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.\(^1\) 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.\(^2,3\) 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.\(^3\) 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.\(^4,5\)

Despite advances in medical treatments and pharmacologic therapies, atherosclerotic cardiovascular disease (ASCVD) remains a leading cause of death worldwide. Dysregulated lipid metabolism is a well-established driver of ASCVD. Unfortunately, even with potent lipid-lowering therapies, ASCVD-related deaths have continued to increase over the past decade, highlighting an incomplete understanding of the underlying risk factors and mechanisms of ASCVD. Accumulating evidence over the past decades indicates a correlation between amino acids and disease state. This review will explore the emerging role of amino acid metabolism in ASCVD, uncovering novel potential biomarkers, causative factors, and therapeutic targets. Specifically, the significance of arginine and its related metabolites, homoarginine and polyamines, branched-chain amino acids, glycine, and aromatic amino acids, in ASCVD will be discussed. These amino acids and their metabolites have been implicated in various processes characteristic of ASCVD, including impaired lipid metabolism, endothelial dysfunction, increased inflammatory response, and necrotic core development. Understanding the complex interplay between dysregulated amino acid metabolism and ASCVD provides new insight that may lead to the development of novel diagnostic and therapeutic approaches. Although further research is needed to uncover the precise mechanisms involved, it is evident that amino acid metabolism plays a role in ASCVD. (Am J Pathol 2024, 19; 1–15; https://doi.org/10.1016/j.ajpath.2023.12.006)

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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.

In the current review, 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, will be discussed. This review will focus on earlier studies on i) arginine as well as later studies exploring the arginine-related metabolites, homoarginine and polyamines. In addition, the accumulating evidence of ii) branched-chain amino acids (BCAAs), iii) glycine, iv) aromatic amino acids (AAAs), their related metabolites, as well as v) selenocysteine in ASCVD will be 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...
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, so for the purposes of this review, the focus will be on the arginine-related metabolites, homoarginine and polyamines.

Synthesized from arginine and lysine by the mitochondrial enzyme arginino glycine amidinotransferase, homoarginine has emerged as a potential biomarker of CVD, where lower circulating levels predict adverse cardiovascular events and mortality.\(^{28-30}\) (Table 1).\(^{14,17,21,27,31-42}\)

Beyond its potential as a biomarker, experimental evidence suggests that homoarginine plays a protective role in CVD and atherosclerosis.\(^{36}\) Homoarginine supplementation to apolipoprotein E–null (Apoe\(^{-/-}\)) mice lowered atherosclerosis and the content of lesional CD\(^{3+}\) T cells by inhibiting T-cell proliferation and their migratory capacity (Table 2).\(^{15,19,21,41-49}\)

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.\(^{50}\) 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 de

| Table 1 | Association of Circulatory Levels of Amino Acids with CVD and Measured Risk Factors in Humans |
|-----------------|---------------------------------|-----------------------------|-----------------------------|
| Amino acidRELATED metabolite | Blood levels | CVD-related parameters |
| Homoaarginine\(^{27}\) | Decrease | Incidences of myocardial infarction, heart failure, and mortality |
| BCAA\(^{14,31-33}\) | Increase | Prevalence of myocardial infarction |
| Glycine\(^{17,21,34-37}\) | Decrease | C, IMT |
| Tryptophan\(^{38}\) | Decrease | LDL-C, ApoB, obesity, hypertension, and diabetes |
| 3-HKYN\(^{39}\) (tryptophan metabolite) | Increase | Atherosclerosis assessed by CCTA |
| Tyrosine\(^{40,41}\) | Increase | Acute myocardial infarction and stenosis |
| Phenylalanine\(^{41,42}\) | Increase | Genetic and observational associations with the risk of coronary heart diseases |

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.
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 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 seemingly contradictory findings requires an understanding...
of the patient populations participating in the studies; VINTAGE MI studies were performed following establishment of CVD, and the National Health and Nutrition Examination Survey study focused on dietary habits over time; however, it is clear the field needs a better molecular understanding of amino acid function to develop more effective clinical therapies.

**Branched-Chain Amino Acids**

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 will be 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 chemotactic 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 have been shown to 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 acylcarcinines, 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 led to inhibition of mitophagy, accumulation of dysfunctional mitochondria, and acceleration of apoptosis and plaque complexity. The essential role for mTOR was demonstrated by the observation that the...
atherogenic effect of high-protein diets was completely abrogated in the absence of macrophage mTOR signaling or autophagy, supporting a specific BCAA-mTORC1-autophagy axis. Human clinical studies on peripheral blood mononuclear cells also found elevated levels of BCAAs leading 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.

The role of BCAAs is not only reported to be deleterious in CVD. Although BCAA supplementation has recently been reported to worsen established atherosclerosis in Apoe<sup>−/−</sup> mice, protective roles for BCAAs in atherosclerotic regression 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 revealed that leucine decreased 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, they reported that peritoneal macrophages isolated from mice supplemented with leucine showed 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 BCAA 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 implied 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 should 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. 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 was genetically associated with lower coronary heart disease risk, and confirmed this finding 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.

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 has been attributed to the hepatic suppression of key glycine biosynthetic enzymes, including SHMT1, SHMT2, and alanine and glyoxylate. Furthermore, lower glycine availability was identified as 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 (ie, 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 increased hepatic steatosis, but also accelerated the formation of atherosclerotic plaques measured by plaque area. Similarly, the loss of alanine and glyoxylate, which catalyze glycine formation from glyoxylate, resulted 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 were 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 blunted lipopolysaccharide- or peptidoglycan polysaccharide-induced translocation of the p65 subunit of NF-kB into the nucleus and the production of tumor necrosis factor-z and superoxide by increasing chloride influx via a glycine-gated chloride channel. Treatment with glycine or a glycine-based therapeutic (DT-109) induced de novo glutathione synthesis in macrophages, and attenuated atherosclerosis in Apoe−/− mice concomitant with decreased superoxide (Table 2). Similarly, glycine supplementation enhanced glutathione formation and lowered superoxide production in a rat model of high-sucrose diet. Moreover, glycine and DT-109 showed 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, there are a limited number of clinical studies. In patients with type 2 diabetes, supplementation with glycine (5 g/day from 3 months) not only improved glycemia, but also decreased proinflammatory cytokines. In patients with metabolic syndrome, treatment with glycine (15 g/day from 3 months) lowered circulating lipid peroxidation and systolic blood pressure. Moreover, in patients with MASLD, supplementation with 200 mg/kg per day of the glycine precursor, serine, lowered 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 CVD, 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 will explore epidemiologic evidence along with basic research on the relationship and potential causative roles of AAAs and their metabolites in ASCVD. The specific focus of this section will be 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 synthesized by microorganisms via the shikimic acid pathway, and the activity of tryptophan synthase A (z-subunit) and tryptophan synthase B (β-subunit). Tryptophan can be metabolized to serotonin and melatonin through hydroxylation and acetylation via tryptophan hydroxylase and arylalkylamine N-acetyltransferase, respectively. The liver is responsible for 90% of tryptophan degradation in the kynurenine pathway through 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. Although low levels of circulating tryptophan have been associated with increased risk of CVD and CAD in various clinical studies, higher levels of tryptophan metabolites, such as kynurenine and 3-hydroxykynurenine, have been shown to predict the incidence of cardiovascular risk in patients with end-stage renal disease and patients with angina pectoris, and have been correlated with subclinical atherosclerosis and CVD (Table 1). These observations indicate that tryptophan metabolism may be dysfunctional in ASCVD and provide a rationale for studying the potential causative role of altered tryptophan-kynurenine metabolism in the pathogenesis of atherosclerosis.

Tryptophan metabolites, such as 3-hydroxyanthranilic acid and indoxyl sulfate, have been shown to be positively associated with proinflammatory and pro-oxidant indexes linked to carotid atherosclerosis in patients with end-stage renal disease. Indoxyl sulfate activates NADPH oxidase, leading to the generation of reactive oxygen species and NF-κB activation, enhancing the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and activation of endothelial cells. Cole et al. found an increase in lesion size and markers of plaque vulnerability, as well as a reduction in IL-10 production, in indoleamine 2,3-dioxygenase-deficient Apoe−/− mice. Moreover, administration of 3,4-dimethoxycinnamoyl anthranilic acid, a synthetic derivative of the tryptophan metabolite anthranilic acid, led to reduced lesion formation and inflammation after collar-induced arterial injury, and reduced cytokine and chemokine production in human atheroma cell cultures. In contrast, Metghalchi et al. showed that indoleamine 2,3-dioxygenase deficiency in Ldlr−/− mice protects against atherosclerosis by decreasing the indoleamine 2,3-dioxygenase-derived metabolite kynurenine acid, which reduces IL-10 production through a CAMP-dependent pathway and inhibition of extracellular signal-regulated kinase 1/2 phosphorylation in myeloid cells, leading to enhanced susceptibility to atherosclerosis and colitis. Kynurenic acid levels in the blood were shown to predict death and recurrent myocardial infarction in patients with CAD. In addition, administration of 3-hydroxyanthranilic acid significantly decreased lesion size in Ldlr−/− mice by lowering plasma cholesterol and triglycerides and inhibiting the uptake of oxidized low-density lipoprotein by macrophages (Table 2).

Together, although these findings indicate dysregulated tryptophan metabolism in ASCVD, it is likely that there are strain-, cell type-, and tryptophan metabolite–specific effects on atherosclerosis. Further investigation is warranted to clarify these discrepancies and evaluate the therapeutic value of targeting tryptophan metabolism for the treatment of ASCVD.

Tyrosine is obtained from the diet and can also be synthesized via phenylalanine hydroxylase, which catalyzes the addition of a hydroxyl group to the 6-carbon aromatic ring of phenylalanine to yield tyrosine. Tyrosine can be metabolized to generate neurotransmitters, such as L-3,4-dihydroxyphenylalanine, dopamine, adrenaline, and noradrenaline, via tyrosine hydroxylase, or it can be 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 were 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, and 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 4′-carbinolamine dehydratase. These can be further metabolized to acetoacetate 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. Phenylacetylglutamine was shown to be associated with elevated risk of CVD, particularly in individuals with type 2 diabetes. Phenylacetylglutamine was shown to enhance platelet activation and increase thrombosis potential in whole blood, isolated platelets, and animal models of arterial injury. Further studies revealed that the microbial porA gene facilitates the conversion of dietary phenylalanine...
into phenylacetic acid, leading to the production of phenylacetylglutamine and phenylacetylglucose, which promotes platelet responsiveness and thrombosis potential through adrenergic receptors.159 Taken together, enhanced circulating phenylalanine is consistently reported to be 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.160,161 Genetic strategies targeting glutathione peroxidases have shown that their deficiency plays a role in atherosclerosis progression.162,163 Overexpression of glutathione peroxidase, which augments vascular reactivity, decreases lesion area in ApoE-/- mice.163 Supplementation of selenium and vitamin E have been demonstrated to decrease lesion size in rabbits fed a high-fat diet, independent of cholesterol levels,164 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.165 This finding was supported by cohort studies reporting high selenium levels are associated with reduced risk of CVD and acute CAD.166 More recently, a meta-analysis found an association between decreased CVD mortality and high selenium intake.167 Despite these associations between selenium levels and cardiovascular health, selenium supplementation has proven insufficient to protect against cardiovascular outcomes in humans.166,168 Potentially because of a defect in the incorporation of selenium into selenoproteins. In support of this idea, the Danish PRECISE trial showed that long-term selenium supplementation increased all-cause mortality.169 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,21,170 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 system113 or the enhanced conversion of glycine to serine via reverse SHMT activity.114 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 trials assessing the efficacy and safety of interventions, such as amino acid supplementation or dietary modifications, are crucial for establishing evidence-based therapeutic strategies. Finally, metabolomics studies have focused on the relationship between the circulating amino acid profile and ASCVD. The emergence of new technologies, including spatial metabolomics and transcriptomics, may allow a better understanding of altered amino acid metabolism within different regions and cell types of the human atherosclerotic plaque. Advancements in these fields hold promise in identifying novel diagnostic and prognostic biomarkers as well as therapeutic approaches for ASCVD.

Disclosure Statement

None declared.

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