Atherosclerosis, a chronic disease of the arteries driven by impaired lipid metabolism and an imbalanced inflammatory response, is the major cause of cardiovascular diseases (CVDs), including myocardial infarction and stroke. The deposition of lipoproteins containing apolipoprotein B in the subendothelial regions of the intima layer in the arterial wall causes a persistent proinflammatory response that drives atherosclerotic plaque formation. As the disease progresses, atherosclerotic plaques accumulate extracellular matrix proteins and calcium minerals, whereas necrotic cores develop as lipoprotein-rich macrophages that accumulate and die. Advanced atherosclerotic plaques can cause ischemia by occluding the artery lumen and impeding blood flow; however, most cardiovascular events are caused by occlusive thrombi from ruptured unstable plaques. Despite advances in medical treatments and pharmacologic therapies, atherosclerotic cardiovascular disease (ASCVD) remains a leading cause of death worldwide. Alarming, a significant increase in deaths due to CVD has been observed during the past decade, highlighting the incomplete understanding of underlying risk.
factors and pathophysiological mechanisms that drive ASCVD.

Dysregulated cholesterol and lipid metabolism plays a central role in the pathogenesis of ASCVD and has been extensively studied for over a century. More recently, mounting evidence has suggested a role for impaired amino acid metabolism. Beyond their obvious role as building blocks of proteins, amino acids play a role in cardiovascular health. The first analysis of amino acid composition in human atherosclerotic plaques was published in 1965 (Figure 1).

Another landmark study, published in 1997, identified the amino acid metabolite, homocysteine, as a predictor of mortality in patients with coronary artery disease (CAD), and sparked interest in the role of amino acid metabolism in atherosclerosis. Today, there is growing interest in amino acid profile and metabolism, not only as potential risk factors and biomarkers for ASCVD, but also as driving factors and interventional targets.

The current review discusses the emerging role of amino acids and the effect of their metabolism on key characteristics of the atherosclerotic plaque, including size, inflammation, and necrotic cores. It focuses on earlier studies on arginine as well as later studies exploring the arginine-related metabolites, homoarginine and polyamines. In addition, the accumulating evidence of branched-chain amino acids (BCAAs), glycine, aromatic amino acids (AAAs), their related metabolites, as well as selenocysteine in ASCVD is discussed.

Arginine and Related Metabolites

The role of arginine metabolism in atherosclerosis has been extensively studied over the past several decades. Arginine influences physiological signaling through nitric oxide (NO) synthase, which catalyzes the conversion of arginine to citrulline, generating NO as a by-product. Metabolism of arginine through this pathway, mediated by endothelial NO synthase, triggers vasodilation, prevents platelet aggregation, lowers leukocyte adhesion, and maintains endothelial cell quiescence. Studies have also identified a correlation between increased arginase activity, which synthesizes ornithine from arginine, and diminished

Figure 1  Landmark findings in the field of amino acids and their metabolism in atherosclerotic cardiovascular disease over the past decades. BCAA, branched-chain amino acid; CAD, coronary artery disease; CHD, coronary heart disease; GWAS, genome-wide association study; IDO, indoleamine 2,3-dioxygenase.
levels of NO, establishing an alternate pathway of arginine metabolism that is part of a phenotypic switch between proinflammatory and proresolving macrophages. NO has been extensively reviewed in the literature, therefore this review focuses on the arginine-related metabolites, homoarginine and polyamines.

Synthesized from arginine and lysine by the mitochondrial enzyme arginine-glycine amidotransferase, homoarginine has emerged as a potential biomarker of CVD, where lower circulating levels predict adverse cardiovascular events and mortality. Beyond its potential as a biomarker, experimental evidence suggests that homoarginine plays a protective role in CVD and atherosclerosis. Homoarginine supplementation to apolipoprotein E-null (Apoe<sup>−/−</sup>) mice lowered atherosclerosis and the content of lesional CD3<sup>+</sup> T cells by inhibiting T-cell proliferation and their migratory capacity (Table 2).

Arginine metabolism into polyamines is a more recent field of study in the context of ASCVD. Polyamines are a family of small, linear polycations composed of putrescine, spermidine, and spermine. The polyamine pathway is initiated by the conversion of arginine into ornithine via arginase 1, which is then decarboxylated by ornithine decarboxylase 1 to produce putrescine. The conversion of putrescine into spermidine and spermine follows linear reactions performed by spermidine synthase and spermine synthase, respectively. These polyamines play pivotal roles in numerous molecular and cellular signaling events, including proliferation, gene transcription, mRNA stability, and protein translation, some of which are aberrant in the pathophysiology of ASCVD.

Interestingly, proinflammatory and proresolving macrophages (both present in the progression and regression of atherosclerosis, respectively) are defined by their expression of arginine-metabolizing enzymes. In proinflammatory macrophages, arginine is used to produce NO, whereas in proresolving macrophages, it is converted into ornithine. Notably, the conversion of arginine into polyamines, facilitated by arginase 1 and ornithine decarboxylase 1, is necessary for the clearance of dead cells and the secretion of the proresolving mediator IL-10 by macrophages after stroke and during atherosclerosis regression, respectively (Table 2).

Among polyamines, spermidine plays a significant role in scavenging reactive oxygen species, thereby reducing oxidative stress. Enhanced spermidine bioavailability is also associated with sustained autophagy, the impairment of which drives atherosclerosis progression. In addition to its antioxidant effects, spermidine supplementation reduces necrotic core formation in Apoe<sup>−/−</sup> mice by promoting cholesterol efflux in a smooth muscle cell autophagy related 7 (Atg7)-dependent manner, and suppresses inflammation by reducing circulating tumor necrosis factor-α levels, which mitigate leukocyte recruitment. Evidence from a prospective community-based cohort study of 829 individuals demonstrated an association between dietary spermidine intake and reduced mortality; this finding was supported by data from the National Health and Nutrition Examination Survey, which

### Table 1: Association of Circulatory Levels of Amino Acids with CVD and Measured Risk Factors in Humans

<table>
<thead>
<tr>
<th>Amino acid/related metabolite</th>
<th>Blood levels</th>
<th>CVD-related parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homoarginine&lt;sup&gt;28&lt;/sup&gt; BCAAs&lt;sup&gt;14,31–33&lt;/sup&gt;</td>
<td>Decrease</td>
<td>• Incidences of myocardial infarction, heart failure, and mortality</td>
</tr>
<tr>
<td>Glycine&lt;sup&gt;17,21,34–37&lt;/sup&gt;</td>
<td>Decrease</td>
<td>• Prevalence of myocardial infarction</td>
</tr>
<tr>
<td>Tyrosine&lt;sup&gt;40,41&lt;/sup&gt; 3-HKYN&lt;sup&gt;39&lt;/sup&gt; (tryptophan metabolite)</td>
<td>Increase</td>
<td>• Prevalence of acute myocardial infarction</td>
</tr>
<tr>
<td>Phenylalanine&lt;sup&gt;41,42&lt;/sup&gt;</td>
<td>Increase</td>
<td>• Systolic blood pressure, LDL-C, triglycerides, and hsCRP</td>
</tr>
</tbody>
</table>

3-HKYN, kynurenine and 3-hydroxykynurenine; ApoB, apolipoprotein B; BCAA, branched-chain amino acid; CCTA, coronary computed tomography angiography; cIMT, carotid intima-media thickness; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.
surveyed individuals from 2003 to 2014 and revealed a strong correlation between dietary spermidine intake and decreased mortality rates, both overall and specific to CVD. This study had several notable strengths, including the identification of specific dietary sources of spermidine and the inclusion of a large and diverse population consisting of 10,942 men and 12,952 women.

A diverse range of clinical and epidemiologic studies have highlighted the complex interplay between arginine metabolism, dietary supplementation, and cardiovascular outcomes. The impact of arginine-mediated NO synthesis on endothelial function, along with the role of arginase in polyamine production and its implications in cellular functions and ASCVD, underlines the multifaceted nature of arginine metabolism. Nevertheless, clinical trials, such as Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) (L-arginine therapy), demonstrated that arginine supplementation after infarction provided no beneficial effects on vascular stiffness or ejection fraction, whereas the findings from the National Health and Nutrition Examination Survey appear to suggest a protective role following spermidine supplementation. Understanding these

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Plaque Characteristics following Amino Acid Treatment in Rodent Models of Atherosclerosis</th>
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<tr>
<td>Treatment</td>
<td>Circulating factors</td>
</tr>
<tr>
<td>Homoarginine&lt;sup&gt;43&lt;/sup&gt;</td>
<td>Decrease: IFN-γ, IL-12p40, NA</td>
</tr>
<tr>
<td>Putrescine&lt;sup&gt;19,44&lt;/sup&gt;</td>
<td>Decrease: NA</td>
</tr>
<tr>
<td>Spermidine&lt;sup&gt;45&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>Leucine&lt;sup&gt;46&lt;/sup&gt;</td>
<td>Increase: HDL-C, Decrease: LDL-C, MCP-1</td>
</tr>
<tr>
<td>BCAAs&lt;sup&gt;47&lt;/sup&gt;</td>
<td>Decrease: MCP-1, TNF-α, IL-1β, IL-6, TC, HDL-C, LDL-C</td>
</tr>
<tr>
<td>Glycine&lt;sup&gt;51&lt;/sup&gt;</td>
<td>Decrease: TC, TG</td>
</tr>
<tr>
<td>3,4-DAA&lt;sup&gt;15&lt;/sup&gt; (tryptophan metabolite)</td>
<td>Decrease: IL-6, TNF-α, GM-CSF, IL-10, CCL1</td>
</tr>
<tr>
<td>3-HAA&lt;sup&gt;49&lt;/sup&gt; (tryptophan metabolite)</td>
<td>Decrease: TG, Cholesterol</td>
</tr>
</tbody>
</table>

3,4-DAA, 3,4-dimethoxycinnamoyl anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; BCAA, branched-chain amino acid; GMCSF, granulocyte-macrophage colony-stimulating factor; HDL-C, high-density lipoprotein cholesterol; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthase; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; MerTK, MER proto-oncogene tyrosine kinase; NA, not applicable; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.
BCAAs include leucine, isoleucine, and valine, all of which are essential amino acids occurring at a high frequency in proteins. Beyond their roles in protein structure and function, BCAAs also regulate metabolic health through their catabolism and regulation of multiple signaling pathways. In this regard, perturbations in BCAA levels have significant associations with atherosclerosis and related cardiometabolic diseases, which are discussed in this section.

There has been a long history of clinical studies demonstrating a positive correlation between BCAAs and atherosclerosis progression as well as risk factors, such as insulin resistance, diabetes, and obesity. More specifically, surrogate markers of atherosclerosis and vascular dysfunction, including carotid intima-media thickness, are directly correlated with circulating BCAA levels (Table 1). The increase in BCAAs likely results from a combination of both lower BCAA uptake by tissues along with reduced activity of the branched-chain keto α-acid dehydrogenase complex, which accounts for the bulk of BCAA degradation, an observation that has been supported by studies in animal models. Furthermore, transcriptomic analysis of plaques at various stages of progression, early (classified as intimal thickening) and advanced (classified as thin or thick fibrous cap atheromas), shows an enrichment of gene networks involved in leucine/isoleucine degradation in early-stage plaques compared with advanced-stage lesions, suggesting that the accumulation of BCAAs occurs in both the plasma and at the tissue level.

Although the preponderance of evidence demonstrates a direct relationship between BCAAs and CVD, there are notable studies that reveal a negative association. For instance, a cross-sectional study of women found the intake of leucine was associated with lower arterial stiffness. In other studies, supplementation of leucine in mouse models led to improved hepatic lipid metabolism and reduced atherosclerotic lesion size, plaque lipid burden, and monocyte chemoattractant protein-1–mediated macrophage infiltration, whereas body weight, food intake, and blood glucose levels remained unchanged. In a second study, supplementing all the BCAAs confirmed these findings, indicating a protective role in atherosclerosis that can be attributed to supplementation BCAA (Table 2). Unfortunately, the leucine supplementation study did not measure plasma levels, making any correlation with human studies and circulating BCAAs difficult. However, the second study showed a trend for increasing levels of valine, leucine, and isoleucine that was significant when combined. Both of these studies suggest a protective role of BCAA supplementation during the initiation and progression of atherosclerosis, which raises the question whether commonly observed elevations in circulating BCAA levels in CVD are simply a biomarker for metabolic dysfunction rather than a promoter of disease. However, supplementation of BCAAs following established atherosclerosis in a murine model has not been performed, making direct comparisons between mice and patients difficult. Currently, the mechanisms of BCAA action are unknown; however, several studies have attempted to address this paucity of information.

Accumulating data from human and animal studies have led to several hypotheses being proposed to explain the effects of BCAAs on cardiovascular risk. A prominent concept is the ability of BCAAs to impair insulin signaling, resulting in systemic insulin resistance, which plays a major role in the development of atherosclerosis. As direct activators of mammalian target of rapamycin (mTOR) signaling [particularly mTOR complex (mTORC) 2], BCAAs enhance phosphorylation of insulin receptor substrate-1 on inhibitory sites, leading to blunted phosphatidylinositol-3-kinase activation and downstream insulin signaling. Although the significance of mTOR has been challenged in other studies, the overall inhibitory role of BCAAs in insulin signaling is a recurring observation. Beyond the regulation of insulin signaling through the mTOR–insulin receptor substrate-1–phosphatidylinositol-3-kinase axis, BCAAs also appear to cause insulin resistance through their catabolic metabolites. For example, the valine metabolite, 3-hydroxyisobutyrate, can activate transendothelial fatty acid transport, causing local tissue lipid accumulation and lipotoxicity, with resultant compromise in insulin signaling. In addition, certain BCAAs and their metabolites inhibit mitochondrial metabolic machinery and function in pancreatic islet β cells, contributing to insulin resistance. Furthermore, BCAAs lead to selective disruption of mitochondrial pyruvate use through inhibition of pyruvate dehydrogenase complex and decreased glucose oxidation. Finally, the role of short-chain acylcarnitines, which are by-products of BCAA metabolism, has a positive correlation with CAD; however, the mechanism remains unknown.

There have been recent reports defining pro-atherogenic roles for BCAAs directly at the level of the atherosclerotic plaque. In mouse studies using atherogenic Apoe−/− mouse models, Zhang et al found that BCAAs, particularly leucine, derived from diets high in protein, are potent activators of mTORC1 signaling in plaque macrophages. Hyperactive mTORC1 leads to inhibition of mitophagy, accumulation of dysfunctional mitochondria, and acceleration of apoptosis and plaque complexity. The essential
role for mTOR is demonstrated by the observation that the atherogenic effect of high-protein diets is completely abrogated in the absence of macrophage mTOR signaling or autophagy, supporting a specific BCAA-mTORC1-autophagy axis. In human clinical studies on peripheral blood mononuclear cells, elevated levels of BCAAs led to increased production of reactive oxygen species via both NADPH oxidase and the mitochondria in an mTOR-dependent manner, indicating that this mechanism may be clinically relevant to the link between BCAAs and cardiovascular risk.

A discussion of dietary protein and BCAAs in CVD would not be complete without an evaluation of gut microbiota. Colonization of germ-free mice with gut microbial communities from lean mice increases expression of genes involved in BCAA catabolism with reduced circulating BCAA levels, whereas the reverse happens on colonization with gut microbiota from obese mice. Similar phenotypes are detected in mice transplanted with microbiota from lean versus obese human participants. In addition, Qiao et al identified the gut symbiont, *Parabacteroides merdae*, as the main effector of atheroprotection in the Apoe<sup>−/−</sup> mouse model. In particular, enhanced BCAA catabolism by *P. merdae* promoted the conversion of BCAAs into branched short-chain fatty acids and inactivation of mTORC1 signaling in plaques. It is interesting that mTOR signaling is implicated in both metabolic dysfunction and atherosclerosis, albeit by completely different mechanisms.

Although BCAA supplementation has recently been reported to worsen established atherosclerosis in Apoe<sup>−/−</sup> mice, protective roles for BCAAs in atherosclerotic progression have also been reported in the context of macrophage lipid handling and foam cell formation. Studies from Rom et al and Grajeda-Iglesias et al indicate that leucine decreases macrophage cholesterol and triglyceride content in a dose-dependent manner by attenuating uptake of triglyceride-rich very low-density lipoprotein and inhibiting triglyceride biosynthesis. In addition, peritoneal macrophages isolated from mice supplemented with leucine show decreased cholesterol content because of reduced cholesterol biosynthesis and enhanced cholesterol efflux. Similar observations were made in cultured macrophages treated with serum from healthy individuals supplemented with leucine. Thus, it is clear that the roles of BCAAs on the immune system and atherosclerosis are complex and context dependent.

Thus far, much of the literature has considered BCAAs in unison when evaluating roles in CVD. However, the distinct roles of leucine, isoleucine, and valine have been largely neglected. Distinct metabolic roles for each of the BCAAs were shown in a recent study of Yu et al, who found that a low isoleucine diet provided several metabolic benefits in mice, including improved hepatic insulin sensitivity, elevated ketogenesis, and increased energy expenditure, which may be mediated by the activation of the fibroblast growth factor 21 uncoupling protein 1 axis. In contrast, reducing dietary valine intake showed modest metabolic effects in mice, and a low leucine diet showed no benefits. Such observations imply that the distinctive effects of BCAAs on ASCVD among studies may be due to the BCAA species and their specific roles. Therefore, when considering the use of BCAAs for diagnosis and therapeutics, the exact amount and function of specific BCAAs needs to be taken into consideration.

**Glycine**

Glycine is a nonessential and the simplest amino acid, which is synthesized from several precursors via interorgan metabolism involving mainly the liver and kidneys. These reactions are catalyzed by key enzymes responsible for glycine formation from serine [serine hydroxymethyltransferase (SHMT) 1 and 2], threonine (threonine dehydrogenase), choline via sarcosine (sarcosine dehydrogenase), and alanine and glyoxylate [alanine-glyoxylate aminotransferase (AGXT)]. Glycine degradation occurs through the glycine cleavage system or its reverse conversion to serine via SHMT, sarcosine via glycine N-methyltransferase, or glyoxylate via D-amino acid oxidase. Emerging studies highlight a key role for glycine and its metabolism in ASCVD and related cardiometabolic diseases, indicating its potential as both a biomarker and an interventional strategy.

Lower circulating glycine is consistently reported to be associated with a higher risk of CVD and cardiometabolic risk factors, including metabolic syndrome, obesity, hypertension, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease (MASLD). In a meta-analysis of genome-wide associations studies, including over half a million participants with and without coronary heart disease, Wittemans et al found that higher circulating glycine is genetically associated with lower coronary heart disease risk. This finding was corroborated through observational studies including 2053 coronary heart disease cases and 11,147 controls. Ding et al found that plasma glycine levels are inversely correlated with the risk of acute myocardial infarction in a cohort of >4000 patients with suspected stable angina pectoris (Table 1).

The consistent reports of lower circulating glycine in cardiometabolic diseases have sparked interest in the metabolic mechanisms behind this phenomenon. In MASLD, which is closely related to ASCVD, the decrease in circulating glycine is attributed to the hepatic suppression of key glycolytic enzymes, including SHMT1, SHMT2, and AGXT. Furthermore, lower glycine availability is a limiting factor for de novo synthesis of the potent antioxidant glutathione. Addressing the inverse association between lower glycine and higher BCAAs in cardiometabolic diseases, White et al applied a stable isotope tracing approach, suggesting that the obesity-associated increase in BCAAs...
activates an interorgan pyruvate-alanine shuttle system that drives glycine depletion. Nevertheless, using comprehensive stable-isotope tracing studies combined with SHMT inhibition, Ghrayeb et al reported that enhanced conversion of glycine to serine via reverse SHMT activity, independent of BCAAs, is the major cause for decreased circulating glycine. Although these studies primarily used dietary mouse models, Tan et al demonstrated that de novo glycine synthesis is inhibited in patients with morbid obesity and enhanced following bariatric surgery. A decrease in the ratios of glycine, its precursors (serine and threonine), and related metabolites (oxalate) was found in serum from patients with significant CAD, supporting the notion of defective glycine biosynthesis or enhanced glycine use in ASCVD. Despite these findings, further metabolic flux studies are warranted to establish whether impaired glycine biosynthesis or enhanced glycine degradation is the cause of reduced circulating glycine in patients with ASCVD, independent of other metabolic risk factors (i.e., obesity and MASLD).

Beyond the association between lower circulating glycine, ASCVD, and cardiometabolic risk factors, accumulating animal studies using dietary, genetic, and pharmacologic approaches support the causative role and therapeutic potential of glycine supplementation. In Apoe−/− mice, dietary glycine restriction not only increases hepatic steatosis, but also accelerates the formation of atherosclerotic plaques measured by plaque area. Similarly, the loss of AGXT, which catalyzes glycine formation from glyoxylate, results in a decreased glycine/oxalate ratio, accelerated MASLD in C57BL/6J mice, and enhanced atherosclerosis in Apoe−/− mice through induction of proinflammatory and proatherogenic chemokines (mainly chemokine C-C motif ligand 5) in the liver, plasma, and atherosclerotic plaque. In contrast, the antiatherogenic, anti-inflammatory, and antioxidant properties of glycine and glycine-based treatments are demonstrated in various in vitro, rodent, and nonhuman primate models. In a murine macrophage model system, glycine had the most potent lipid-lowering effects among all amino acids through suppression of very low-density lipoprotein uptake and triglyceride biosynthesis.

In splenic and alveolar macrophages as well as Kupffer cells, glycine blunts lipopolysaccharide- and superoxide by increasing chloride efflux via a glycine-gated chloride channel. Treating with glycine or a glycine-based therapeutic (DT-109) induces de novo glutathione synthesis in macrophages, and attenuates atherosclerosis in Apoe−/− mice concomitant with decreased superoxide (Table 2). Similarly, glycine supplementation enhances glutathione formation and lowers superoxide production in a rat model of high-sucrose diet. Glycine and DT-109 show additional cardiometabolic benefits by lowering ASCVD risk factors, including obesity, blood pressure, and MASLD, in rodents and nonhuman primates.

Although the antiatherogenic properties and cardiometabolic benefits of glycine-based treatments have been consistently demonstrated in animal studies, the number of clinical studies have been limited. In patients with type 2 diabetes, supplementation with glycine (5 g/day from 3 months) not only improves glycemia, but also decreases proinflammatory cytokines. In patients with metabolic syndrome, treatment with glycine (15 g/day from 3 months) lowers circulating lipid peroxidation and systolic blood pressure. Moreover, in patients with MASLD, supplementation with 200 mg/kg per day of the glycine precursor, serine, lowers hepatic steatosis and circulating transaminases. Despite the success of these studies, the potential benefits of glycine-based treatments in patients with ASCVD have not been systematically addressed and warrant further clinical evaluation.

**Aromatic Amino Acids and Related Metabolites**

The AAAs, tryptophan, tyrosine, and phenylalanine, are essential nutrients in the human diet and play a role in various physiological processes. They are also precursors for important neurotransmitters and hormones, including serotonin, dopamine, and thyroid hormones. The epidemiology of AAAs in relation to atherosclerosis is complex, with some studies suggesting that elevated levels of specific AAAs are associated with an increased risk of CAD, atherosclerosis progression, and development. One major limitation of the previous studies investigating the link between AAAs and atherosclerosis is that they are mostly observational and cross-sectional, which makes it difficult to establish a causal relationship. Although there is limited evidence of causation, potential mechanisms behind these associations include altered immune function, increased oxidative stress, and endothelial dysfunction. This section explores epidemiologic evidence along with basic research on the relationship and potential causative roles of AAAs and their metabolites in ASCVD. It specifically focuses on the effects of AAAs and their related metabolites on endothelial dysfunction, inflammation, and oxidative stress, key factors in the pathogenesis of atherosclerosis.

Tryptophan is an essential amino acid, which can be synthetized by microorganisms via the shikimic acid pathway and the activity of tryptophan synthase A (α-subunit) and tryptophan synthase B (β-subunit). Tryptophan can be metabolized to serotonin and melatonin through hydroxylation and acetylation via tryptophan hydroxylase and aroylalanine N-acetyltransferase, respectively. The liver is responsible for 90% of tryptophan degradation in the kynurenine pathway via the conversion of tryptophan to kynurenine by tryptophan 2,3-dioxygenase. The remaining degradation of tryptophan in the kynurenine pathway occurs through indoleamine 2,3-dioxygenase in the brain, gastrointestinal tract, and liver. Tryptophan and kynurenine metabolites have emerged as intriguing players associated with atherosclerotic plaque progression. Low levels of tryptophan are associated with increased cardiovascular disease risk. It is plausible that low levels of tryptophan, especially among patients with metabolic syndrome, could be a contributor to the increased cardiovascular disease risk. In addition, the decreased levels of tryptophan and serotonylation, which is a consequence of increased kynurenine production, could explain the increased cardiovascular disease risk among patients with metabolic syndrome.
catalyzed by thyroperoxidase to produce hormones, such as thyroxine and triiodothyronine. Clinical studies have shown an association between elevated serum levels of tyrosine and an increased risk of atherosclerosis and cardiovascular events (Table 1). In individuals at risk of developing CVD, elevated tyrosine levels are associated with an increased carotid intima-media thickness, a marker of early atherosclerosis. Nuclear magnetic resonance–based metabolomic profiling revealed association of serum concentrations of tyrosine with increased 3-month mortality in patients with acute heart failure. Furthermore, reduction in tyrosine aminotransferase, which catalyzes the conversion of tyrosine to 4-hydroxyphenylpyruvate, or administration of tyrosine lowers glutathione and enhances oxidative stress in endothelial cells and the brain. Clearly, altered tyrosine metabolism is associated with ASCVD; however, further research is needed to establish a potential causative role.

Phenylalanine is also synthesized through the shikimate pathway. Its degradation to tyrosine occurs primarily in the liver by a complex system composed of phenylalanine hydroxylase, the pterin coenzyme tetrahydrobiopterin (BH4), and several enzymes involved in BH4 regeneration, including dihydropteridine reductase and pterin 4α-carbinolamine dehydratase. These can be further metabolized to acetoacetic acid and fumaric acid via hydroxyphenylpyruvic acid. Elevated circulating levels of phenylalanine are positively correlated with multiple risk factors for ASCVD, including systolic blood pressure, low-density lipoprotein cholesterol, triglycerides, and high-sensitivity C-reactive protein. Furthermore, increased circulating phenylalanine is significantly linked to CAD, ischemic stroke, and CVD events, as well as unexpected cardiac failure. Although phenylalanine, tyrosine, or their ratio was not significantly different between patients with or without CAD, the phenylalanine/tyrosine ratio was associated with higher levels of serum neopterin or C-reactive protein in a large cohort of 3316 patients. BH4 preserves vascular function by regulating NO synthase activity and maintaining endothelial function, whereas BH4 deficiency promotes oxidative stress, endothelial dysfunction, reduced NO bioavailability, increased vascular inflammation, and the progression of atherosclerosis. Oral administration of BH4 to Apoe−/− mice restores NO-dependent endothelial function and attenuates the progression of atherosclerosis. Interestingly, phenylacetylglutamine, a gut microbial metabolite of phenylalanine, is associated with elevated risk of CVD, particularly in individuals with type 2 diabetes. Phenylacetylglutamine enhances platelet activation and increases thrombosis potential in whole blood, isolated platelets, and animal models of arterial injury. The microbial porA gene facilitates the conversion of dietary phenylalanine into phenylacetic acid, leading to the production of phenylacetylglutamine and phenylacetylglycine, which promotes platelet responsiveness and thrombosis potential through adrenergic receptors. Taken together, enhanced circulating phenylalanine is consistently elevated in CVD; however, further studies...
are needed to identify the underlying metabolic mechanisms, and the potential causative role of dysregulated phenylalanine in ASCVD.

Selenocysteine

Selenocysteine, recognized as the 21st protein-encoding amino acid, is notably present in certain enzymes that contribute to oxidation-reduction homeostasis, such as thioredoxin reductases, glutathione peroxidases, and glycine reductases. This amino acid is a variant of cysteine in which selenium, typically obtained from dietary sources, replaces sulfur. Interestingly, selenocysteine possesses a more potent reducing capacity than that of cysteine. This property enables proteins incorporating selenocysteine, referred to as selenoproteins, to perform antioxidant functions.

The glutathione peroxidase family of antioxidant selenoproteins offers protection against lipid peroxidation and oxidative damage. Genetic strategies targeting glutathione peroxidases have shown that their deficiency plays a role in atherosclerosis progression. Overexpression of glutathione peroxidase, which augments vascular reactivity, decreases lesion area in Apoe−/− mice. Supplementation of selenium and vitamin E decreases lesion size in rabbits fed a high-fat diet, independent of cholesterol levels, highlighting the importance of selenoproteins. Human studies have demonstrated similar findings, with a meta-analysis revealing an inverse correlation between serum selenium levels and cardiovascular events. This finding was supported by cohort studies reporting high selenium levels are associated with reduced risk of CVD and acute CAD. More recently, a meta-analysis found an association between decreased CVD mortality and high selenium intake. Despite these associations between selenium levels and cardiovascular health, selenium supplementation has proven insufficient to protect against cardiovascular outcomes in humans, potentially because of a defect in the incorporation of selenium into selenoproteins. In support of this idea, the Denmark PREvention of Cancer by Intervention with Selenium (PRECISE) trial showed that long-term selenium supplementation increased all-cause mortality. Future studies should focus on the role of selenium and selenoprotein synthesis and activity to better inform future therapies for individuals at risk for ASCVD.

Concluding Remarks and Future Directions

This review explored the accumulating evidence over the past several decades on amino acid metabolism in relation to ASCVD, covering epidemiologic, clinical, animal, and in vitro studies on arginine and its metabolites, BCAAs, glycine, AAAs, and selenocysteine. When combining the observational clinical studies, there is a unique signature of an altered amino acid profile associated with ASCVD that includes elevated circulating BCAAs with a concurrent reduction in circulating glycine (Table 1). Although the observational studies are relatively consistent with regard to the altered amino acid profile associated with ASCVD risk, animal studies addressing their causative role in atherosclerosis are often contradictory, specifically those focusing on BCAA and AAA metabolism. Currently, direct comparisons between human data and rodent studies are difficult as there is a wide variation in the rodent models. Most human studies measure amino acids in the circulation of patients with CVD, whereas a significant portion of animal studies center around administering amino acids in conjunction with diets that induce atherosclerosis, hindering direct side-by-side comparisons. In addition, most of the human and animal studies have also relied on cross-sectional designs, limiting the understanding of the temporal relationships between amino acid levels and ASCVD progression.

Another limitation of the research on amino acids and ASCVD so far, which offers new avenues of investigation, is a unifying approach to study the interactions among amino acid metabolism, disease progression, and severity. Most studies have primarily focused on individual amino acids or metabolites, and little is known about their interactions or combined effects in the context of atherosclerosis. So far, such interactions have been addressed in the context of altered glycine metabolism in human ASCVD, where lower ratios of glycine and its precursors, serine and threonine, have been associated with disease severity, suggesting impaired glycine availability in relation to ASCVD progression. Currently, only two animal studies have explored such interactions, indicating that glycine depletion could be the result of either enhanced BCAAs that activate an interorgan pyruvate-alanine shuttle system or the enhanced conversion of glycine to serine via reverse SHMT activity. However, these studies were performed in mouse models of obesity and MASLD rather than atherosclerosis. Therefore, to establish a unifying understanding of impaired amino acid metabolism in ASCVD, a comprehensive investigation of the interactions among amino acid metabolism in relation to the pathogenesis of ASCVD is needed and can be achieved through a combination of clinical and animal studies applying metabolomics and tracing techniques.

Finally, the insufficient evidence from human studies is another limitation that warrants further investigation. Longitudinal studies with large and diverse populations are warranted to investigate the association between amino acid levels, their metabolites, and the incidence, progression, and outcomes of ASCVD. Such studies should also consider potential confounding factors, including dietary patterns, lifestyle factors, and coexisting diseases. Moreover, although arginine intervention has been studied in earlier clinical trials of CVD, further clinical evidence on the effects of other amino acids on ASCVD is lacking. Intervention studies are needed to determine the effects of targeted modulation of amino acids or their metabolites on ASCVD progression and outcomes. Randomized controlled
Amino Acid Metabolism and ASCVD


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