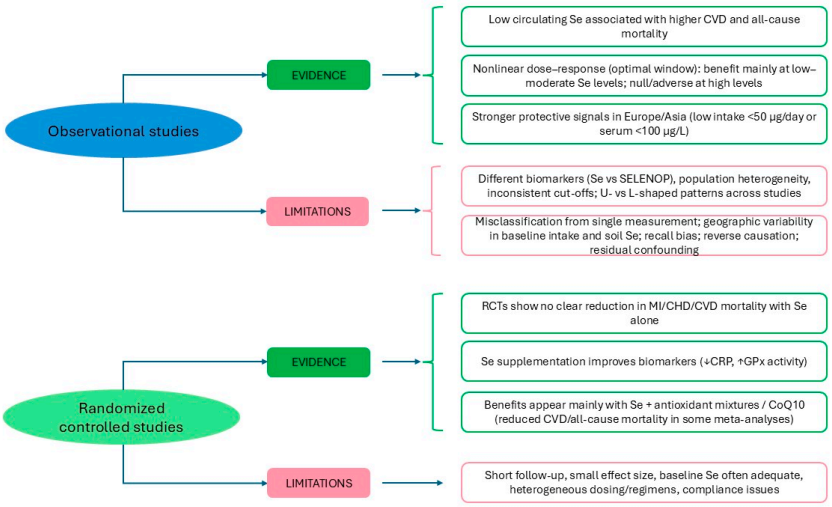

Figure 4. Critical appraisal of the association between selenium and cardiovascular disease. Abbreviations: CHD: coronary heart disease; CoQ10: coenzyme Q10; CRP: C-reactive protein; CVD: cardiovascular disease; GPx: glutathione peroxidase; MI: myocardial infarction; RCT: randomized controlled trial; Se: selenium; SELENOP: selenoprotein P.

3.3. Cellular and Molecular Mechanisms of Selenium in Preventing Atherosclerosis

As previously mentioned, when present in appropriate concentration, Se exerts its beneficial effects, playing a key role in protecting against atherosclerosis. Se contribution is summarized in Figure 5 and was proved by several in vitro and in vivo studies, hereby described, that demonstrated how this trace element can influence multiple and interconnected pathways of atherosclerotic cascade, including maintenance of redox balance, control of inflammation, and prevention of DNA damage, cellular senescence and endothelial dysfunction.

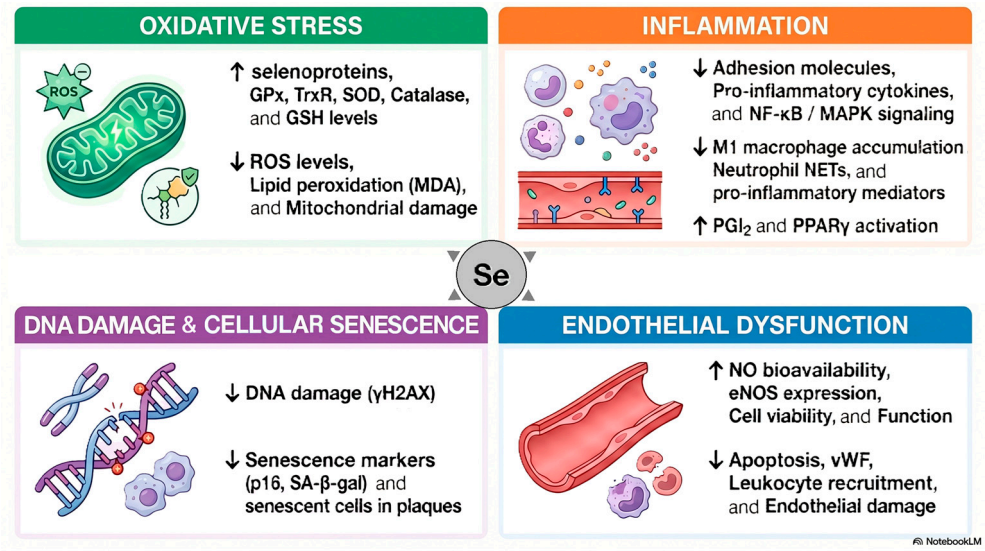

Figure 5. Selenium mechanisms in contrasting key processes of atherosclerotic disease. Image partially generated with Notebook LM. Abbreviations: eNOS: endothelial nitric oxide synthase; γH2AX: phosphorylated H2A

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histone family member X; GPx: glutathione peroxidase; GSH: glutathione; MAPK: mitogen-activated protein kinase; MDA: malondialdehyde; NETs: neutrophil extracellular traps; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NO: nitric oxide; PGI2: prostaglandin I2; PPARγ: peroxisome proliferator activated receptor-gamma; ROS: reactive oxygen species; SA-β-gal: senescence-associated beta-galactosidase; Se: selenium; SOD: superoxide dismutase; TrxR: thioredoxin reductase; vWF: von Willebrand factor.

3.3.1. Selenium in Contrasting Oxidative Stress

The status of oxidative stress, characterized by the imbalance between oxidants levels (in particular ROS) and ineffective antioxidant defense, is strongly linked to atherosclerosis. This association is due to the enhanced production of these reactive species, caused by all established cardiovascular risk factors, including hypercholesterolemia, hypertension, diabetes mellitus, and smoking. As described in Section 2, vascular regions presenting this condition of oxidative stress provoke a disturbance in blood flow, thus becoming preferential sites for atherosclerotic plaques formation [129].

In this scenario, every contribution to antioxidant defenses can exert an essential role. Se directly participates in the suppression of oxidative stress, by associating in selenoproteins and scavenging damaging oxidant species, as well as bolstering endogenous antioxidant defense systems [130]. Indeed, several studies demonstrated that Se not only takes part in constituting selenoproteins but also increases levels and activity of multiple antioxidant mediators. Already in 1998 Rosenblat & Aviram provided evidence that Se increases GSH content and GPx activity by 2-fold in murine macrophage-like cells, resulting in a 30% reduction in macrophage-mediated LDL oxidation, a key early event in atherogenesis [131]. Other in vitro evidence, using the same model, showed that organic compounds containing Se, diphenyl diselenide and disubstituted diaryl diselenides, can reduce ROS generation, foam cell formation, NF-κB activation, and mitochondrial dysfunction through GPx-like and TrxR-like activities [132,133]. In rat VSMCs, pre-treatment with sodium selenite prevents the oxidative stress induced by oxysterol, preserving of TrxR and GPx expression levels, sustaining the activity of this latter and superoxide dismutase (SOD) and maintaining the total antioxidant capacity, with the inhibition of ROS generation [134]. In human umbilical vein endothelial cells, HUVECs, Se pre-treatment, in the form of sodium selenite, confers protection against chemically induced oxidative damage, by stimulating TrxR expression and increasing the activity of this enzyme, as well as that of two other selenoproteins, GPx1 and GPx4, by 3-4 fold [135]. More recently, Se contained in an integrated cascade nanozyme (MSe_1), with SOD- and GPx-like activities, by reducing ROS levels in HUVECs and RAW264.7 macrophages [136].

The first in vivo evidence about antioxidant properties of selenium were observed in the 90s and in rabbit, in a synergic combination with vitamin E [137,138] and in hamster in association with glutathione [139]. More recent studies better characterized actions of this element. Nanoscale selenium showed protective effects against mitochondrial oxidative damage and apoptosis in hyperhomocysteinemia conditions, by hampering downregulation of GPx1 and GPx4 in rats' vascular endothelial cells [140]. Similarly, human umbilical vein cell line, EA.hy926, pre-treated with selenium nanoparticles (SeNPs) are less susceptible to H_2O_2, as demonstrated by lower levels of malondialdehyde (MDA), key product of lipid peroxidation and marker of oxidative stress, and increased activity of GPx and SOD. Enhanced activity of these enzymes, along with catalase, following treatment with SeNPs was confirmed in vivo, in apolipoprotein E-deficient ($ApoE^{-/-}$) mice fed a high-fat diet. Moreover, in this model, SeNPs increase the expression levels of other six selenoproteins, TrxR1, TrxR2, SelP, SelR, SelS and Sep15. As result of these alterations, these mice present lower levels of serum and hepatic MDA [141]. Analogous results were obtained by Xiao et al. with sodium selenite and SeNPs stabilized with chitosan [142]. Another animal study conducted in spontaneously hypertensive rat models, proved that Se supplementation mitigates overall redox status of the aortic wall, by enhancing GPx1 activity and reducing lipid peroxidation, eNOS expression, and markers of advanced glycation end products [143]. In addition, a recent study reported that a selenium-containing confined cascade nanozyme system exerts potent antioxidant

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effects in endothelial cell models and in ApoE$^{-/-}$ mice, by promoting ROS scavenging through catalytic cascade reactions, consequently leading to reduced intracellular ROS levels, decreased lipid peroxidation, and attenuation of oxidative stress–related vascular damage, supporting the role of selenium in redox homeostasis [144].

3.3.2. Selenium in Regulating Inflammatory Mediators

Atherosclerosis is characterized by a persistent inflammation of the vascular wall, where endothelial activation, leukocyte recruitment, and cytokine production drive lesion development and progression [145].

Remarkably, Se was reported to influence several cellular and molecular mediators of this cascade, thus directly and indirectly mitigating activation and amplification of inflammation. In HUVECs, in inflammatory conditions, Se contrasts the expression of three important adhesion molecules, E-selectin, VCAM-1, and ICAM-1 [146]. At macrophages level, in murine RAW 264.7 cells, it was observed that Se triggers the increase of 15d-PGJ2, a prostaglandin that suppresses the expression of pro-inflammatory mediators, IL-6, TNF-α, and NO, and promotes the activation of peroxisome proliferator-activated receptor-gamma (PPARγ), important transcription factor and nuclear receptor that regulates lipid homeostasis, dampens inflammatory responses, and limits plaque development [147]. Additional evidence about selenium relevance in controlling atherosclerosis-related inflammation is provided by the study of Cao et al. in bovine mammary endothelial cells maintained in selenium-deficient media. Without this element, arachidonic acid metabolism shifts toward a pro-inflammatory and pro-thrombotic profile with reduced production of vasodilatory and anti-aggregatory prostacyclin (PGI_2) and other protective prostaglandins ($PGF_2\alpha$, PGE_2), and significant increase in pro-aggregatory thromboxane A_2 (TXA_2) and the pro-inflammatory lipid hydroperoxide 15-HPETE [148]. Moreover, Se can indirectly limit vascular inflammation, by upregulating GPx1/GPx4. Indeed, when these enzymes are experimentally downmodulated in endothelial cells, in addition to ROS accumulation, there is also an increase in the expression of adhesion molecules (ICAM-1, VCAM-1), and leukocyte recruitment via activation of NF-κB and MAPK pathways, whereas their upregulation or pharmacological mimicking attenuates these responses [18].

Anti-inflammatory properties of selenium are further supported by some in vivo studies. Specifically, in 1992 Meydani et al. were among the first to demonstrate in an animal model (F344 rats) that selenium deficiency specifically decreases aortic PGI_2 synthesis and shifts the TXB_2/PGI_2 ratio unfavorably, directly linking dietary selenium to vascular wall prostacyclin production relevant to atherogenesis [149]. Additionally, in male Sprague-Dawley rats, it was observed that serum selenium is inversely correlated to cholesterol level and its supplementation, besides reducing ROS levels, downregulates CD36 expression, a scavenger receptor involved in oxLDL uptake, foam cell formation, and vascular inflammation [150]. Coherently, in ApoE$^{-/-}$ mice fed a high-fat diet, selenomethionine reduces atherosclerotic plaque development by modulating inflammatory cell behavior, with decreased M1 macrophage accumulation in lesions and reduced neutrophil extracellular trap formation, with the confirmation of these effects also ex vivo in human neutrophils [151]. In the same mouse model, Xiao et al. demonstrated that both SeNPs and selenite forms prevent vascular inflammation, revealing decreased levels of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, reduced NF-κB signaling in the vessel wall, and absence of macrophages infiltration and VSMCs migration [141].

Emerging human evidence supports anti-inflammatory power of Se, in fact a recent epidemiological study found that selenium concentration is inversely associated with pro-inflammatory markers, TNF-α, IL-6, MCP-1 [152].

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3.3.3. Selenium in Preventing DNA Damage and Cellular Senescence

DNA damage that accumulates in vascular and resident cells is recognized as a causal factor in the initiation and progression of atherosclerosis, triggering an overwhelmed response that promotes cellular senescence, apoptosis, and plaque instability [153].

Adequate selenium status is reported to counteract several hallmarks of aging that are also implicated in atherogenesis, including oxidative DNA damage, telomere attrition, and cellular senescence [130]. For these properties scientists are testing this element, included in nanozymes, to assess its potential as preventive strategy in atherosclerotic disease. Remarkably, Liu et al. developed a Se-containing integrated cascade nanozyme (MSe_1), with SOD- and GPx-like activities, that shows antisenescence effects in HUVECs, reducing levels of γH2AX, marker of DNA damage, senescence marker p16 and the amount of SA-β-gal in these cells. Consistently, lower signals of the two markers were revealed by staining plaque areas of $ApoE^{-/-}$ mice treated with MSe_1 [136]. Similar results were obtained by Huang et al., who recently demonstrated that selenium-doped copper formate (Cuf-Se) nanozyme, endowed with SOD- and GPx-like activities, besides scavenging ROS and inhibiting foam cell formation, reduces cellular senescence of HUVECs, with dampened level of γH2AX. Moreover, these results were confirmed in $ApoE^{-/-}$ mice, where a significant decrease in senescent cells number was observed in aortic root sections after treatment with Cuf-Se [154].

3.3.4. Selenium in Attenuating Endothelial Dysfunction

The loss of normal endothelial functions, including impaired vasodilation, increased oxidative stress, and a pro-inflammatory, pro-thrombotic state characterize the condition known as endothelial dysfunction, a hallmark of atherosclerosis. Indeed, this altered phenotype creates a permissive environment for lipid accumulation and vascular inflammation, promoting macrophages migration and plaque development [155].

Increasing studies indicate that Se can attenuate endothelial dysfunction through several mechanisms. From a study with HUVECs exposed to homocysteine as model of vascular injury, it emerged that selenium not only reduces apoptosis, but also improves cell viability, preserves NO bioavailability, and dampens oxidative stress through activation of the AKT pathway, effects that collectively support the restoration of endothelial function. These actions were further confirmed in male Sprague-Dawley rats that show increased plasma NO levels, and reduced von Willebrand factor (vWF), marker of endothelial damage, after Se supplementation [156]. Consistently, nanoscale Se protects endothelial cells and hyperhomocysteinemic rats from vascular damage [140]. Huang et al. proved protective effects of selenium in Wistar rats, in which adequate selenium intake markedly mitigates aortic endothelial damage, present in Se-deficient group, that conversely shows crater-like surface defects, endothelial cell necrosis, platelet adhesion, and smooth muscle cell migration toward the intima, besides reduced GPx activity and altered prostacyclin/thromboxane balance [157].

In addition to in vitro and animal studies, a cross-sectional study including 191 adults revealed an inverse correlation between serum selenium levels and carotid intima-media thickness, a structural marker of endothelial dysfunction and subclinical atherosclerosis, and endothelial adhesion molecules (VCAM-1, ICAM-1, E-selectin) that mediate leukocyte recruitment to dysfunctional endothelium, corroborating the presence of a link with this element and atherogenic alterations [152].

4. Conclusions

Available evidence supports a multifaceted role for Se in preserving vascular integrity and counteracting key processes involved in atherosclerosis development. Se contributes to the control of oxidative stress and inflammation, two central drivers of vascular injury, while also modulating autophagy and cellular homeostasis. These effects collectively mitigate endothelial dysfunction, a pivotal early event in atherogenesis, and help delay the progression of vascular aging. In addition to these well-established mechanisms, emerging evidence points to a role for Se in maintaining genomic

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stability. Se-dependent enzymes and nuclear selenoproteins can limit oxidative DNA damage, reduce chromosomal instability, and support telomere integrity. Given that DNA damage promotes cellular senescence and amplifies pro-inflammatory signaling, this pathway represents a promising but still underexplored component of Se's vasculoprotective effects.

However, both deficiency and excess can disrupt redox balance, enhance oxidative stress, and impair endothelial function. Low Se status is consistently associated with higher cardiovascular risk, particularly for CVD incidence and CVD mortality, as shown by multiple observational meta-analyses. However, Se appears to be protective only within an optimal physiological range, while very high circulating levels or excessive intake may attenuate benefits or increase risk, supporting a nonlinear dose–response relationship. This U-shaped association likely contributes to the heterogeneity observed across clinical studies and underscores the importance of maintaining optimal, rather than supra-physiological, selenium levels.

Despite significant mechanistic and preclinical support, translation into clinical practice remains challenging. RCTs do not provide strong evidence of reduced CHD/CVD events or mortality with Se supplementation alone, despite suggestive trends in some analyses. Nevertheless, evidence from observational studies and targeted interventions suggests that benefits may be more evident in individuals with low baseline Se status, whereas supplementation in Se-replete populations may confer little advantage or potential adverse effects. Evidence also suggests that Se-related benefits may depend on synergistic interactions with other antioxidants (e.g., vitamins A/C/E, zinc, β-carotene, coenzyme Q10), since mortality reductions have been observed mainly in combined supplementation strategies.

These findings highlight the need for a personalized approach to Se in cardiovascular prevention, taking into account baseline nutritional status, individual risk profiles, and possibly genetic variability in selenoprotein function. The potential toxicity threshold and upper safe range for cardiovascular outcomes remains poorly defined, warranting further investigation of high-normal and excessive selenium exposure. Future research should prioritize well-designed, stratified clinical trials and mechanistic studies to better define dose–response relationships and identify reliable biomarkers of Se activity beyond circulating levels. In particular, further investigation into selenium's role in DNA damage and repair pathways in vascular cells may uncover novel therapeutic targets. The role of combined antioxidant regimens (Se plus co-antioxidants) should be evaluated in factorial trial designs to disentangle Se-specific effects from synergistic supplementation effects.

Overall, selenium emerges as a promising yet complex modulator of vascular health. A balanced, evidence-based approach will be essential to harness its protective effects while minimizing risks, ultimately contributing to more effective strategies for the prevention and management of atherosclerotic cardiovascular disease.

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Abbreviations

The following abbreviations are used in this manuscript:

Ang II	Angiotensin II
CHD	Coronary heart disease
Cuf-Se	Selenium-doped copper formate
CVD	Cardiovascular disease
DC	Dendritic cell
DDR	DNA damage responses
EM	Extracellular matrix
GPx	Glutathione peroxidase
GSH	Glutathione
HUVECs	Human umbilical vein endothelial cells
IL-6	Interleukin 6
LDL	Low-density lipoprotein
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MI	Myocardial infarction
MMP	Matrix metalloproteinase
OxLDL	Oxidized low-density lipoprotein
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
PPARγ	Peroxisome proliferator-689 activated receptor-gamma
ROS	Reactive Oxygen Species
SASP	Senescence-associated secretory phenotype
Se	Selenium
SELENOP	Selenoprotein P
SeMet	Selenomethionine
SeNPs	Selenium nanoparticles
SOD	Superoxide dismutase
TGF-β	Transforming growth factor-beta
TrxR	Thioredoxin reductase
TNF-α	Tumor necrosis factor-alpha
TXA_2	Thromboxane A_2
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells
vWF	von Willebrand factor
WSS	Wall shear stress

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