L-Homoarginine and cardiovascular disease

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Purpose of review
An increasing number of reports indicate that low levels of the endogenous amino acid L-homoarginine are linked to cardiovascular disease. In this article, we review the current findings regarding L-homoarginine metabolism and (patho-)physiology with a focus on its clinical impact.

Recent findings
Recent clinical and epidemiological studies revealed a strong association of low circulating L-homoarginine with cardiovascular outcomes and mortality. Human and murine studies identified L-arginine:glycine amidinotransferase (AGAT) as the responsible enzyme for endogenous L-homoarginine formation, suggesting a further important function of AGAT apart from its involvement in creatine and energy metabolism. Further studies related L-homoarginine to smoking and hypertension, and metabolic phenotypes.

Summary
AGAT deficiency results in diminished intracellular energy stores (i.e., ATP and phosphocreatine), as well as a lack of L-homoarginine, and has been linked to an improved metabolic risk profile, but also to impaired cardiac and cerebrovascular function. L-homoarginine's structural similarity to L-arginine suggested physiological interference with L-arginine pathways (e.g., nitric oxide). Animal experiments and clinical trials are needed to improve knowledge on the physiology of L-homoarginine and differentiate its role as marker and mediator in cardiovascular disease.

Keywords
cardiovascular disease, creatine, L-homoarginine, L-arginine:glycine amidinotransferase, nitric oxide

INTRODUCTION

Diseases of the heart and circulatory system [cardiovascular disease (CVD)] are still the leading cause of death in Europe. According to the European Society of Cardiology, each year CVD causes over 4 million deaths in Europe (46% of all deaths) [1]. The main forms of CVD are stroke and coronary heart disease, conditions in which insufficient blood reaches the brain and heart muscle due to atherosclerotic narrowing of arteries. In the heart, this manifests as angina or life-threatening myocardial infarction, conditions which, over time may lead to heart failure. Currently, treatment strategies to improve survival and reduce recurrent events focus on risk factor management. Despite differences in vascular anatomy and physiology, atherosclerotic diseases of the heart, brain, aorta, and limbs share common risk factors [2]. Most cardiovascular and cerebrovascular events are attributable to high blood pressure, smoking, abnormal blood glucose, hypercholesterolemia, and obesity. Primary and secondary prevention aims at optimizing these metabolic risk factors. Correspondingly, pharmacological interventions include antihypertensive medication, statins, and antidiabetic drugs. Most of these drugs are known for decades. Insights of so far unknown metabolic pathways and molecular mechanisms are needed to develop novel diagnostic and therapeutic strategies.

During the last couple of years, the endogenous and nonproteinogenic amino acid L-homoarginine has emerged as a promising biomarker for cardiovascular and cerebrovascular outcome [3,4]. In this context, new light has been shed on the enzyme L-arginine:glycine amidinotransferase (AGAT), which was shown to be responsible for L-homoarginine synthesis in humans and mice [5▪]. Until

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KEY POINTS

- L-homoarginine is a marker of cardiovascular and cerebrovascular outcome in humans.
- AGAT synthesizes L-homoarginine and creatine precursors in humans and mice.
- L-homoarginine has the potential to interfere with L-arginine/NO metabolism *in vivo*.

recently, the only known function of AGAT was synthesis of guanidinoacetate – the intermediate product of creatine synthesis [6]. In this review, we discuss the current findings regarding L-homoarginine synthesis and sources, L-homoarginine physiology, and clinical impact of L-homoarginine.

L-HOMOARGININE SYNTHESIS AND SOURCES

L-Homoarginine is a nonproteinogenic amino acid structurally related to L-arginine. Circulating L-homoarginine concentrations have been linked to single-nucleotide polymorphisms (SNPs) in several population-based studies. Genome-wide association studies from the Gutenberg Health Study first identified an association between L-homoarginine plasma concentrations and SNPs related to the *AGAT* gene on chromosome 15 [5▪]. The leading SNP rs1288775 encodes for the exchange of A -> T, resulting in the missense mutation Gln110His within the second exon of the *AGAT* gene. Of notice, carriers of two AA alleles showed higher plasma concentrations, that is, 2.24 (1.75, 2.95) μmol/l [mean (interquartile range)], whereas L-homoarginine was lower in AT carriers [2.08 (1.55, 2.54) μmol/l] and lowest in TT carriers [1.80 (1.40, 2.28) μmol/l], representing a gene dose-dependent decrease by 16 and 24%, respectively. The minor allele frequency was 6% for AA carriers. Genome-wide association from patients of the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study and from participants of the Young Finns Study confirmed the strong association between circulating L-homoarginine and SNPs related to the *AGAT* gene [7]. Until recently, AGAT was known as the first and rate-limiting enzyme of creatine synthesis. In kidney, AGAT catalyzes the transfer of L-arginine's

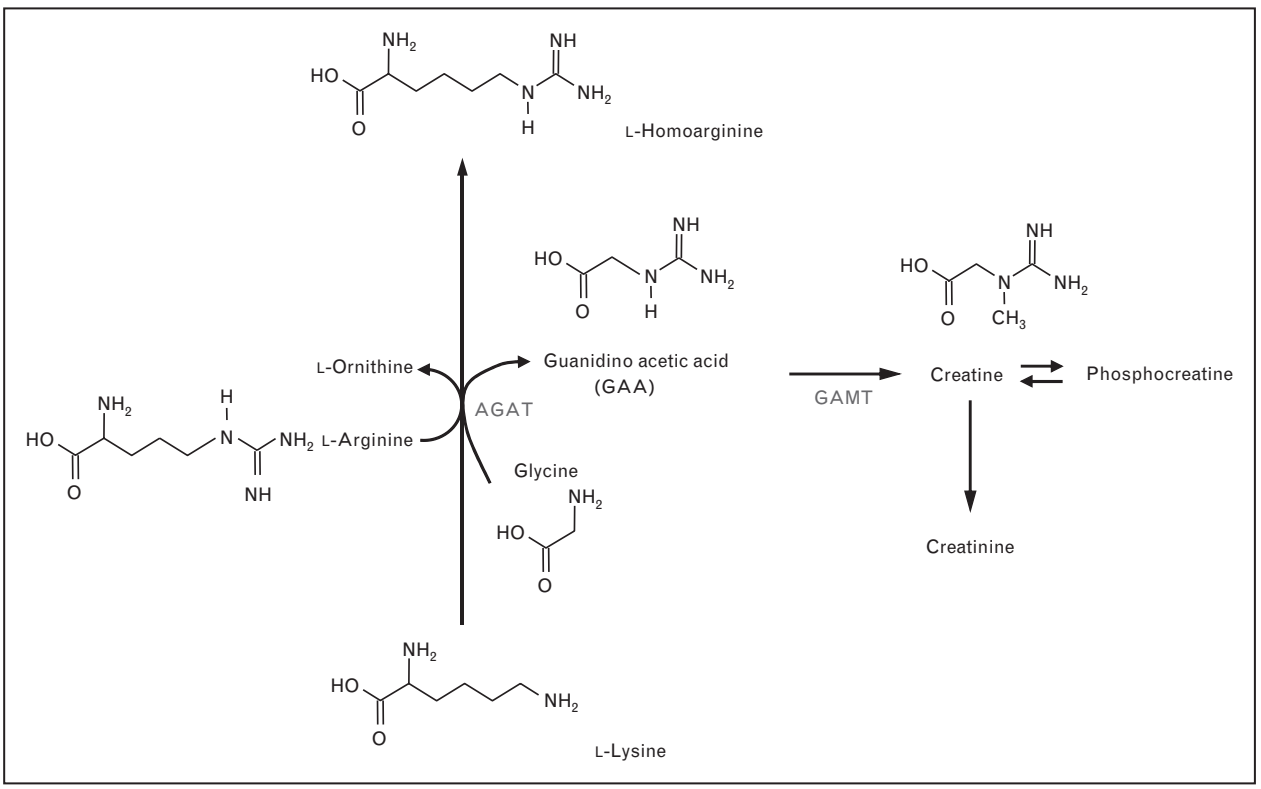

FIGURE 1. Scheme of creatine and homoarginine metabolism. Creatine synthesis is a two-step enzymatic process consisting of AGAT and GAMT. AGAT transfers the amidino group of L-arginine to glycine producing guanidino acetic acid. GAMT methylates guanidinoacetate resulting in creatine formation. Homoarginine synthesis involves AGAT, which transfers the amidino group of L-arginine to lysine producing homoarginine. AGAT, L-arginine:glycine amidinotransferase; GAMT, guanidinoacetate N-methyltransferase.

guanidino group to glycine producing guanidinoacetate and ornithine (Fig. 1). In the liver, methylation of guanidinoacetate is catalyzed by guanidinoacetate N-methyltransferase (GAMT, EC 2.1.1.2), resulting in creatine formation [6]. Creatine itself serves as a rapidly available energy buffer especially in heart and brain. AGAT knockout mice are lacking creatine and guanidinoacetate. AGAT-deficient mice exhibit a lean phenotype with muscular dystrophy, which is completely reversible on creatine supplementation [8▪,9]. Further metabolic analysis revealed that humans and mice with AGAT deficiency are inefficient at synthesizing L-homoarginine from L-arginine and L-lysine [5▪,10]. Tellingly, AGAT is able to transfer the guanidino group from L-arginine not only to glycine but also to L-lysine, thereby producing not only guanidinoacetate but also L-homoarginine, respectively (Fig. 1). These findings confirm studies by Ryan *et al.* [11] from the 1960s, demonstrating that an amidinotransferase in kidney tissue catalyzes L-homoarginine synthesis from L-lysine.

At the same time, L-homoarginine was identified as an ingredient of different species of *Lathyrus* (grass pea). *Lathyrus* spp. *L. cicera* and *L. sativus* as well as *Lens culinaris* (lenti) contain 1 to 2% of L-homoarginine [12]. In contrast to *L. sativus*, which also contains a relatively high amount of an AMPA agonist and thereby causes neurolathyrism, *L. culinaris* does not harm humans [13].

L-HOMOARGININE PHYSIOLOGY

Early functional studies in the 1970s have identified L-homoarginine as inhibitor of human liver and bone alkaline phosphatase [3,14]. But, more recent studies have suggested an involvement in vascular function and disease. Given its structural similarity with L-arginine, L-homoarginine most likely interferes with L-arginine pathways. Probably the most important function of L-arginine is to serve as substrate for nitric oxide (NO) synthesis. Several studies showed that L-homoarginine can serve as an alternative substrate for NO synthase (NOS) [15,16]. Whereas maximal activity (V_{max}) for NOS-dependent oxidation was similar between L-arginine and L-homoarginine, binding affinity (K_m) was 10-fold to 20-fold lower for homoarginine than L-arginine [16]. Therefore, L-homoarginine is generally considered a weak substrate for NOS. Consistently, mouse studies [17] revealed lower maximal NO levels, but more sustained NO formation after L-homoarginine supplementation compared with arginine. Circulating nitrate concentrations – an indirect measure for NOS activity – were increased even 8 h after L-homoarginine treatment, whereas nitrate levels in arginine supplemented mice returned to baseline after 4 h [17]. Back in the 1990s, Radomski *et al.* studied the physiological role of L-homoarginine in human platelets. In this context, L-homoarginine was found to inhibit aggregation of human platelets stimulated with collagen similar to L-arginine [18]. However, the physiological relevance of these findings needs further validation because L-homoarginine was effective only in supraphysiological concentrations. In addition to serving as a substrate for NOS, several groups have shown inhibition of arginase by L-homoarginine, suggesting increased L-arginine levels and subsequently increased NO formation [15,19]. In comparison to L-arginine, L-homoarginine plasma concentrations *in vivo* are relatively low, and high concentrations were required *in vitro* that is for arginase inhibition. Therefore, further investigations are mandatory to elucidate L-homoarginine's physiological significance as an endogenous metabolite of the NO pathway.

CLINICAL IMPACT OF L-HOMOARGININE AND EXPERIMENTAL EVIDENCE

In healthy humans, circulating concentrations of L-homoarginine are about 2 to 3 μmol/l with material age-related and sex-related differences. Reference ranges for serum L-homoarginine obtained from healthy participants of the population-based Study of Health in Pomerania were 1.41–5.00 and 1.20–5.53 μmol/l (2.5th; 97.5th percentile), for men and women, respectively [20▪]. Within the last 5 years, the number of publications investigating associations between circulating L-homoarginine and diverse laboratory or clinical phenotypes substantially increased. In 2010, März *et al.* [3] published data from the LURIC and 4D (Die Deutsche Diabetes Dialyse) studies, showing a strong association between low L-homoarginine serum levels and increased cardiovascular and all-cause mortality (Figs. 2 and 3). Cross-sectional analyses of these studies related L-homoarginine to markers of endothelial function (i.e., intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), suggesting that L-homoarginine might improve endothelial function. Recent findings from the Hoorn study [21▪] confirmed the association of L-homoarginine with overall mortality and cardiovascular death in a population of older age (Figs. 2 and 3). Further studies tried to allocate subtypes of cardiovascular death. Low L-homoarginine was shown to predict fatal strokes in the LURIC study and was associated with sudden cardiac death or death due to heart failure in the 4D study [4,22] (Fig. 3). To elucidate the clinical phenotypes involved, circulating L-homoarginine was associated with angiographic ejection fraction and laboratory

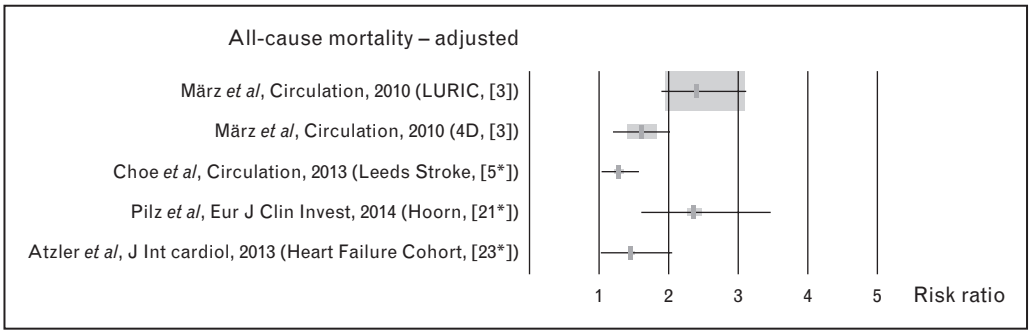

FIGURE 2. Forest plot of L-homoarginine levels and all-cause mortality risk (adjusted risk ratios). Decreased L-homoarginine levels were associated with all-cause mortality in the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study, Die Deutsche Diabetes Dialyse (4D) study, Hoorn study, Leeds Stroke Cohort, and Heart Failure Cohort (adjusted risk ratios). Studies are plotted according to the last name of the first author followed by the journal name, publication year, and name of the study in parentheses. Horizontal lines represent 95% confidence intervals. Each square represents the proportional weight of the study.

parameters of heart failure, specifically N-terminal pro B-type natriuretic peptide, suggesting a positive correlation between L-homoarginine and myocardial function. A similar correlation was found between L-homoarginine and N-terminal pro B-type natriuretic peptide levels in another cohort of 282 heart failure patients [23▪]. In addition to angiographic and laboratory parameters of myocardial dysfunction, this study also associated low L-homoarginine levels with the clinical stages of heart failure [4,23▪]. Patients suffering from moderate or severe heart failure (i.e., New York Heart Association class 3 and 4) exhibited decreased L-homoarginine levels. L-homoarginine plasma levels did not differ between dilatative and ischemic cardiomyopathy, and analysis did not reveal a significant correlation with heart failure etiology [23▪]. Taken together, angiographic, laboratory, and clinical parameters of heart failure are associated with L-homoarginine levels, but the underlying pathophysiological mechanism remains unclear. Some authors suggest that low L-homoarginine levels might indicate reduced intracellular energy stores, which is a hallmark of heart failure [24]. Clinical studies have confirmed cellular and molecular causal associations of L-homoarginine with metabolites of energy metabolism – namely creatine [4,8▪]. As described, phosphorylated creatine serves as a spatial and temporal energy buffer. And, key components of the creatine:phosphocreatine system are downregulated in the failing heart [24]. In line with this observation, a moderate elevation of intracellular creatine protects mice from acute myocardial infarction [25]. Murine studies and cell culture experiments have shown that levels of both creatine and homoarginine are dependent on AGAT and, therefore, positively correlate with each other [5▪,8▪]. Association studies in humans have validated this finding [5▪,7]. Therefore, it was hypothesized that circulating L-homoarginine might indicate the status of intracellular energy stores in heart failure. However, recent results suggest that the association of creatine and heart failure is not as trivial as presumed. In this context, Phillips *et al.* [26] reported that chronically increased myocardial creatine leads

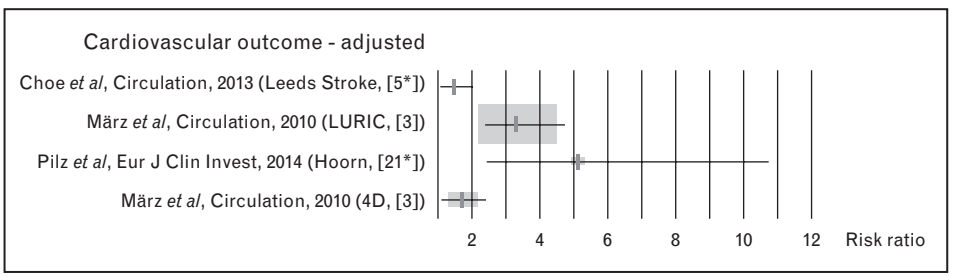

FIGURE 3. Forest plot of homoarginine levels and risk of cardiovascular outcome (adjusted risk ratios). Decreased homoarginine levels were associated with cardiovascular outcome in the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study, Die Deutsche Diabetes Dialyse (4D) study, Leeds Stroke Cohort, Hoorn study, and Heart Failure Cohort (adjusted risk ratios). Studies are plotted according to the last name of the first author followed by the journal name, publication year, and name of the study in parentheses. Horizontal lines represent 95% confidence intervals. Each square represents the proportional weight of the study.

to the development of progressive hypertrophy and heart failure, suggesting rather a bell-shaped association of creatine. Lygate *et al.* [27] furthermore reported that creatine-deficient mice revealed an unaltered response to chronic myocardial infarction, suggesting that creatine might be a dispensable metabolite for left ventricular remodeling and development of chronic heart failure following myocardial infarction. Thus, the relevance of creatine and L-homoarginine within cardiac energetics in heart failure remains unclear.

Other studies have suggested an increased risk of cerebrovascular disease in patients with chronic kidney disease [28]. A large meta-analysis revealed that the overall risk ratio for patients with reduced estimated glomerular filtration rate of less than 60 ml/min/1.73m^2 was 1.43 (confidence interval 1.31–1.57, $P < 0.001$) [29]. Cross-sectional analyses of the Leeds Stroke cohort, LURIC study, and 4D study revealed positive correlations of circulating L-homoarginine with parameters of kidney function, that is creatinine levels and estimated glomerular filtration rate [3,4,5▪]. Given that AGAT is mainly expressed in the kidney, renal dysfunction and damage might be associated with reduced AGAT expression and, therefore, reduced L-homoarginine production. But, involvement of L-homoarginine in renal NO metabolism might also be an explanation for the association of L-homoarginine with renal function. NOS, which is expressed in the kidney, regulates renal hemodynamics and is regulated in response to injury [30]. Therefore, interplay between L-homoarginine, renal hemodynamics, kidney damage, and finally mortality is highly relevant [31].

Major modifiable vascular risk factors are arterial hypertension and smoking, which double the risk of cardiovascular and cerebrovascular disease. In a recent cross-sectional study of 231 healthy men, smoking was associated with decreased L-homoarginine [32]. Similar associations have been described in the LURIC and Hoorn studies [4,21▪]. It is tempting to speculate that the increased risk for vascular events in smokers is partly mediated by decreased L-homoarginine levels. In the Hoorn cohort of older participants as well as in patients undergoing coronary angiography (LURIC), circulating L-homoarginine positively correlated with systolic and diastolic blood pressure [21▪,33]. An increase of L-homoarginine levels by about 0.5 μmol/l was associated with an increase of systolic blood pressure by 3.9 mmHg. Given that an increase of systolic blood pressure by 20 or 10 mmHg diastolic is associated with doubling of cardiovascular mortality [34], it is very unlikely that blood pressure changes are involved in mediating the beneficial effects of L-homoarginine in vascular disease. In addition to smoking and hypertension, associations between L-homoarginine and metabolic parameters have been described. Positive correlations were found with BMI and triglyceride levels, but not with low-density lipoprotein or high-density lipoprotein cholesterol levels. In the LURIC study, no correlation was found between L-homoarginine and parameters of glucose metabolism (i.e., hemoglobin A1c and diabetes), whereas results from the Hoorn study [33] indicated a positive correlation between L-homoarginine with hemoglobin A1c. Given the discrepancy between the studies, at present, it remains unclear whether L-homoarginine has a causal effect on blood pressure and metabolic parameters or whether these effects are mediated by creatine metabolism.

CONCLUSION

L-Homoarginine is a naturally occurring amino acid endogenously synthesized by AGAT catalysis, suggesting a further important function of AGAT in addition to its involvement in the creatine/energy metabolism. Low circulating L-homoarginine has been independently associated with cardiovascular and all-cause mortality in older individuals and patients at cardiovascular risk. AGAT deficiency results in diminished intracellular energy stores (i.e., ATP and phosphocreatine) as well as lack of L-homoarginine and is characterized by an improved metabolic risk profile, but also by impaired cardiac and cerebrovascular function. Therefore, animal experiments and randomized clinical trials are necessary to differentiate in which cases L-homoarginine is just a marker or in which other cases a causal mediator in CVD.

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Conflicts of interest

There are no conflicts of interest.

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