Received: 1 September 2022 | Accepted: 9 December 2022

DOI: 10.1111/fcp.12858

REVIEW ARTICLE

Fundamental & Clinical Pharmacology | WILEY

Homoarginine in the cardiovascular system: Pathophysiology and recent developments

Vitali Koch[1,2] | Leon D. Gruenewald[1] | Tatjana Gruber-Rouh[1] | Katrin Eichler[1] | David M. Leistner[1] | Scherwin Mahmoudi[1] | Christian Booz[1] | Simon Bernatz[1] | Tommaso D'Angelo[3] | Moritz H. Albrecht[1] | Leona S. Alizadeh[1] | Nour-Eldin A. Nour-Eldin[1] | Jan-Erik Scholtz[1] | Ibrahim Yel[1] | Thomas J. Vogl[1] | Winfried März[4] | Stefan E. Hardt[2] | Simon S. Martin[1]

[1]Goethe University Hospital Frankfurt, Frankfurt am Main, Germany

[2]Department of Cardiology, Angiology, and Pulmonology, University Hospital Heidelberg, Heidelberg, Germany

[3]Department of Biomedical Sciences and Morphological and Functional Imaging, University Hospital Messina, Messina, Italy

[4]Fifth Department of Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Correspondence
Vitali Koch, Goethe University Hospital Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany.
Email: vitali-koch@gmx.de and vitali.koch@kgu.de

Funding information
This research project did not receive any funding.

Abstract

Upcoming experimental and epidemiological data have identified the endogenous non-proteinogenic amino acid L-homoarginine (L-hArg) not only as a novel biomarker for cardiovascular disease but also as being directly involved in the pathogenesis of cardiac dysfunction. The association of low L-hArg levels with adverse cardiovascular events and mortality has proposed the idea of nutritional supplementation to rescue pathways inversely associated with cardiovascular health. Subsequent clinical and experimental studies contributed significantly to our knowledge of potential effects on the cardiorenal axis, acting either as a biomarker or a cardiovascular active agent. In this review article, we provide a comprehensive summary of the L-hArg metabolism, pathophysiological aspects, and current developments in the field of experimental and clinical evidence in favor of protective cardiovascular effects. Establishing a reliable biomarker to identify patients at high risk to die of cardiovascular disease represents one of the main goals for tackling this disease and providing individual therapeutic guidance.

KEYWORDS
amino acids, cardiovascular, homoarginine, mortality, nitric oxide

1 | INTRODUCTION

Accounting for more than 6 million deaths every year, cardiovascular diseases remain the leading cause of death in the European Union.[1] Recent data estimate costs of more than €200 billion a year, representing a high socioeconomic burden for healthcare systems worldwide.[2]

Current research focuses on identifying novel biomarkers and treatment targets to improve the survival of patients with cardiovascular disorders and establish new therapeutic approaches. Over the last decades, amino acids increasingly attracted scientific attention because of their proposed interactions with pathways in metabolic and cardiovascular pathophysiology. In this context, several previous studies have shown that low concentrations of L-homoarginine (L-hArg) are associated with myocardial dysfunction, an increased risk of major cardiovascular events, and stroke.[3–5] The underlying mechanisms remain to be further elucidated but accumulating evidence suggests that L-hArg may modulate the metabolism of nitric oxide (NO) due to its

structural similarities with arginine by serving as an alternative substrate for the NO synthase.[6] Apart from its effects on the NO pathway, new light has been shed on the connection of L-hArg to energy and creatine metabolism. Arginine-glycine amidinotransferase (AGAT) is not only the first and rate-limiting key enzyme in the synthesis of L-hArg from lysine but is also critically involved in the generation of the energy metabolites guanidinoacetate and creatine.[4] It has also been shown that dietary supplementation with L-hArg seems to directly influence the cause of heart failure in different scenarios, including a murine model of post-myocardial infarction heart failure,[7] aortic banding,[8] or renal insufficiency.[9] Establishing a reliable biomarker to identify patients at high risk to die of cardiovascular disease represents one of the main goals for tackling this disease and providing individual therapeutic guidance.

In this review article, we aim to summarize current findings and developments regarding molecular pathways and clinical applications of L-hArg in the cardiorenal system.

2 | ENDOGENOUS SYNTHESIS OF L-HOMOARGININE AND EXOGENOUS SOURCES

The synthesis of L-hArg is known to take place in different organs of humans and animals (e.g., small intestine, kidney, liver, or brain) using mainly arginine and lysine as substrates.[6] Previously, two large genome-wide association studies (GWAS) have independently identified associations of L-hArg with single nucleotide polymorphisms (SNPs) for the gene encoding the enzyme AGAT, which is known to catalyze the generation of L-hArg from its key substrates arginine and lysine in animals and humans.[4,10] The reaction comprises the transfer of the amidino group from L-arginine to the amino group of L-lysine to finally generate L-hArg with ornithine being a co-product.[11] Consequently, lymphoblasts of a patient with AGAT deficiency were not able to synthesize L-hArg.[12] In an animal study, deficiency of AGAT in mice caused a substantial reduction in systemic L-hArg levels, indicating the important role of this enzyme in the regulation of L-hArg homeostasis.[4]

Another metabolic route for L-hArg formation includes enzymes of the urea cycle.[13,14] In this pathway, homocitrulline is generated from lysine by ornithine transcarbamoylase (OTC), transformed to homoargininosuccinate by argininosuccinate synthase (ASS), and finally converted into L-hArg by argininosuccinate lyase (ASL).[15] However, especially the role of the second metabolic route involving OTC should be elucidated regarding its contribution to endogenous L-hArg production. Additionally, L-hArg may be directly generated through transamidination of lysine by glycine transamidinase.[16,17]

Plants and especially seeds contain a magnitude of naturally occurring non-proteinogenic amino acids.[18] Moreover, L-hArg was identified as the major free non-proteinogenic amino acid in different sorts of grain legumes, such as Lathyrus species (grass pea seeds) that contain approximately 1–2% of L-hArg.[19]

3 | PHYSIOLOGY OF L-HOMOARGININE

L-hArg [n6-(aminoiminomethyl)-L-lysine] is an endogenous, non-proteinogenic amino acid derivative structurally related to L-arginine and present in human plasma at levels of 1 to 2 μmol/L.[20] In a study on rats with isoprenaline-induced takotsubo cardiomyopathy, intraperitoneally injected L-hArg at doses of 20, 220, or 440 mg/kg body weight had an elimination half-life of 20 to 40 min.[16,21] In another study on 20 healthy young volunteers, the half-life period of supplemented hArg could not be determined.[22] Regarding its special chemical characteristics, it is known to be solid, slightly water-soluble and has a melting point of 213–215°C at a molecular weight of 188.2 g/mol.[23] It is produced from its precursor lysine by the mitochondrial enzyme AGAT, which is expressed in the kidney or liver, as well as tissues with high energy requirements like striated muscle.[4,15,24,25] Apart from its role in L-hArg synthesis, AGAT participates in energy metabolism by creating creatine through the final methylation of guanidinoacetate by guanidinoacetate N-methyltransferase (GAMT).[20] Metabolic studies have shown that humans and mice with AGAT deficiency are not able to generate L-hArg, consequently developing muscular dystrophy with complete reversal upon creatinine supplementation.[4,12] However, creatine at presumably high concentrations may inhibit AGAT-mediated L-hArg formation as demonstrated in AGAT$^{+/+}$ mice and previously published work using tissues from chicken and rats.[4,26–28] Contrary, the administration of sodium nitrate ($NaNO_3$) has been shown to influence the homeostasis of L-hArg with remarkably higher plasma concentrations compared to placebo-treated healthy volunteers.[29] It seems to shift AGAT-mediated reactions toward L-hArg synthesis by decreasing N-methyltransferase-catalyzed synthesis of guanidinoacetic acid (GAA) and subsequently creatine.

In the case of dietary intake, L-hArg can be incorporated from the lumen of the small intestine via cationic amino acid transporters.[30] Subsequently, it is not only supposed to affect plasma concentrations and synthesis of endogenous L-hArg but also those from its metabolites arginine, asymmetric dimethylarginine (ADMA), creatine, GAA, and NO.[21] Animal studies confirmed dose-dependent increasements in L-hArg plasma concentrations after supplementation of dietary

L-hArg or L-arginine.[8,31] Both genetic differences of AGAT and miscellaneous conditions were supposed to be responsible for variances in plasma L-hArg concentrations. While fasting was reported to go along with increased serum L-hArg concentrations in humans and rats,[32,33] smokers showed decreased L-hArg levels.[34]

Possible molecular mechanisms by which L-hArg might act in cardiovascular disorders were proposed in several previous in vitro and in vivo studies.[4,13,16,22,35–38] To date, three different pathways for the catabolism of L-hArg were reported.[13] Given its structural similarity to L-arginine with an additional methylene group (CH_2) in its main chain, L-hArg is supposed to act as an alternative substrate for NO synthase and to be directly involved in the generation of the potent vasodilator NO.[16,39–41] Apart from being a precursor of NO, it may increase its availability by inhibiting arginase, which leads to increased plasma concentrations of L-arginine, the main substrate for NO synthase.[42] In this context, NO is synthesized from arginine in a two-stage reaction carried out by NO synthase. First, NO synthase hydroxylates L-arginine to Nω-hydroxy-L-arginine. In the second step, NO synthase oxidizes Nω-hydroxy-L-arginine to L-citrulline and NO.[43,44] Asymmetric dimethylarginine (ADMA), NG-monomethyl-L-arginine (L-NMMA), and symmetric dimethylarginine (SDMA) are methylated analogs of arginine and important modulators of the NO pathway.[45] For example, ADMA is a naturally occurring endogenous inhibitor of NO synthase.[46] The methyl groups are provided from the methyl donor S-adenosylmethionine involving the enzymes protein arginine methyltransferase type 1 and 2 (PRMT1, PRMT2). While PRMT1 catalyzes the generation of L-NMMA and ADMA, PRMT2 is involved in the formation of SDMA and L-NMMA.[46] Despite its similarity to L-hArg, several previous cardiovascular studies on the long-term effects of L-arginine supplementation failed to prevent cardiac remodeling and heart failure.[47–52]

NO exerts well-investigated cardioprotective effects and takes part in the regulation and maintenance of cell viability, endothelial function, and vascular homeostasis.[53] However, the importance of L-hArg as a direct substrate for NO synthase has been questioned considering its lower catalytic efficiency and the approximately 20 times lower concentrations in biological fluids and tissue (plasma concentration of approximately 2 μmol/L) compared to L-arginine (plasma levels of 100–250 μmol/L).[13,40,54] On the other side, experimental data in mice revealed a prolonged NO activity with NO concentrations being elevated even 8 h after L-hArg supplementation in comparison to L-arginine treated animals going to baseline levels after 4 h.[55] Furthermore, binding affinity (K_m) as one essential kinetic parameter for the NO synthase-dependent oxidation is known to be higher for L-hArg than for L-arginine (e.g., for NO synthase-I 23 ± 5 vs. 2.7 ± 0.5 μmol/L).[40] Vmax values of 4380 ± 140 and 34 ± 3 μmol/min/mg protein for L-arginine and L-hArg, respectively, were reported for rat liver arginase.[56] Regarding its action as a weak competitive inhibitor of arginase,[3,57] the relevance of possible arginine elevations as causal mechanisms of L-hArg-induced cardiovascular safeguards seems uncertain. This was also underlined by a study that investigated the inhibition of the two arginase isoforms in mammals, arginase 1 and arginase 2, by L-hArg.[42] Whereas arginase 1 is predominantly expressed in blood and liver cells, arginase 2 plays an important role in extra-hepatic tissues, such as the kidneys.[58] The authors observed L-hArg-mediated arginase inhibition at concentrations that are significantly higher than those observed in plasma and found no association between plasma L-hArg concentrations and the L-arginine/ornithine ratio in healthy older adults.[42] They concluded that arginase inhibition is unlikely to represent a key mechanism in the putative cardioprotective effects of L-hArg. Available studies on the affection of arginase activity by L-hArg are contradictory since stimulatory as well as inhibitory effects have been reported.[42,56,57,59–61]

Another catabolic pathway of L-hArg is catalyzed by L-arginase hydrolyzing L-hArg to urea and lysine.[62] However, the ability of L-hArg to serve as an alternative substrate for arginase requires further research.

The last and quite recently identified catabolic route involves alanine-glyoxylate aminotransferase 2 (AGXT-2) which converts L-hArg to 6-guanidino-2-oxocaproic acid (GOCA).[13] In vivo murine studies revealed increasements in intracellular L-hArg concentrations in the scenario of AGXT-2 deficiency, proposing that this mitochondrial enzyme seems to be also required for the maintenance of systemic L-hArg levels by changing its expression. Interestingly, administration of labeled L-hArg in mice resulted in similarly high plasma levels of labeled GOCA as the concentrations of the other labeled L-hArg metabolites L-homocitrulline and L-lysine. This observation under experimental conditions suggests that the proposed enzymatic clearance of L-hArg through AGXT-2 seems to be comparable to previously reported metabolic pathways of L-hArg that encompass arginase or NO synthase. Genome-wide association studies confirmed these findings by showing a correlation between SNPs located at AGXT-2 and L-hArg levels in humans.[4,10] Individuals from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study being heterozygous or homozygous for the AGXT-2 rs37369 T-allele showed elevated L-hArg plasma concentrations by 8% and 12%, respectively.[10,13]

Since L-hArg is not degraded by enzymes in animal tissues in the presence of physiological concentrations of other amino acids, the in vivo catabolism of the amino acid seems limited.[31] Several studies have reported on the importance of the kidneys in the regulation and maintenance of L-hArg concentrations.[11,24,63–65] It is

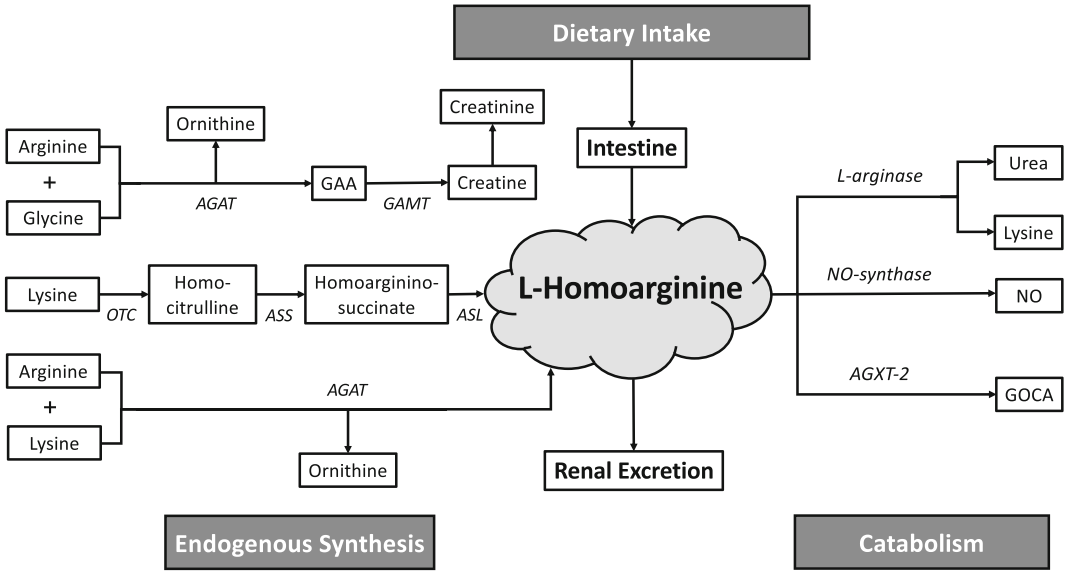

FIGURE 1 Illustration of the L-hArg metabolism. L-hArg is either ingested or intrinsically formed from lysine and arginine by the enzyme AGAT. Other metabolic routes include the formation of L-hArg through enzymes of the urea cycle or through the transamidination of lysine by glycine transamidinase. The amino acid derivative serves as a substrate for L-arginase but can also be catabolized by NO-synthase to NO, or by AGXT-2 to GOCA. Abbreviations: AGAT, arginine-glycine amidinotransferase; AGXT-2, alanine-glyoxylate aminotransferase 2; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; GOCA, 6-guanidino-2-oxocaproic acid; L-hArg, L-homoarginine; NO, nitric oxide; OTC, ornithine transcarbamylase

supposed that approximately 95% of orally administered L-hArg is excreted in the urine of pigs and rats, respectively.[31] Therefore, renal excretion of L-hArg might be a reliable indicator of dietary intake and intrinsic production.

In addition to the involvement of L-hArg in the cardiovascular systems, early investigations reported inhibitory effects on alkaline phosphatase isoenzymes in the liver, intestine, or bone.[66–68] However, previous studies have demonstrated inhibitory effects of L-hArg on the activity of alkaline phosphatases in the millimolar range,[69,70] such as in smooth muscle cells at supraphysiological concentrations of 10 mmol/L.[71] Therefore, physiological L-hArg concentrations might be insufficient to induce a significant suppression.

A simplified illustration of the metabolic pathways of L-hArg is provided in Figure 1.

4 | EPIDEMIOLOGICAL ASSOCIATIONS WITH L-HOMOARGININE DEFICIENCY

With the observation of flow-mediated vasodilatation upon increased plasma concentrations during the second and third trimenon of pregnancy (4.8 ± 1.7 and 5.3 ± 1.5 μmol/L, respectively), L-hArg has been supposed to be involved in the maintenance of endothelial function.[72] Furthermore, L-hArg was found to be inversely associated with the endothelial adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in a cohort of 3305 subjects referred for coronary angiography (LURIC study).[3]

In 2010, Maerz et al. were the first to describe that L-hArg plasma levels are independently associated with cardiovascular and all-cause mortality in patients referred for coronary angiography and in patients undergoing haemodialysis.[3] Their prospective study investigated the correlation between L-hArg concentrations and cardiovascular outcomes in two large cohorts of 3305 subjects referred for coronary angiography (LURIC study) and 1255 diabetic patients undergoing haemodialysis (4D study). Especially in patients with impaired renal function, L-hArg levels were found to be reduced and inversely associated with markers of endothelial dysfunction.

Studies in the following years revealed an inverse correlation of L-hArg with cardiovascular events and mortality in miscellaneous clinical scenarios, such as heart failure,[73,74] acute chest pain,[38] stroke,[4] peripheral artery disease,[75] or critically ill patients in general.[76] However, data from patients with chronic kidney disease (CKD) are inconsistent. While several studies demonstrated the ability of L-hArg to predict cardiovascular mortality and progression to dialysis in patients with renal insufficiency,[63,77,78] another publication did not observe a significant relationship between L-hArg concentrations and mortality.[65] Previous studies have demonstrated an organ-specific pattern of AGAT expression with the highest levels in renal tissue from patients with end-stage heart failure.[25] Consistent with these findings, L-hArg levels were positively correlated

to left ventricular ejection fraction and pronounced in patients with decreased compared to those with normal kidney function. Due to the association of AGAT with energy metabolism, patients with diminished kidney function and consequently lower AGAT activity might be more vulnerable to myocardial energy depletion.[77] A study on the association of L-hArg with cardiovascular outcomes in 1255 diabetic haemodialysis patients identified L-hArg as a strong risk factor for sudden cardiac death and death due to heart failure.[64] In this context, patients in the lowest L-hArg quintile showed a more than twofold and threefold increased risk of sudden cardiac death or death due to heart failure, respectively, than patients in the highest quintile. Furthermore, low L-hArg concentrations were not only found to be lower at decreased kidney function but also to be associated with the progression of chronic kidney disease.[78]

Considering the participation of AGAT in creatine formation, L-hArg is supposed to play a role in heart failure pathophysiology.[25,79] Cullen et al evaluated the expression and role of AGAT in 12 patients with nonischemic cardiomyopathy requiring the implantation of a left ventricular assist device (LVAD) due to deteriorating clinical status despite inotropic support and 10 patients with stable end-stage heart failure (ESHF).[25] The study showed that myocardial concentrations of AGAT mRNA were increased in response to heart failure in both patient populations (4.3-fold [$p < 0.001$] and 2.7-fold [$p < 0.005$] in LVAD and ESHF relative to donors, respectively), as well as in rat myocardia. Interestingly, after receiving a combination therapy consisting of mechanical support and standard pharmacological therapy, followed by the administration of clenbuterol to stimulate hypertrophy and improve cardiac function, AGAT activity returned to baseline levels. The findings indicate that the myocardium might compensate for the energy loss in the context of heart failure by the induction of locally elevated AGAT mRNA expression to normalize intracellular creatine levels. Another study investigating patients with takotsubo cardiomyopathy also revealed reduced plasma L-hArg concentrations in comparison to healthy subjects (1298 ± 112 vs. 2094 ± 321 nmol/L, respectively).[79]

Whereas epidemiological evidence suggests a strong relationship between low L-hArg concentrations and adverse cardiovascular outcomes, a recently published Mendelian randomization analysis revealed no causal relationship between L-hArg and any of the studied cardiometabolic outcomes.[80] The study investigated three different SNPs located in GATM (glycine amidinotransferase), CPS1 (carbamoyl-phosphate synthase 1), and AGXT-2 (alanine-glyoxylate aminotransferase 2) that were all associated with serum L-hArg concentrations in a recent genome-wide association study (GWAS) of 5143 individuals.[10] However, the study had several limitations that are general to all Mendelian randomization analyses. One assumption that might influence studies with this principle is that endpoints for outcome prediction should be independent of confounders. However, all three genes exercise certain regulatory functions that are known to be independent of L-hArg. Another possible explanation is that other metabolites related to L-hArg might be responsible for the poor outcome associated with low L-hArg plasma concentrations. In this context, several other metabolites with a strong correlation to increased cardiovascular risk and poor outcomes have been reported. A summary is provided in Table 1. Finally, the study cohort consisted of patients aged 24–39 years who typically did not suffer from cardiovascular diseases or had associated risk factors. In light of the many study limitations, the role of L-hArg and its association with poor outcomes still needs to be further explored.

5 | EXPERIMENTAL EVIDENCE

Apart from several epidemiological investigations on L-hArg as a cardiovascular risk marker, the amino acid has been tested in different animal studies for potential direct effects on cardiac function and remodeling. Cardiac remodeling represents a complex adaptation process in response to miscellaneous pathological stimuli aiming at maintaining cardiac output.[88,89] It is accompanied by an increase in myocardial size and interstitial fibrosis on a microscopic level and a reduction of cardiac function, compliance, and elasticity on a macroscopic level.[90] At later stages, the cardiac remodeling process flows into replacement fibrosis and cell death which are considered major drivers of the transition to symptoms, heart failure, and adverse cardiovascular events.[91] The loss of cardiomyocytes is irreversible as evidenced by the persistence of focal replacement fibrosis seen on cardiac magnetic resonance through the detection of LGE (late gadolinium enhancement).[92] Since cellular hypertrophy and diffuse fibrosis are plastic processes with a certain reversibility—as seen in patients after valve replacement—L-hArg attracted a lot of interest as a potential new drug therapy acting as a protective factor in cardiovascular and renal diseases.[93]

Mice with homozygous AGAT knockout ($AGAT^{-/-}$) and consequently L-hArg and creatine deficiency showed decreased stroke volumes and neurological deficits after supplementation with L-hArg in a model of experimental stroke, while the normalization of brain creatine had no neuroprotective effects.[4]

Compared to wild-type mice with normal AGAT enzyme activity, $AGAT^{-/-}$ mice present a haemodynamic phenotype that is characterized by lower LV systolic pressure with impaired contractility and relaxation.[94] In contrast to creatine supplementation,

TABLE 1 Illustration of arginine metabolites and their association with cardiovascular outcome

Amino acid metabolite	Function	Pathway	Association with cardiovascular outcome
ADMA[46]	Analogue of L-arginine	Endogenous inhibitor of NO synthase, leading to endothelial dysfunction and cardiovascular events	Increased ADMA levels in atherosclerosis, hypertension, chronic heart failure, hypercholesterolemia, diabetes mellitus, and chronic renal failure
L-arginine[81]	Nitrogen source for NO synthesis	NO is synthesized from arginine in a two-stage reaction carried out by NO synthases. First, NO synthase hydroxylates L-arginine to Nω-hydroxy-L-arginine. In a second step, NO synthase oxidizes Nω-hydroxy-L-arginine to L-citrulline and NO	No improvement in cardiovascular outcomes after arginine supplementation. No correlation with the prevalence of CAD
L-citrulline[82]	Intermediate non-proteinogenic amino acid in the arginine pathway	Arginine is metabolized by NO synthase to NO and L-citrulline	High L-citrulline levels were associated with the development of CAD and an increased risk for major adverse cardiovascular events
GOCA[83]	Transamination product of L-hArg	Transamination of L-hArg catalyzed by AGXT-2 leads to the formation of GOCA	GOCA levels in the highest tertile (≥2.13 nmol/L) were associated with increased renal and cardiovascular risk.
GAA[84]	Naturally-occurring derivative of glycine and direct metabolic precursor of creatine	Formed by glycine and arginine through AGAT	Increased GAA levels were found to be associated with an unfavorable cardiometabolic risk profile
L-hArg[3]	Cationic amino acid derived from lysine	L-hArg is mainly synthesized from lysine and arginine by AGAT	Inversely associated with cardiovascular and all-cause mortality
Homocitrulline[85]	Metabolite of ornithine in mammalian metabolism	Homocitrulline originates from isocyanic acid binding to ε-amino groups of lysine residues	Serum homocitrulline levels were elevated in patients with CAD and positively associated with disease severity
L-NMMA[86]	Arginine analogue	Synthesized by methylation of arginine. Selective inhibitor of NO synthase	Associated with vascular endothelial dysfunction by inhibiting endothelial NO synthase
SDMA[87]	Endogenous methylated arginine analogue. Structural isomer of the endogenous NO synthase inhibitor asymmetric dimethylarginine	Formed by PRMT5 and 7 (both type II methyltransferases). Inhibitor of arginine transport at supraphysiological concentrations	Associated with cardiovascular disease and mortality in different populations, with the strongest associations observed in the general population
Ornithine[82]	Non-proteinogenic amino acid	Beside L-citrulline the major catabolic product of arginine, representing the first step of creatine synthesis	Positively associated with the prevalence of CAD

Abbreviations: ADMA, asymmetric dimethylarginine; AGXT-2, alanine-glyoxylate aminotransferase 2; CAD, coronary artery disease; GAA, guanidinoacetic acid; GOCA, 6-guanidino-2-oxocaproic acid; L-hArg, L-homoarginine; L-NMMA, NG-monomethyl-L-arginine; NO, nitric oxide; PRMT, protein-arginine methyltransferase; SDMA, symmetric dimethylarginine.

which was not able to fully rescue this cardiac phenotype, administration of L-hArg via drinking water at a concentration of 14 mg/L for 10 days normalized all inotropic and lusitropic parameters. On the cardiomyocyte level, L-hArg deficiency per se resulted in impaired cardiac function with reduced myocyte shortening and relengthening velocities.[94]

Using a murine model of post-myocardial infarction heart failure, another study investigated possible protective effects of L-hArg by supplementing adult female mice with 14 mg/L L-hArg for 4 weeks before myocardial infarction surgery and for another 6 weeks follow-up.[7] Dietary L-hArg supplementation has led to preserved contractile reserve and improved diastolic indices under stimulated conditions. However, remodeling of the left ventricle and the global function as assessed by magnetic resonance imaging differed not between controls and HA-treated animals.

A further study on this topic utilized a different model of heart failure induced by aortic constriction to investigate L-hArg mediated attenuation of cardiac remodeling in rats.[8,37] The authors reported dose-dependent improvements in ejection fraction and a decreased morphological and molecular response to pressure overload as evidenced by reduced hypertrophy and fibrosis. Co-administration of standard heart failure medication resulted in a more pronounced attenuation of adverse morphological changes without any side effects. The cardioprotective effects of L-hArg on cardiac remodeling hold true in rats undergoing 5/6 nephrectomy with induced cardiorenal syndrome.[9] Using this classic model of renal insufficiency, L-hArg treatment was found to increase LV contractility and attenuate cardiac remodeling processes.

Dellera et al investigated the effects of in vivo L-hArg supplementation on smooth muscle cell proliferation after arterial balloon angioplasty with balloon-induced injury of the left carotid artery in rats.[95] Intravenously administered L-hArg via the right jugular vein at a concentration of 30 mg/kg/day over 14 days was able to reduce neointimal hyperplasia as evidenced by a significant decrease of the intima/media ratio.

A recently published study on female ApoE-deficient mice supplemented with L-hArg (14 mg/L) in drinking water reported a molecular explanation for the proposed atheroprotective effects of L-hArg.[96] Suggesting a T-cell-related mechanism of action due to a substantial reduction of CD3+ T-cells in atherosclerotic lesions, the authors observed a profoundly modulated spatial organization of the T-cell actin cytoskeleton and increased filopodia formation. Further analysis revealed inhibition of T-cell proliferation and impaired migratory capacities of T-cells due to chemokine release by L-hArg.

Since the first report on the role of L-hArg as a non-competitive inhibitor of tissue-nonspecific alkaline phosphatases (TNAP),[66] several studies have demonstrated an association of elevated TNAP expression with accelerated vascular calcification and mortality.[97–103] TNAP is found in miscellaneous tissues, including the liver, kidney, endothelium, and bone.[104] Experimental findings in a murine model, in which coronary atherosclerosis was induced by endothelium-specific transgenic overexpression of TNAP (eTNAP), atherogenic diet, and a mutation in the LDL receptor gene, showed that 4-week HA treatment preserved LV ejection fraction, protected from LV dilatation, and decreased myocardial fibrosis.[35] Beyond those effects, no changes in coronary artery calcification and atherosclerosis were registered when compared to placebo animals without lipid abnormalities. However, the exact pathways underlying TNAP-mediated cardiovascular changes remain elusive and require further research. Using a similar experimental setting with diet-induced obese mice, glucose-lowering effects of L-hArg have been reported, suggesting a compensatory response under conditions of severely impaired glucose homeostasis and in patients with type 2 diabetes mellitus.[105]

Considering the proposed interactions of L-hArg with NO metabolism, one could infer that L-hArg might also affect blood pressure. Literature about the association between L-hArg and blood pressure is still controversial at present. Whereas cohort studies with patients referred for coronary angiography (LURIC) or elderly participants (Hoorn) reported positive correlations of L-hArg with systolic and diastolic blood pressure,[5,106–108] it has also been shown that salt-sensitive hypertensive rats experienced lowered blood pressure following intravenous L-hArg infusions together with an increased urinary excretion pointing toward an enhanced NO synthesis.[109] In addition, another experimental setting in rats undergoing aortic banding with subsequent 4-week L-hArg treatment reported lowered blood pressure from 108 ± 3 to 96 ± 3 mmHg following a dose of 800 $mg \cdot kg^{-1} \cdot day^{-1}$.[8]

To summarize, the majority of aforementioned experimental studies corroborate clinical evidence of an association between L-hArg levels and cardiovascular health, fueling the hypothesis of direct protective effects rather than being only a biomarker for risk assessment. Furthermore, they underline the potential use of L-hArg as a therapeutic option for patients with cardiorenal disorders.

6 | APPLICATION OF L-HOMOARGININE IN CLINICAL TRIALS

A clinical study investigated the kinetic and dynamic properties of an orally applied dose of 125-mg L-hArg in 20 young healthy volunteers.[22] Once daily administered over 4 weeks, L-hArg plasma concentrations were increased sevenfold over baseline levels compared to a fourfold increase after a single dose. The study group of young individuals (mean age of 35 years) showed mean L-hArg concentrations of 2.87 ± 0.91 μmol/L at baseline, whereas a previous study from 786 healthy individuals (aged from 35 to 54 years) reported plasma concentrations of 1.88 μmol/L L-hArg [25th and 75th percentile 1.47 and 2.41 μmol/L].[110] The higher L-hArg concentration at baseline might be attributed to the younger age and normal kidney function of participants but HA levels might also be affected by sex or AGAT SNPs.[111] L-hArg supplementation was well tolerated without vascular or neurological abnormalities or any other significant side effects.

These promising study findings provide a clear rationale to conduct prospective studies with more study participants and longer treatment periods to examine mechanistic pathways and the effects of L-hArg in patients with miscellaneous cardiovascular

diseases and metabolic disorders. A randomized double-blind placebo-controlled study has been registered to examine the oral supplementation of L-hArg in patients with acute ischemic stroke (ClinicalTrials.gov identifier: NCT03692234).

7 | CONCLUSION

Results from clinical and experimental studies increasingly move the naturally occurring amino acid L-hArg into the spotlight of cardiovascular prevention. Despite associations between low L-hArg levels and increased cardiovascular and all-cause mortality in patients at cardiovascular risk, the exact pathophysiological mechanisms remain to be elucidated. However, the hypothesized direct cardioprotective effects of dietary L-hArg supplementation in different animal models are quite encouraging and promising to investigate its effects in larger randomized human trials. To date, clinical and experimental data have reported good tolerability of dietary intake without causing any significant side effects. Further research will result in a better characterization of mechanistic pathways and strengthen our understanding by which L-hArg exerts its direct protective effects in different patient populations including those with renal insufficiency.

ACKNOWLEDGEMENTS

Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

C.B. received speaking fees from Siemens Healthineers. M.H.A. received speaking fees from Siemens Healthineers and Bracco. I.Y. received a speaking fee from Siemens Healthineers. The other authors have no potential conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

All authors have contributed significantly to this work, participating in the conception, design, analysis, interpretation of data, and writing/editing. V.K. wrote the manuscript and designed the research. L.D.G., T.G.R., K.E., W.M., S.E.H., N.-E.A.N.-E., J.-E.S., and C.B. performed research. T.D'A., L.S.A., I.Y., M.H.A., S.B., S.M., and T.J.V. analyzed data. S.S.M. and D.M.L. performed research and revised the manuscript.

DATA AVAILABILITY STATEMENT

All data and information used are included in this manuscript.

ORCID

Vitali Koch https://orcid.org/0000-0001-6915-5906
Tommaso D'Angelo https://orcid.org/0000-0003-0004-6378

REFERENCES

1. Townsend N, Kazakiewicz D, Lucy Wright F, et al. Epidemiology of cardiovascular disease in Europe. *Nat Rev Cardiol*. 2022; 19(2):133-143. doi:10.1038/s41569-021-00607-3
2. Movsisyan NK, Vinciguerra M, Medina-Inojosa JR, Lopez-Jimenez F. Cardiovascular diseases in central and Eastern Europe: a call for more surveillance and evidence-based health promotion. *Ann Glob Health*. 2020;86(1):21. doi:10.5334/aogh.2713
3. März W, Meinitzer A, Drechsler C, et al. Homoarginine, cardiovascular risk, and mortality. *Circulation*. 2010;122(10):967-975. doi:10.1161/CIRCULATIONAHA.109.908988
4. Choe CU, Atzler D, Wild PS, et al. Homoarginine levels are regulated by L-arginine:glycine amidinotransferase and affect stroke outcome: results from human and murine studies. *Circulation*. 2013;128(13):1451-1461. doi:10.1161/CIRCULATIONAHA.112.000580
5. Pilz S, Meinitzer A, Tomaschitz A, et al. Low homoarginine concentration is a novel risk factor for heart disease. *Heart*. 2011; 97(15):1222-1227. doi:10.1136/hrt.2010.220731
6. Pilz S, Meinitzer A, Gaksch M, et al. Homoarginine in the renal and cardiovascular systems. *Amino Acids*. 2015;47(9):1703-1713. doi:10.1007/s00726-015-1993-2
7. Atzler D, McAndrew DJ, Cordts K, et al. Dietary supplementation with homoarginine preserves cardiac function in a murine model of post-myocardial infarction heart failure. *Circulation*. 2017; 135(4):400-402. doi:10.1161/CIRCULATIONAHA.116.025673
8. Koch V, Gruenewald LD, Gruber-Rouh T, et al. Homoarginine treatment of rats improves cardiac function and remodeling in response to pressure overload. *Fundam Clin Pharmacol*. 2022; 36(6):992-1004. doi:10.1111/fcp.12808
9. Koch V, Weber C, Riffel JH, et al. Impact of homoarginine on myocardial function and remodeling in a rat model of chronic renal failure. *J Cardiovasc Pharmacol Ther*. 2022;27: 10742484211054620. doi:10.1177/10742484211054620
10. Kleber ME, Seppälä I, Pilz S, et al. Genome-wide association study identifies 3 genomic loci significantly associated with serum levels of homoarginine: the AtheroRemo Consortium. *Circ Cardiovasc Genet*. 2013;6(5):505-513. doi:10.1161/CIRCGENETICS.113.000108
11. Tsikas D, Wu G. Homoarginine, arginine, and relatives: analysis, metabolism, transport, physiology, and pathology. *Amino Acids*. 2015;47(9):1697-1702. doi:10.1007/s00726-015-2055-5
12. Davids M, Ndika JD, Salomons GS, Blom HJ, Teerlink T. Promiscuous activity of arginine:glycine amidinotransferase is responsible for the synthesis of the novel cardiovascular risk factor homoarginine. *FEBS Lett*. 2012;586(20):3653-3657. doi:10.1016/j.febslet.2012.08.020
13. Rodionov RN, Oppici E, Martens-Lobenhoffer J, et al. A novel pathway for metabolism of the cardiovascular risk factor homoarginine by alanine:glyoxylate aminotransferase 2. *Sci Rep*. 2016;6(1):35277. doi:10.1038/srep35277
14. Karetnikova ES, Jarzebska N, Markov AG, Weiss N, Lentz SR, Rodionov RN. Is homoarginine a protective cardiovascular risk factor? *Arterioscler Thromb Vasc Biol*. 2019;39(5):869-875. doi:10.1161/ATVBAHA.118.312218
15. Ryan WL, Wells IC. Homocitrulline and homoarginine synthesis from lysine. *Science*. 1964;144(3622):1122-1127. doi:10.1126/science.144.3622.1122
16. Adams S, Che D, Qin G, Farouk MH, Hailong J, Rui H. Novel biosynthesis, metabolism and physiological functions of L-homoarginine. *Curr Protein Pept Sci*. 2019;20(2):184-193. doi:10.2174/1389203719666181026170049
17. Kato T, Sano M, Mizutani N, Hayakawa C. Homocitrullinuria and homoargininuria in hyperargininaemia. *J Inherit Metab Dis*. 1988;11(3):261-265. doi:10.1007/BF01800367
18. Rao SL. A look at the brighter facets of β-N-oxalyl-l-α,-β-diaminopropionic acid, homoarginine and the grass pea. *Food*

Chem Toxicol. 2011;49(3):620-622. doi:10.1016/j.fct.2010.06.054
19. Nunn PB, Bell EA, Watson AA, Nash RJ. Toxicity of non-protein amino acids to humans and domestic animals. *Nat Prod Commun*. 2010;5(3):485-504. doi:10.1177/1934578X1000500329
20. Atzler D, Schwedhelm E, Choe CU. L-homoarginine and cardiovascular disease. *Curr Opin Clin Nutr Metab Care*. 2015;18(1):83-88. doi:10.1097/MCO.0000000000000123
21. Gunes DN, Kayacelebi AA, Hanff E, Lundgren J, Redfors B, Tsikas D. Metabolism and distribution of pharmacological homoarginine in plasma and main organs of the anesthetized rat. *Amino Acids*. 2017;49(12):2033-2044. doi:10.1007/s00726-017-2465-7
22. Atzler D, Schönhoff M, Cordts K, et al. Oral supplementation with L-homoarginine in young volunteers. *Br J Clin Pharmacol*. 2016;82(6):1477-1485. doi:10.1111/bcp.13068
23. Kim S, Thiessen PA, Bolton EE, et al. PubChem substance and compound databases. *Nucleic Acids Res*. 2016;44(D1):D1202-D1213. doi:10.1093/nar/gkv951
24. Ryan WL, Johnson RJ, Dimari S. Homoarginine synthesis by rat kidney. *Arch Biochem Biophys*. 1969;131(2):521-526. doi:10.1016/0003-9861(69)90425-1
25. Cullen ME, Yuen AH, Felkin LE, et al. Myocardial expression of the arginine:glycine amidinotransferase gene is elevated in heart failure and normalized after recovery: potential implications for local creatine synthesis. *Circulation*. 2006;114(1 Suppl):I16-I20. doi:10.1161/CIRCULATIONAHA.105.000448
26. Choe CU, Nabuurs C, Stockebrand MC, et al. L-arginine:glycine amidinotransferase deficiency protects from metabolic syndrome. *Hum Mol Genet*. 2013;22(1):110-123. doi:10.1093/hmg/dds407
27. Walker JB, Hannan JK. Creatine biosynthesis during embryonic development. False feedback suppression of liver amidinotransferase by N-acetimidoylsarcosine and 1-carboxymethyl-2-iminoimidazolidine (cyclocreatine). *Biochemistry*. 1976;15(12):2519-2522. doi:10.1021/bi00657a004
28. Roberts JJ, Walker JB. Higher homolog and N-ethyl analog of creatine as synthetic phosphagen precursors in brain, heart, and muscle, repressors of liver amidinotransferase, and substrates for creatine catabolic enzymes. *J Biol Chem*. 1985;260(25):13502-13508. doi:10.1016/S0021-9258(17)38750-1
29. Tsikas D, Maassen N, Thorns A, et al. Short-term supplementation of sodium nitrate vs. sodium chloride increases homoarginine synthesis in young men independent of exercise. *Int J Mol Sci*. 2022;23(18):10649. doi:10.3390/ijms231810649
30. Banjarnahor S, Rodionov RN, Konig J, Maas R. Transport of L-arginine related cardiovascular risk markers. *J Clin Med*. 2020;9(12):3975. doi:10.3390/jcm9123975
31. Hou Y, Hu S, Jia S, et al. Whole-body synthesis of L-homoarginine in pigs and rats supplemented with L-arginine. *Amino Acids*. 2016;48(4):993-1001. doi:10.1007/s00726-015-2145-4
32. Marescau B, Deshmukh DR, Kockx M, et al. Guanidino compounds in serum, urine, liver, kidney, and brain of man and some ureotelic animals. *Metabolism*. 1992;41(5):526-532. doi:10.1016/0026-0495(92)90213-T
33. May M, Kayacelebi AA, Batkai S, Jordan J, Tsikas D, Engeli S. Plasma and tissue homoarginine concentrations in healthy and obese humans. *Amino Acids*. 2015;47(9):1847-1852. doi:10.1007/s00726-015-1922-4
34. Sobczak A, Prokopowicz A, Szula M, et al. Do homoarginine and asymmetric dimethylarginine act antagonistically in the cardiovascular system? *Circ J*. 2014;78(8):2096. doi:10.1253/circj.cj-14-0586
35. Rodionov RN, Begmatov H, Jarzebska N, et al. Homoarginine supplementation prevents left ventricular dilatation and preserves systolic function in a model of coronary artery disease. *J am Heart Assoc*. 2019;8(14):e012486. doi:10.1161/JAHA.119.012486
36. Faller KME, Atzler D, McAndrew DJ, et al. Impaired cardiac contractile function in arginine:glycine amidinotransferase knockout mice devoid of creatine is rescued by homoarginine but not creatine. *Cardiovasc Res*. 2018;114(3):417-430. doi:10.1093/cvr/cvx242
37. Bahls M, Atzler D, Markus MRP, et al. Low-circulating homoarginine is associated with dilatation and decreased function of the left ventricle in the general population. *Biomolecules*. 2018;8(3):63. doi:10.3390/biom8030063
38. Atzler D, Baum C, Ojeda F, et al. Low homoarginine levels in the prognosis of patients with acute chest pain. *J am Heart Assoc*. 2016;5(4):e002565. doi:10.1161/JAHA.115.002565
39. Hecker M, Walsh DT, Vane JR. On the substrate specificity of nitric oxide synthase. *FEBS Lett*. 1991;294(3):221-224. doi:10.1016/0014-5793(91)81434-A
40. Moali C, Boucher JL, Sari MA, Stuehr DJ, Mansuy D. Substrate specificity of NO synthases: detailed comparison of L-arginine, homo-L-arginine, their N omega-hydroxy derivatives, and N omega-hydroxynor-L-arginine. *Biochemistry*. 1998;37(29):10453-10460. doi:10.1021/bi980742t
41. Bretscher LE, Li H, Poulos TL, Griffith OW. Structural characterization and kinetics of nitric-oxide synthase inhibition by novel N5-(iminoalkyl)- and N5-(iminoalkenyl)-ornithines. *J Biol Chem*. 2003;278(47):46789-46797. doi:10.1074/jbc.M306787200
42. Tommasi S, Elliot DJ, Da Boit M, Gray SR, Lewis BC, Mangoni AA. Homoarginine and inhibition of human arginase activity: kinetic characterization and biological relevance. *Sci Rep*. 2018;8(1):3697. doi:10.1038/s41598-018-22099-x
43. Noble MA, Munro AW, Rivers SL, et al. Potentiometric analysis of the flavin cofactors of neuronal nitric oxide synthase. *Biochemistry*. 1999;38(50):16413-16418. doi:10.1021/bi992150w
44. Stuehr D, Pou S, Rosen GM. Oxygen reduction by nitric-oxide synthases. *J Biol Chem*. 2001;276(18):14533-14536. doi:10.1074/jbc.R100011200
45. Eryavuz Onmaz D, Abusoglu S, Yaglioglu H, Abusoglu G, Unlu A. Developing a robust, fast and reliable measurement method for the analysis of methylarginine derivatives and related metabolites. *J Mass Spectrom Adv Clin Lab*. 2021;19:34-45. doi:10.1016/j.jmsacl.2021.02.002
46. Sibal L, Agarwal SC, Home PD, Boger RH. The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease. *Curr Cardiol Rev*. 2010;6(2):82-90. doi:10.2174/157340310791162659
47. Brooks WW, Conrad CH, Robinson KG, Colucci WS, Bing OH. L-arginine fails to prevent ventricular remodeling and heart failure in the spontaneously hypertensive rat. *Am J Hypertens*. 2009;22(2):228-234. doi:10.1038/ajh.2008.334
48. Bednarz B, Jaxa-Chamiec T, Maciejewski P, et al. Efficacy and safety of oral l-arginine in acute myocardial infarction. Results of the multicenter, randomized, double-blind, placebo-controlled ARAMI pilot trial. *Kardiol pol*. 2005;62(5):421-427.
49. Blum A, Hathaway L, Mincemoyer R, et al. Oral L-arginine in patients with coronary artery disease on medical management. *Circulation*. 2000;101(18):2160-2164. doi:10.1161/01.cir.101.18.2160
50. Dudek D, Legutko J, Heba G, et al. L-arginine supplementation does not inhibit neointimal formation after coronary stenting in human beings: an intravascular ultrasound study. *Am Heart J*. 2004;147(4):E12. doi:10.1016/j.ahj.2003.10.025
51. Schulman SP, Becker LC, Kass DA, et al. L-arginine therapy in acute myocardial infarction: the Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *Jama*. 2006;295(1):58-64. doi:10.1001/jama.295.1.58
52. Walker HA, McGing E, Fisher I, et al. Endothelium-dependent vasodilation is independent of the plasma L-arginine/ADMA ratio in men with stable angina: lack of effect of oral L-arginine on

endothelial function, oxidative stress and exercise performance. *J am Coll Cardiol*. 2001;38(2):499-505. doi:10.1016/s0735-1097(01)01380-8
53. Bauersachs J, Widder JD. Endothelial dysfunction in heart failure. *Pharmacol Rep*. 2008;60(1):119-126.
54. Wu G, Bazer FW, Davis TA, et al. Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*. 2009;37(1):153-168. doi:10.1007/s00726-008-0210-y
55. Pentyala JRS. Sustained nitric oxide generation with L-homoarginine. *Res Commun Biochem Cell Mol Biol*. 1999;3:223-232.
56. Reczkowski RS, Ash DE. Rat liver arginase: kinetic mechanism, alternate substrates, and inhibitors. *Arch Biochem Biophys*. 1994;312(1):31-37. doi:10.1006/abbi.1994.1276
57. Hrabak A, Bajor T, Temesi A. Comparison of substrate and inhibitor specificity of arginase and nitric oxide (NO) synthase for arginine analogues and related compounds in murine and rat macrophages. *Biochem Biophys Res Commun*. 1994;198(1):206-212. doi:10.1006/bbrc.1994.1029
58. Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginases. *Comp Biochem Physiol B Biochem Mol Biol*. 1996;114(1):107-132. doi:10.1016/0305-0491(95)02138-8
59. Beruter J, Colombo JP, Bachmann C. Purification and properties of arginase from human liver and erythrocytes. *Biochem J*. 1978;175(2):449-454. doi:10.1042/bj1750449
60. Ikemoto M, Tabata M, Miyake T, et al. Expression of human liver arginase in *Escherichia coli*. Purification and properties of the product. *Biochem J*. 1990;270(3):697-703. doi:10.1042/bj2700697
61. Michel T. R is for arginine: metabolism of arginine takes off again, in new directions. *Circulation*. 2013;128(13):1400-1404. doi:10.1161/CIRCULATIONAHA.113.005924
62. Baskal S, Dimina L, Tsikas SA, et al. Lysine and homoarginine are closely interrelated metabolites in the rat. *Amino Acids*. 2022;54(6):967-976. doi:10.1007/s00726-022-03158-0
63. Ravani P, Maas R, Malberti F, et al. Homoarginine and mortality in pre-dialysis chronic kidney disease (CKD) patients. *PLoS ONE*. 2013;8(9):e72694. doi:10.1371/journal.pone.0072694
64. Drechsler C, Meinitzer A, Pilz S, et al. Homoarginine, heart failure, and sudden cardiac death in haemodialysis patients. *Eur J Heart Fail*. 2011;13(8):852-859. doi:10.1093/eurjhf/hfr056
65. Hov GG, Aasarod KI, Sagen E, Asberg A. Arginine, dimethylated arginine and homoarginine in relation to cardiovascular risk in patients with moderate chronic kidney disease. *Clin Biochem*. 2015;48(10-11):646-651. doi:10.1016/j.clinbiochem.2015.03.012
66. Rufo MB, Fishman WH. L-homoarginine, a specific inhibitor of liver-type alkaline phosphatase, applied to the recognition of liver-type enzyme activity in rat intestine. *J Histochem Cytochem*. 1972;20(5):336-343. doi:10.1177/20.5.336
67. Romanul FC, Bannister RG. Localized areas of high alkaline phosphatase activity in the terminal arterial tree. *J Cell Biol*. 1962;15(1):73-84. doi:10.1083/jcb.15.1.73
68. Savinov AY, Salehi M, Yadav MC, Radichev I, Millan JL, Savinova OV. Transgenic overexpression of tissue-nonspecific alkaline phosphatase (TNAP) in vascular endothelium results in generalized arterial calcification. *J am Heart Assoc*. 2015;4(12):e002499. doi:10.1161/JAHA.115.002499
69. Collin P, Nefussi JR, Wetterwald A, et al. Expression of collagen, osteocalcin, and bone alkaline phosphatase in a mineralizing rat osteoblastic cell culture. *Calcif Tissue Int*. 1992;50(2):175-183. doi:10.1007/BF00298797
70. Suzuki K, Yoshimura Y, Hisada Y, Matsumoto A. Sensitivity of intestinal alkaline phosphatase to L-homoarginine and its regulation by subunit-subunit interaction. *Jpn J Pharmacol*. 1994;64(2):97-102. doi:10.1254/jjp.64.97
71. Alesutan I, Feger M, Tuffaha R, et al. Augmentation of phosphate-induced osteo-/chondrogenic transformation of vascular smooth muscle cells by homoarginine. *Cardiovasc Res*. 2016;110(3):408-418. doi:10.1093/cvr/cvw062
72. Valtonen P, Laitinen T, Lyyra-Laitinen T, et al. Serum L-homoarginine concentration is elevated during normal pregnancy and is related to flow-mediated vasodilatation. *Circ J*. 2008;72(11):1879-1884. doi:10.1253/circj.CJ-08-0240
73. Atzler D, Rosenberg M, Anderssohn M, et al. Homoarginine—an independent marker of mortality in heart failure. *Int J Cardiol*. 2013;168(5):4907-4909. doi:10.1016/j.ijcard.2013.07.099
74. Pilz S, Edelmann F, Meinitzer A, et al. Associations of methylarginines and homoarginine with diastolic dysfunction and cardiovascular risk factors in patients with preserved left ventricular ejection fraction. *J Card Fail*. 2014;20(12):923-930. doi:10.1016/j.cardfail.2014.09.004
75. Jud P, Hafner F, Verheyen N, et al. Homoarginine/ADMA ratio and homoarginine/SDMA ratio as independent predictors of cardiovascular mortality and cardiovascular events in lower extremity arterial disease. *Sci Rep*. 2018;8(1):14197. doi:10.1038/s41598-018-32607-8
76. Lee TF, Bersten AD, Heilbronn LK, et al. ADMA and homoarginine independently predict mortality in critically ill patients. *Nitric Oxide*. 2022;122-123:47-53. doi:10.1016/j.niox.2022.03.002
77. Tomaschitz A, Meinitzer A, Pilz S, et al. Homoarginine, kidney function and cardiovascular mortality risk. *Nephrol Dial Transplant*. 2014;29(3):663-671. doi:10.1093/ndt/gft512
78. Drechsler C, Kollerits B, Meinitzer A, et al. Homoarginine and progression of chronic kidney disease: results from the Mild to Moderate Kidney Disease Study. *PLoS ONE*. 2013;8(5):e63560. doi:10.1371/journal.pone.0063560
79. Kayacelebi AA, Nguyen TH, Neil C, Horowitz JD, Jordan J, Tsikas D. Homoarginine and 3-nitrotyrosine in patients with takotsubo cardiomyopathy. *Int J Cardiol*. 2014;173(3):546-547. doi:10.1016/j.ijcard.2014.03.080
80. Seppälä I, Oksala N, Jula A, et al. The biomarker and causal roles of homoarginine in the development of cardiometabolic diseases: an observational and Mendelian randomization analysis. *Sci Rep*. 2017;7(1):1130. doi:10.1038/s41598-017-01274-6
81. Tang WH, Shrestha K, Wang Z, Troughton RW, Klein AL, Hazen SL. Diminished global arginine bioavailability as a metabolic defect in chronic systolic heart failure. *J Card Fail*. 2013;19(2):87-93. doi:10.1016/j.cardfail.2013.01.001
82. Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J am Coll Cardiol*. 2009;53(22):2061-2067. doi:10.1016/j.jacc.2009.02.036
83. Martens-Lobenhoffer J, Emrich IE, Zawada AM, et al. L-Homoarginine and its AGXT2-metabolite GOCA in chronic kidney disease as markers for clinical status and prognosis. *Amino Acids*. 2018;50(10):1347-1356. doi:10.1007/s00726-018-2610-y
84. Ostojic SM, Vranes M, Loncar D, Zenic N, Sekulic D. Guanidinoacetic acid and creatine are associated with cardiometabolic risk factors in healthy men and women: a cross-sectional study. *Nutrients*. 2018;10(1):87. doi:10.3390/nu10010087
85. Jaisson S, Kerkeni M, Santos-Weiss IC, Addad F, Hammami M, Gillery P. Increased serum homocitrulline concentrations are associated with the severity of coronary artery disease. *Clin Chem Lab Med*. 2015;53(1):103-110. doi:10.1515/cclm-2014-0642
86. Jamwal S, Sharma S. Vascular endothelium dysfunction: a conservative target in metabolic disorders. *Inflamm Res*. 2018;67(5):391-405. doi:10.1007/s00011-018-1129-8
87. Schlesinger S, Sonntag SR, Lieb W, Maas R. Asymmetric and symmetric dimethylarginine as risk markers for total mortality and cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *PLoS ONE*. 2016;11(11):e0165811. doi:10.1371/journal.pone.0165811

88. Lund O, Erlandsen M. Changes in left ventricular function and mass during serial investigations after valve replacement for aortic stenosis. *J Heart Valve Dis*. 2000;9(4):583-593.
89. Elahi MM, Chuang A, Ewing MJ, Choi CH, Grant PW, Matata BM. One problem two issues! Left ventricular systolic and diastolic dysfunction in aortic stenosis. *Ann Transl Med*. 2014;2(1):10. doi:10.3978/j.issn.2305-5839.2013.06.05
90. Villari B, Campbell SE, Hess OM, et al. Influence of collagen network on left ventricular systolic and diastolic function in aortic valve disease. *J am Coll Cardiol*. 1993;22(5):1477-1484. doi:10.1016/0735-1097(93)90560-N
91. Dahl JS, Magne J, Pellikka PA, Donal E, Marwick TH. Assessment of subclinical left ventricular dysfunction in aortic stenosis. *JACC Cardiovasc Imaging*. 2019;12(1):163-171. doi:10.1016/j.jcmg.2018.08.040
92. Treibel TA, Kozor R, Schofield R, et al. Reverse myocardial remodeling following valve replacement in patients with aortic stenosis. *J am Coll Cardiol*. 2018;71(8):860-871. doi:10.1016/j.jacc.2017.12.035
93. Krayenbuehl HP, Hess OM, Monrad ES, Schneider J, Mall G, Turina M. Left ventricular myocardial structure in aortic valve disease before, intermediate, and late after aortic valve replacement. *Circulation*. 1989;79(4):744-755. doi:10.1161/01.CIR.79.4.744
94. Faller KME, Atzler D, McAndrew DJ, et al. Impaired cardiac contractile function in AGAT knockout mice devoid of creatine is rescued by homoarginine but not creatine. *Cardiovasc Res*. 2017;114(3):417-430. doi:10.1093/cvr/cvx242
95. Dellera F, Ganzetti GS, Froio A, et al. L-homoarginine administration reduces neointimal hyperplasia in balloon-injured rat carotids. *Thromb Haemost*. 2016;116(2):400-402. doi:10.1160/TH15-10-0831
96. Nitz K, Lacy M, Bianchini M, et al. The amino acid homoarginine inhibits atherogenesis by modulating T-cell function. *Circ Res*. 2022;131(8):701-712. doi:10.1161/CIRCRESAHA.122.321094
97. Ndrepepa G, Xhepa E, Braun S, et al. Alkaline phosphatase and prognosis in patients with coronary artery disease. *Eur J Clin Invest*. 2017;47(5):378-387. doi:10.1111/eci.12752
98. Park JB, Kang DY, Yang HM, et al. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. *Eur Heart J*. 2013;34(12):920-931. doi:10.1093/eurheartj/ehs419
99. Ryu WS, Lee SH, Kim CK, Kim BJ, Yoon BW. Increased serum alkaline phosphatase as a predictor of long-term mortality after stroke. *Neurology*. 2010;75(22):1995-2002. doi:10.1212/WNL.0b013e3181ff966a
100. Beddhu S, Baird B, Ma X, Cheung AK, Greene T. Serum alkaline phosphatase and mortality in hemodialysis patients. *Clin Nephrol*. 2010;74(2):91-96. doi:10.5414/cnp74091
101. Wannamethee SG, Sattar N, Papcosta O, Lennon L, Whincup PH. Alkaline phosphatase, serum phosphate, and incident cardiovascular disease and total mortality in older men. *Arterioscler Thromb Vasc Biol*. 2013;33(5):1070-1076. doi:10.1161/ATVBAHA.112.300826
102. Tonelli M, Curhan G, Pfeffer M, et al. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. *Circulation*. 2009;120(18):1784-1792. doi:10.1161/CIRCULATIONAHA.109.851873
103. Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: a meta-analysis of prospective cohort studies. *Atherosclerosis*. 2014;236(1):7-17. doi:10.1016/j.atherosclerosis.2014.06.006
104. Mornet E, Stura E, Lia-Baldini AS, Stigbrand T, Menez A, Le Du MH. Structural evidence for a functional role of human tissue nonspecific alkaline phosphatase in bone mineralization. *J Biol Chem*. 2001;276(33):31171-31178. doi:10.1074/jbc.M102788200
105. Stockebrand M, Hornig S, Neu A, et al. Homoarginine supplementation improves blood glucose in diet-induced obese mice. *Amino Acids*. 2015;47(9):1921-1929. doi:10.1007/s00726-015-2022-1
106. Pilz S, Teerlink T, Scheffer PG, et al. Homoarginine and mortality in an older population: the Hoorn study. *Eur J Clin Invest*. 2014;44(2):200-208. doi:10.1111/eci.12208
107. van der Zwan LP, Davids M, Scheffer PG, Dekker JM, Stehouwer CD, Teerlink T. L-homoarginine and L-arginine are antagonistically related to blood pressure in an elderly population: the Hoorn study. *J Hypertens*. 2013;31(6):1114-1123. doi:10.1097/HJH.0b013e32836037fb
108. Mokhaneli MC, Botha-Le Roux S, Fourie CMT, Boger R, Schwedhelm E, Mels CMC. Homoarginine and blood pressure: a 10-year prospective relationship in normotensives. *J Hum Hypertens*. 2022;36(2):135-143. doi:10.1038/s41371-020-00449-5
109. Chen PY, Sanders PW. Role of nitric oxide synthesis in salt-sensitive hypertension in Dahl/Rapp rats. *Hypertension*. 1993;22(6):812-818. doi:10.1161/01.HYP.22.6.812
110. Atzler D, Appelbaum S, Cordts K, et al. Reference intervals of plasma homoarginine from the German Gutenberg Health Study. *Clin Chem Lab Med*. 2016;54(7):1231-1237. doi:10.1515/cclm-2015-0785
111. Kleist CJ, Choe CU, Atzler D, et al. Population kinetics of homoarginine and optimized supplementation for cardiovascular risk reduction. *Amino Acids*. 2022;54(6):889-896. doi:10.1007/s00726-022-03169-x

How to cite this article: Koch V, Gruenewald LD, Gruber-Rouh T, et al. Homoarginine in the cardiovascular system: Pathophysiology and recent developments. *Fundam Clin Pharmacol*. 2023;37(3):519-529. doi:10.1111/fcp.12858