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Homocysteine, Inflammation, and Oxidative Stress: Reassessing a Controversial Biomarker in Cardiovascular Diseases

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Abstract

Homocysteine, a sulfur-containing amino acid, has long been studied as a potential risk factor for cardiovascular disease. While initial studies found strong correlations between elevated serum homocysteine concentrations and cardiovascular events, interventional trials aimed at reducing its levels through vitamin B supplementation failed to demonstrate improved outcomes. In this review, we highlight the experimental studies demonstrating homocysteine's effects on oxidative stress, immune activation, and endothelial dysfunction, all of which are key contributors to atherogenesis. We also emphasize homocysteine's interactions with other risk factors and its reinterpretation as a biomarker of vascular diseases rather than a direct therapeutic target. Importantly, incorporation of homocysteine measurement into broader cardiovascular risk profiles may improve risk prediction, particularly in patients with concomitant metabolic or inflammatory conditions. Thus, evidence on the impact of anti-inflammatory and antioxidant strategies on homocysteine-related pathways is also explored. In this manner, we suggest that homocysteine's clinical utility lies in its ability to signal underlying inflammatory and oxidative stress. Future research should prioritize modulation of downstream oxidative and inflammatory pathways rather than isolated homocysteine reduction

Keywords: Homocysteine; Cardiovascular diseases; Atherosclerosis; Inflammation; Oxidative Stress

Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, caused partly by chronic systemic inflammation and endothelial dysfunction (1). In addition to well-established risk factors like hypertension, hyperlipidemia, and smoking, there is growing interest in identifying nontraditional biomarkers that have the potential to provide deeper insights into and alter the management of vascular pathologies. One of these biomarkers is homocysteine, a sulfur-containing amino acid involved in methionine metabolism. Although homocysteine has been extensively studied, it remains a controversial biomarker in vascular disease, and it is still unclear whether it acts as a causal driver or merely as a bystander reflecting vascular stress.

Epidemiological studies from the late 20th century initially linked elevated plasma homocysteine levels with increased risks of myocardial infarction, stroke, and peripheral vascular disease (2, 3). This raised the question of whether homocysteine might be a causal factor in atherosclerosis,

acting through mechanisms such as oxidative stress, reduced nitric oxide bioavailability, and vascular inflammation. (4, 5) However, subsequent studies dampened this enthusiasm after revealing that vitamin supplementation (B6, B9, B12) lowered homocysteine levels without producing substantial improvements in cardiovascular outcomes (6, 7). Nevertheless, considering homocysteine as a biochemical marker of endothelial stress and inflammation, instead of a standalone therapeutic target reignited interest in homocysteine's effect on vascular disease and treatment. Studies continue to demonstrate its capacity to promote vascular dysfunction (8) while emerging strategies focus more on mitigating its downstream effects like oxidative damage and immune activation instead of lowering homocysteine itself (9).

This review revisits homocysteine's role in cardiovascular disease with an emphasis on its pro-inflammatory mechanisms and the clinical significance of modifying the homocysteine-inflammation pathway. By examining both historical and recent evidence, we aim to clarify homocysteine's role in CVD pathophysiology and to assess its relevance within the context of current anti-inflammatory cardiovascular therapies.

Methodology of Literature Search

This article is designed as a narrative review aiming to synthesize current evidence on the biochemical, experimental, and clinical aspects of homocysteine in cardiovascular disease. A comprehensive, non-systematic literature search was conducted in PubMed, Scopus, and Google Scholar databases to identify relevant publications between January 1990 and September 2025. The search strategy combined the following key terms: "homocysteine" AND ("inflammation" OR "oxidative stress" OR "atherosclerosis" OR "cardiovascular disease").

Both basic science (in vitro and in vivo) and clinical studies were included to ensure mechanistic and translational coverage. Additional references were identified through backward citation tracking of the included papers.

Selection of the studies was based on relevance, methodological quality, and contribution to current understanding of homocysteine-related inflammatory and oxidative pathways.

Homocysteine: Biochemistry and Metabolism:

Homocysteine is an amino acid that plays an important part in the methionine cycle. It is produced when methionine, an essential amino acid found in sources like red meat, fish, and dairy products is demethylated. This multi-step process is where methionine is first converted to S-adenosylmethionine (SAM). SAM then transfers a methyl group, becoming S-adenosylhomocysteine (SAH). The SAM: SAH ratio is a crucial indicator of the cell's methylation capacity. An imbalance in this ratio can affect DNA methylation and other methylation-dependent processes (10).

Homocysteine accumulation normally does not occur in healthy individuals since it is processed through two major pathways: remethylation and transsulfuration. In the remethylation pathway, homocysteine is converted back to methionine with the help of the enzyme methionine synthase, which needs vitamin B12 (cobalamine) and vitamin B9 (folate) as cofactors. Another important enzyme in this pathway is methylenetetrahydrofolate reductase (MTHFR) (11). This enzyme

converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is the form of folate used for the remethylation.

The transsulfuration pathway converts homocysteine into cystathionine and further to cysteine, with cystathionine β-synthase and cystathionase enzymes respectively. This pathway depends on vitamin B6 (pyridoxine) as a cofactor. Glutathione, an important intracellular antioxidant, is also generated from this pathway through cysteine, signifying the pathway's importance in limiting the body's ability to neutralize free radicals.

Disruptions in either pathway can lead to hyperhomocysteinemia, a condition caused by elevated homocysteine levels in plasma. This is most importantly caused by nutritional deficiencies of folate, B6 or B12. Other factors include renal impairments, aging, alcohol consumption, hypothyroidism, and medications like methotrexate and antiepileptic drugs. The genetic mutation of methylenetetrahydrofolate reductase (MTHFR) can also play a part in this serum imbalance (9). While homocysteine does not play a part in the protein production itself, its accumulation can cause devastating effects on various cellular processes, the main one being oxidative stress. Homocysteine auto-oxidizes in plasma, producing reactive oxygen species (ROS) that have the potential to damage endothelial cells, oxidize lipoproteins, and initiate inflammatory signaling. This imbalance is one of the earliest pathophysiological events observed in homocysteine-related vascular disease (10).

Homocysteine thiolactone which is one of the byproducts of this auto-oxidation process, is especially destructive in the condition of homocysteinemia. In normal serum homocysteine levels, its production is low. However, in hyperhomocysteinemic states, it can acylate lysine residues on proteins and low-density lipoproteins (LDL) in a process called protein homocysteinylation (10). Additionally, homocysteine thiolactone has been shown to trigger apoptosis in endothelial cells through a caspase-independent mechanism (11).

These various reactive oxygen species produced by homocysteine are also associated with decreased endothelial nitric oxide through inhibition of endothelial nitric oxide synthase and degradation of nitric oxide, causing vasoconstriction, platelet activation, and monocyte adhesion, which is what is thought to be the culprit of homocysteine-induced vascular dysfunction and consequently, atherosclerosis (12).

In summary, homocysteine is more than a passive metabolic intermediate. Its accumulation reflects a disruption in various mechanisms vital to oxidative balance, endothelial function, and inflammatory signaling. Understanding its biochemical effects is crucial for appreciating its controversial but biologically plausible role in the development and progression of cardiovascular diseases.

Mechanisms Linking Homocysteine to Inflammation:

Homocysteine has long been associated with vascular disease; however, the mechanistic links between Hcy and inflammation have been clarified by biochemical, cellular, and animal studies. (13). Homocysteine promotes inflammation through multiple, interconnected pathways involving oxidative stress, endothelial function, and immune system activation that contribute to a pro-atherogenic environment.

Oxidative Stress and Redox Imbalance

When present in elevated concentrations, homocysteine is highly prone to auto-oxidation in plasma. This then leads to the generation of reactive oxygen species (ROS) like superoxide anions and hydrogen peroxide. These ROS can damage lipids, proteins, and DNA. They also stimulate redox-sensitive transcription factors like NF-κB, which regulate the expression of pro-inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) (14, 15). Several studies have demonstrated that oxidative stress generated by homocysteine precedes detectable inflammation in endothelial and vascular tissues (16,17).

Endothelial Dysfunction

The vascular endothelium is both a barrier and an active regulator of inflammation and homocysteine was found to induce endothelial dysfunction through various pathways (18). Homocysteine reduces the bioavailability of nitric oxide (NO), a potent vasodilator and anti-inflammatory molecule, consequently impairing endothelial function. This process occurs through various mechanisms: inhibition of endothelial nitric oxide synthase (eNOS) (18), increased degradation of NO by superoxide radicals, and upregulation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthesis (19). These changes promote a pro-inflammatory state in the endothelium, indicated by increased expression of adhesion molecules (VCAM-1, ICAM-1 eg.) that facilitate leukocyte adhesion and transmigration (20) (Figure 1). Evidence from various studies confirms that homocysteine exposure increases endothelial permeability and monocyte recruitment in vitro and in vivo (21, 22).

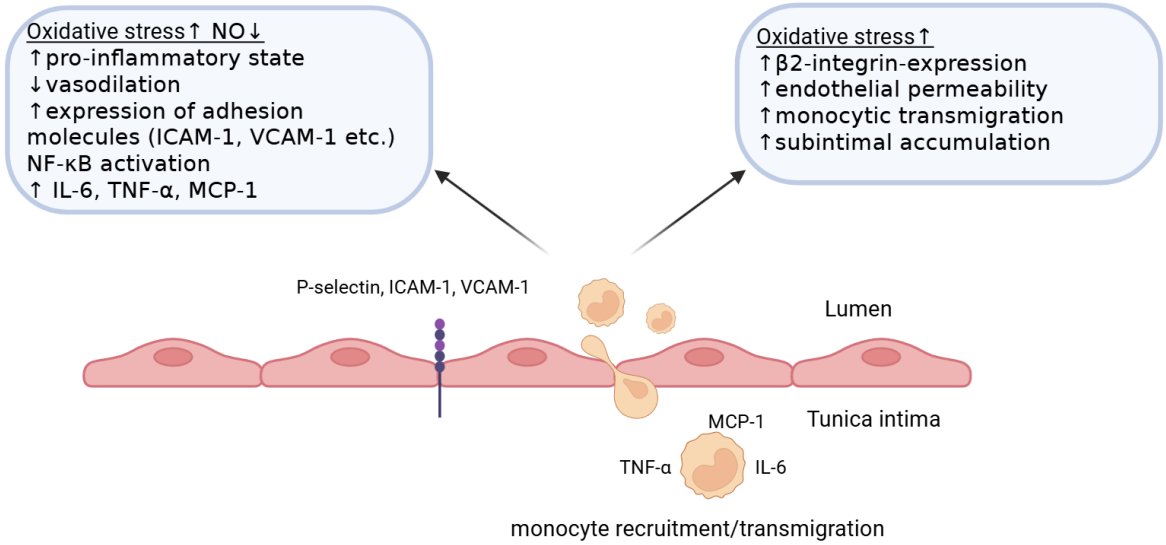

Figure 1. Endothelial dysfunction in hyperhomocysteinemia: Increased oxidative stress reduces nitric oxide (NO) bioavailability, upregulates adhesion molecules (ICAM-1, VCAM-1, P-selectin), and activates NF-κB signaling. These changes enhance cytokine release (IL-6, TNF-α, MCP-1), increase β2-integrin expression, and promote monocyte adhesion and transmigration, contributing to vascular inflammation and atherogenesis. *NO, nitric oxide; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NF-κB, nuclear factor kappa B; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1.

Immune Activation and Cytokine Signaling

Homocysteine also modulates cell behavior in addition to its effects on redox balance and vascular tone. It activates both innate and adaptive immune cells via multiple pro-inflammatory pathways. In macrophages, it stimulates the production of neopterin, a marker of cellular immune activation, mediated by interferon-γ signaling (23). Whereas in the adaptive arm, homocysteine promotes the differentiation of naïve CD4 T cells into T-helper 17 (Th17) cells by upregulating transcription factors such as RAR-related orphan nuclear receptor gamma t (RORγt) and enhancing IL-17 secretion (24). The immune effects of these processes create an inflammatory environment, contributing to vascular injury and supporting the development of atherosclerotic lesions. Moreover, homocysteine thiolactone can homocysteinylate proteins and make them immunologic, triggering autoimmune responses (10).

Crosstalk with Other Risk Factors

Homocysteine often acts synergistically with other cardiovascular risk factors such as hyperlipidemia, hypertension, metabolic syndrome, and gene polymorphisms (25-28). For example, homocysteine has been shown to enhance low-density lipoprotein (LDL) oxidation which is a key step in foam cell formation and early plaque development (29). This emphasizes the importance of homocysteine not only as a lone participant but as a disease amplifier in the context of systemic inflammation.

Homocysteine in Cardiovascular Disease:

There has been extensive research on the role of homocysteine on cardiovascular disease (CVD), including coronary artery disease (CAD), stroke, and peripheral artery disease as a potential biomarker and mediator (30, 31). Its relevance was first established through epidemiologic studies that associated elevated plasma homocysteine levels with increased cardiovascular risk (2, 5, 32). Clinical trials attempting to lower homocysteine levels through vitamin supplementation, however, have yielded mixed results, opening up the debate about its causal role (6, 7).

Epidemiological Evidence

Early observational studies showed a strong correlation between serum homocysteine levels and cardiovascular events (2, 33). For instance, patients who were in the highest homocysteine quartile were shown to have a 2-to-3-fold increased risk of myocardial infarction and stroke (3, 34). These findings suggested that homocysteine could be proposed as an independent risk factor. Some studies also noted homocysteine's correlation with arterial stiffness and carotid intima-media thickness which are the surrogate markers for subclinical atherosclerosis (35, 36).

Recent cohort studies and meta-analyses continue to support a positive correlation between elevated serum homocysteine levels and cardiovascular outcomes. Even slight increases in plasma homocysteine have been linked with increased coronary heart disease, showing a dose-dependent relationship (37). Similarly, in cerebrovascular disease, increased homocysteine levels have been associated with both stroke and ischemic stroke (38). New studies also suggest a specific relationship between homocysteine and large artery atherosclerotic stroke (39),

reinforcing homocysteine's relevance as a predictive biomarker across multiple forms of cardiovascular disease.

Mechanistic Support from Experimental Studies

While the clinical and observational studies were at the forefront of the inflammatory effects of homocysteine, experimental studies have also played an important role in demonstrating the mechanisms behind these processes. In vitro, studies have shown that homocysteine promotes extracellular matrix remodeling and vascular smooth muscle cell proliferation that contribute to plaque formation and vessel wall thickening. These effects seem to be mediated by enzymes involved in vascular remodeling called matrix metalloproteinases (MMPs) (40). Animal models of hyperhomocysteinemia also support homocysteine's pathogenic role. Various studies showed that mice with elevated homocysteine levels develop intimal hyperplasia, arterial stiffness, and increased expression of vascular adhesion molecules (41, 42). Some recent studies have also reported structural disruptions in the arterial wall such as elastin fragmentation and smooth muscle cell disorganization (43, 44). Some studies revealed that homocysteine thiolactone, one of the auto-oxidation products of homocysteine that we've discussed before, has been shown to form in endothelial cells and modify vascular proteins in a way that compromises their structure and function. These modifications take part in the early pathogenesis of atherosclerosis, causing endothelial dysfunction, supporting the view that homocysteine actively alters the vascular environment to promote disease progression (45, 46).

Intervention Trials and Controversy

Despite strong observational and mechanistic support, randomized controlled trials that tried to show that reducing homocysteine levels would improve cardiovascular outcomes have failed to do so (47). Several large studies, including the HOPE-2 and NORVIT trials, investigated whether vitamin B6, vitamin B9, and B12 supplementation could reduce cardiovascular risk by reducing serum homocysteine levels (6, 7). Even though these supplementations lowered homocysteine levels, they did not meaningfully affect event rates. These findings led to skepticism regarding homocysteine's causal role and raised the thought that homocysteine might be functioning more as a marker of vascular injury than a direct target.

Lessons from HOPE-2 and NORVIT

The skepticism surrounding homocysteine's causal role stemmed from various studies, and two of those are HOPE-2 and NORVIT trials. They are both large and well-dxesigned studies, that sought to determine whether vitamin B6, vitamin B9, and vitamin B12 supplementation could reduce cardiovascular event rates in high-risk populations.

Over 5.500 patients participated in the HOPE-2 trial. Participants had risk factors of vascular disease or diabetes and were randomized to receive vitamin-B supplementation or placebo. Even though supplementation led to a ~25% reduction in homocysteine levels, it did not produce a significant reduction in myocardial infarction, stroke, or cardiovascular death rates. Possible explanations for this result might include the high prevalence of concurrent therapies (such as statins and ACE inhibitors), the rate of folate food fortification in participant countries, and revised estimates suggesting that homocysteine-lowering might only return small cardiovascular

benefits. These findings support the growing idea that homocysteine might act more as a marker for vascular disease than a modifiable therapeutic target (6).

Similarly, the NORVIT trial evaluated more than 3.700 patients shortly after acute MI. They found that despite lowering homocysteine levels by nearly 28%, B-vitamin therapy did not reduce event rates. More interestingly, the group receiving all three vitamins had a slightly increased risk of adverse events, more particularly stroke and unstable angina. These results raised some concerns about the potential adverse effects of high-dose vitamin supplementation. It also highlighted the complex role of homocysteine in vascular disease (7).

Together, HOPE-2 and NORVIT reflect the contradictory state between epidemiologic associations and interventional outcomes. This reinforces the idea that while homocysteine remains a relevant biomarker, its role in cardiovascular pathology as a causal agent is far from definitive.

Reinterpretation in Light of Inflammatory Context

More recent interpretations suggest that there might be a deeper complexity behind the failure of these supplementation trials. Homocysteine may not be a primary cause of atherosclerosis but a modulator of inflammatory and oxidative pathways that are already active in high-risk individuals (48, 49). Homocysteine's role might be particularly relevant in subgroups with systemic inflammation, impaired renal function, or genetic polymorphisms (e.g., MTHFR mutation). These populations that were often underrepresented in earlier trials might give us a better idea on homocysteine's role in vascular disease (50-52).

Homocysteine as a Risk Marker in Practice

While current clinical guidelines no longer recommend routine screening for homocysteine in the general population (53) it continues to be of interest in research and specific clinical scenarios (54-56). Homocysteine may additionally serve as a complementary marker in individuals with premature vascular disease, unclear risk profiles, or those with high oxidative or inflammatory burden (57, 58). Various studies have also explored whether homocysteine could help reclassify patients in intermediate-risk categories (59, 60).

Anti-Inflammatory Therapies and Their Impact on Homocysteine:

When considering the overlapping roles of inflammation and endothelial dysfunction in cardiovascular disease, therapies targeting inflammatory pathways have cultivated substantial interest (61, 62). While homocysteine is not typically the target of these treatments, its interactions with inflammation could suggest that anti-inflammatory agents might indirectly modulate its effects or plasma levels (63, 64). This section explores how commonly used pharmacologic and nutritional anti-inflammatory strategies affect homocysteine metabolism and function.

Corticosteroids and Immunomodulators

Corticosteroids are a class of hormones that can be used as strong anti-inflammatory agents across a wide range of conditions, including dermatologic or autoimmune diseases. However,

their effects on homocysteine remain uncertain. Some studies have reported elevated homocysteine levels in patients who have endogenous corticosteroid production or undergoing chronic corticosteroid therapy, contributing to the increased cardiovascular risk in these patients (65,66). Contrarily, corticosteroids' ability to reduce systemic inflammation including IL6 (67) might dampen downstream vascular consequences (68) associated with hyperhomocysteinemia (69). This dual effect underlines the complexity of interpreting homocysteine levels in inflammatory disease settings.

Statins

Statins are a class of lipid-lowering medications that inhibit HMG-CoA reductase which is a rate-limiting enzyme in cholesterol biosynthesis. They are widely used to prevent and manage cardiovascular diseases by decreasing low-density lipoprotein (LDL) levels. Beyond their lipid-lowering properties, statins have pleiotropic effects which include anti-inflammatory and endothelial stabilizing properties (70, 71). Statins were found to reduce CRP levels independently of LDL (70,72) and have been shown to improve endothelial function in patients with elevated serum homocysteine (73, 74). Even though some earlier studies suggested that statin may reduce homocysteine concentrations only modestly with unclear clinical significance (75), the more recent meta-analyses have demonstrated that statin therapy produced a statistically and clinically meaningful reduction in homocysteine concentrations (76, 77). Notably, evidence from the PROSPER trial indicates that statins provide cardiovascular benefits in patients with elevated serum homocysteine levels, even when homocysteine levels remain unchanged. In a post hoc analysis, participants with the highest baseline homocysteine had the greatest absolute risk reduction in coronary heart disease events when treated with pravastatin (78). These findings hint that the protective effects of statins in cardiovascular diseases might arise through mechanisms unrelated to homocysteine metabolism and mechanism, such as inflammation modulation and endothelial stabilization.

Vitamin B Supplementation

The vitamin B family, including B6, B9, and B12—on which homocysteine metabolism heavily relies—has been central to trials attempting to lower homocysteine levels (79,80). While supplementation reliably reduces plasma homocysteine concentrations, most large-scale trials have failed to demonstrate cardiovascular benefit, as discussed in Lessons from HOPE-2 and NORVIT section (6, 7). B-vitamin therapy may also lack efficacy in reducing inflammation itself, limiting its therapeutic value in inflammatory vascular disease. In patients with peripheral arterial occlusive disease, while vitamin supplementation significantly reduced homocysteine levels, it had no impact on key inflammatory markers such as CRP, IL-6, or MCP-1 (81). However, in some subpopulations such as those with severe deficiencies or high baseline homocysteine levels, benefits may still exist (82, 83).

Dietary Interventions and Antioxidants

Dietary strategies rich in anti-inflammatory nutrients like the Mediterranean diet have been associated with lower homocysteine levels and reduced cardiovascular risk (84-86). Higher dietary intake of B-vitamins like folate and vitamin B6 has been linked with improved

homocysteine metabolism and reduced cardiovascular risk (87). Additionally, diets rich in antioxidants like vitamin C, vitamin E, and polyphenols might help counteract the oxidative effects of homocysteine (88), even without lowering its plasma concentration (89) Studies like the ATTICA epidemiologic study suggests that dietary indices like the Food Compass Score (FCS) may be inversely related to both inflammatory biomarkers and homocysteine levels (90).

Insights from the ATTICA Study

The ATTICA epidemiologic study provides valuable information on the connection between diet, homocysteine levels, and cardiovascular risk factors in a general population. This broad study conducted in Greece has investigated various nutritional and inflammatory parameters among its participants (91). One analysis from the ATTICA cohort identified that greater adherence to the Mediterranean diet was inversely related to serum homocysteine levels and was associated with reduced 5-year incidence of cardiovascular disease (92). These findings strengthen the idea that anti-inflammatory dietary strategies may lower both homocysteine levels and cardiovascular risk. Other findings from ATTICA have demonstrated that high-density lipoprotein (HDL) which is a protective lipid marker is inversely related to both CRP and homocysteine levels, highlighting the interconnection between lipid metabolism, inflammation, and homocysteine in the pathogenesis of cardiovascular disease (93). These studies, together, underline the role of diet content and quality in modulating both traditional and emerging cardiovascular risk factors. Consequently, they proved a robust epidemiologic basis for dietary recommendations aimed at reducing homocysteine and inflammation-related vascular damage.

Taken all these together, Table 1 summarizes homocysteine's metabolic routes and highlights mechanistic links to vascular injury described in this review (Table 1).

Table 1. Homocysteine metabolism and pathophysiologic processes.

Pathway	Core Reaction	Key Enzyme (s)	Cofactor(s)	Product / Intermediate	Biological Consequence	Clinical Implication
Methionine Cycle	Methionine → S-adenosylmethionine (SAM) → S-adenosylhomocysteine (SAH) → Homocysteine; cellular methylation capacity reflected by SAM:SAH ratio[10]	Methionine adenosyltransferase; SAH hydrolase	ATP	SAM, SAH, Homocysteine	Governs methylation capacity (SAM:SAH)[10]	DNA hypomethylation; epigenetic dysregulation [10]
Remethyla	Homocysteine-	Methion	Vitamin	Methion	Replenishes	MTHFR variants or

tion	Methionine[11]	ine synthase; MTHFR[11]	B12 (cobalamin), Vitamin B9 (folate)[11]	ine	methionine; sustains methylation reactions[11]	folate/B12 deficiency-hyperhomocysteinemia[9,11]
Transsulfuration	Homocysteine -Cystathionine - Cysteine	Cystathionine β-synthase (CBS); Cystathionase	Vitamin B6 (pyridoxine)	Cysteine; Glutathione (GSH)	Supports antioxidant defense via GSH synthesis	B6 deficiency -increased ROS and endothelial injury[8,9]
Auto-oxidation & Derivatives	Homocysteine auto-oxidation - ROS and homocysteine thiolactone formation[10]	Spontaneous oxidation	Oxidizing milieu (O_2)	Superoxide; H_2O_2; Homocysteine thiolactone[10]	Protein N-homocysteinylation; oxidative stress; endothelial apoptosis (caspase-independent)[10,11]	LDL modification; atherogenic remodeling[30,45,46]
Effects on NO Bioavailability	eNOS inhibition; ↗ADMA; NO degradation[18,19]	eNOS; pathways regulating ADMA	NADPH; BH4	↙NO	Vasoconstriction; platelet activation; leukocyte adhesion[18-22]	Endothelial dysfunction; hypertension[18-22]
Inflammatory Signaling	NF-κB activation-↗IL-6, TNF-α, MCP-1[14,15]	NF-κB; MAPK cascades	ROS-dependent	Pro-inflammatory cytokines	Monocyte adhesion/transmigration; vascular inflammation[20-22]	Plaque initiation/progression; ↗endothelial permeability [20-22]
Immune Modulation	Th17 polarization; ↗RORγt; ↗IL-17[24]	RORγt; STAT3 axis	---	IL-17 ↗	Skewing toward pro-inflammatory adaptive responses[24]	Inflammatory vascular milieu [10,24]
Oxidative-Stress–Mediated Vascular Injury	ECM remodeling; elastin fragmentation; ↗MMP activity[39-43]	MMP-2; MMP-9	Zn^{2+}-dependent proteases	ECM degradation	Arterial stiffening; intimal thickening[41-44]	Atherosclerosis progression; increased arterial stiffness[41-44]
Antioxidant / Diet Link	One-carbon vitamin balance; GSH synthesis; antioxidant nutrients[84,87-90]	CBS; MS; MTHFR	Folate, B6, B12; vitamins C/E; polyphenols[85,88-91]	GSH; Methionine	ROS buffering; redox homeostasis[84,87-90]	Dietary insufficiency amplifies vascular injury risk[84,87-90]
Clinical Biomarke	Elevated plasma	---	---	---	Biochemical indicator of	Associated with CAD, stroke, PAD; utility in

r Role	homocysteine (total Hcy) associated with CVD phenotypes[2,3,36-40,60,61]	endothelial/inflammatory stress	risk stratification/reclassification[35-39,59,60]

Abbreviations: ADMA, asymmetric dimethyl-L-arginine; BH4, tetrahydrobiopterin; CAD, coronary artery disease; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; GSH, reduced glutathione; Hcy, homocysteine; IL-6, interleukin-6; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MTHFR, methylenetetrahydrofolate reductase; NF-κB, nuclear factor kappa B; NO, nitric oxide; PAD, peripheral artery disease; RORγt, RAR-related orphan receptor gamma t; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor alpha.

Targeting Downstream Effects

Since there was limited cardiovascular benefit in homocysteine-lowering trials (94), attention has turned towards mitigating homocysteine's downstream vascular effects like ROS generation, protein homocysteinylation, and endothelial dysfunction (88,89, 95). Therapies that help enhance nitric oxide bioavailability (96), protect mitochondrial function (97), or inhibit NF-κB (98) seem to be promising. While such strategies are not yet in routine clinical use, they indicate a broader shift toward targeting disease mechanisms rather than isolated biomarkers.

Recent studies indicate that Hcy contributes to oxidative stress, mitochondrial dysfunction, and endothelial injury beyond its role as a biomarker. In particular, a novel study highlighted that targeting downstream pathways, such as enhancing hydrogen sulfide (H_2S) bioavailability and supporting transsulfuration metabolism, may mitigate these effects (97). However, it is crucial to note that such interventions, including mitochondrial protection and nitric oxide enhancement, remain largely preclinical, with limited clinical evidence of efficacy to date.

It is crucial to acknowledge that the majority of these interventions, such as NF-κB inhibition, mitochondrial protection, and nitric oxide enhancement, are still in preclinical or early translational phases, with scant evidence of clinical efficacy thus far.

Discussion and Future Directions

The role of homocysteine in cardiovascular disease remains incomplete despite years of research. While early enthusiasm put homocysteine as a promising player in terms of therapeutic target, subsequent clinical trials, particularly after B-vitamin supplementation failed to produce meaningful effects in cardiovascular events (6,7), tempered expectations. However, growing recognition that homocysteine might be more of a marker of inflammatory and endothelial stress rather than the sole causal agent helped reevaluate its relevance in modern cardiovascular medicine (31,32). Consistent with our main thesis, homocysteine should be interpreted primarily as a marker of endothelial and inflammatory stress rather than a direct therapeutic target. Clinical management should therefore focus on modulating the downstream oxidative and inflammatory cascades it represents.

This review highlights the intricate relationship between homocysteine, vascular inflammation, and cardiovascular pathology. Experimental studies demonstrate that homocysteine exerts pro-inflammatory effects through oxidative stress, endothelial injury, and immune modulation (24, 41, 47). These effects of homocysteine might be especially pronounced and relevant in certain

populations like those with chronic kidney disease or genetic polymorphisms (52, 53). Various studies also suggest that homocysteine does not act in isolation, but interacts synergistically with other risk factors, amplifying vascular injury (26-28).

A key limitation of earlier intervention trials could have been their narrow focus on homocysteine concentrations and basing the therapeutic effect on lowering homocysteine itself rather than its downstream biological effects. Future therapies might be more effective if they target these downstream effects (64, 75). Approaches that focus on enhancing nitric oxide bioavailability (96), protecting mitochondrial function (97), or inhibiting NF-κB (98) may offer novel strategies to mitigate homocysteine's impact without requiring its direct reduction in plasma.

Another underexplored area seems to be the heterogeneity of patient response. Stratified approaches based on baseline inflammation, comorbid conditions, or genetic background may reveal subsets of patients who are more likely to benefit from homocysteine-lowering strategies (52, 53). Additionally, combining homocysteine with other biomarkers might enhance mortality risk stratification and inform treatment decisions in individuals with peripheral arterial disease (PAD) (99,100).

From a research standpoint, further studies are needed to:

- Clarify homocysteine's role as a marker versus mediator by means of prospective trials
- Assess the temporal relationship between homocysteine and inflammation via longitudinal human cohort studies
- Explore the anti-inflammatory therapies' influence on homocysteine's vascular effects without altering homocysteine's plasma concentration
- Analyze the place of homocysteine in cardiovascular risk stratification among multimarker predictive models

To conclude, homocysteine's role as an amplifier of vascular inflammation deserves renewed focus, even if it is no longer considered a sole therapeutic target. In summary, targeting the downstream inflammatory and oxidative consequences of homocysteine, rather than homocysteine itself, may represent a more promising strategy for cardiovascular disease prevention and treatment.

Compliance with Ethical Standards

Conflict of Interest: All authors declare that there is no conflict of interest.

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