Homoarginine in health and disease

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Purpose of review
Homoarginine (hArg) is an endogenous, nonproteinogenic amino acid. It is enzymatically synthesized from L-arginine and L-lysine. Low hArg concentrations appear to be a risk factor in the renal and cardiovascular systems. This review discusses advances in-vitro and in-vivo experimental and clinical research on hArg in health and disease.

Recent findings
Recent studies indicate that low circulating and low urinary concentrations of hArg are associated with morbidity and worse outcome. Although the biological activities of hArg remain still unexplored, hArg supplementation is intensely investigated as a strategy to increase hArg concentration to reach normal levels in cases of low hArg concentrations. The greatest changes in circulating hArg concentrations are observed during pregnancy and after delivery. In healthy adults, a daily dose of 125 mg hArg seems to be optimum to normalize circulating levels. Short-term supplementation of inorganic nitrate enhances hArg biosynthesis in healthy young men. Apart from hArg supplementation, dietary L-arginine and L-citrulline appear to be a promising alternative.

Summary
Considerable progress has been made in recent years, but hArg remains still enigmatic. Further research is required to explore the biological activities of hArg. Supplementation of hArg or its precursors L-citrulline/L-arginine seem to be promising strategies to prevent and overcome altered hArg synthesis.

Keywords
drugs, lysine, pregnancy, supplementation, transamidination

INTRODUCTION

L-Homoarginine (hArg), the methylene analog of L-arginine (Arg), is a natural compound, both in plants and in mammals (Fig. 1) [1]. In the recent two decades, hArg attracted great interest from different scientific disciplines including chemistry, biochemistry and medicine. Research in these areas resulted in new analytical methods for the analysis of hArg in biological samples, novel findings on its biology and chemistry, and unexpected observations from experimental, epidemiological and clinical studies. Systematic reviews and meta-analyses of articles on hArg appeared in the first decade of the revived hArg research suggest that hArg is an important clinical risk marker in the renal and cardiovascular systems [2–7,8▪].

METHODS OF ANALYSIS OF HOMOARGININE

Several different analytical methods based on electrophoresis, gas chromatography (GC) liquid chromatography (LC), mass spectrometry (MS) and tandem mass spectrometry (MS/MS) have been developed, validated and used for the quantitative analysis of hArg and related amino acids, including Arg and its dimethylated metabolites in various biological samples, including plasma, serum and urine [1,9–14]. These analytical approaches were useful in studying origin, biochemistry, determining reference values and intervals, the clinical significance as well as the pharmacological potential of hArg. These issues are discussed in the sections that follow.

ORIGIN, METABOLISM AND POTENTIAL BIOLOGICAL ACTIVITIES OF HOMOARGININE

hArg is a natural compound known since several decades [1]. Yet, hArg has been an overlooked amino

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KEY POINTS

- Recent advances in several aspects of homoarginine research are reviewed.
- Low blood and urine homoarginine concentrations are risk markers in cardiovascular diseases.
- Kidney is a major origin and regulator of homoarginine metabolism.
- Normal pregnancy and preeclampsia are associated with elevated homoarginine synthesis.
- Supplementation of homoarginine in conditions of low homoarginine concentrations is investigated.

acid. Despite intense research, many aspects of hArg, especially including its biochemistry and physiology, are incompletely understood. The enzyme arginine: glycine amidinotransferase (AGAT; EC 2.1.4.1) catalyzes the formation of hArg and L-ornithine (Orn) from Arg and L-lysine (Lys) [1] (Fig. 1). Arginase hydrolyzes hArg to Lys, yet the activity of arginase is several orders of magnitude lower than the hydrolysis of Arg to Orn [15]. hArg and Lys are closely interrelated metabolites in humans [15] and rats [16▪▪]. Yet, it seems that Lys is metabolically favoured. Supplementation of Lys to increase hArg has not been considered thus far. hArg serves as a substrate for nitric oxide synthase (NOS) to produce nitric oxide and L-homocitrulline. The affinity of hArg to NOS isozymes and the concentration of hArg in biological fluids are much lower than that of Arg. With respect to NOS, hArg seems to be rather a competitor of Arg, with high hArg concentrations leading even to decreased NOS activity. This adds to the paradoxes of the NOS activity inhibitors asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) [17].

hArg has been used as an inhibitor of alkaline phosphatase and human arginase activity at mmol/L-concentrations [18]. Given the low μmol/L-hArg concentrations in blood and tissue, these activities of hArg are rather of experimental and pharmacological interest. The opposite behaviour of hArg (i.e. substrate) and ADMA (i.e. inhibitor) against NOS has led to the hypothesis that hArg and ADMA may act antagonistically in the cardiovascular system.

The distribution and the metabolism of pharmacological hArg at high doses has been investigated in the takotsubo cardiomyopathy model in anaesthetized rats [19]. hArg was distributed throughout the body and was eliminated with an elimination half-life of about 30 min. Pharmacological hArg caused considerable changes in amino acid homeostasis in rat organs [20▪▪]. The pharmacokinetics of hArg has been thoroughly investigated in healthy humans [21,22▪▪]. The optimum dose of hArg needed to achieve reduction of cardiovascular risk was elaborated to be 25 mg/day [22▪▪].

Generally, hArg is considered a nonproteinogenic amino acid. Occurrence and concentration of physiological peptides and proteins in biological samples is uncertain [11]. Chemical guanidination of Lys residues in peptides and proteins, for instance by *O*-isothiourea, is well known to convert Lys residues into hArg residues. Yet, it is unknown whether such a PTM occurs. hArg peptides such as phaseolotoxin, that is, *N*-sulphodiaminophosphinyl-ornithyl-alanyl-homoarginine, occur in plants and they are produced by bacteria such as *Pseudomonas syringae pv. phaseolicola*. The temperature-mediated biosynthesis of the phytotoxin phaseolotoxin by *P. syringae pv. phaseolicola* depends on the autoregulated expression of the phtABC genes. The biological properties of natural and synthetic hArg-containing peptides such as eptifibatide, a clinically used antiplatelet drug, differ from the Lys and Arg homologs [23▪▪]. A new modification of Lys residues in peptides and proteins has been shown to include chemical reaction of the ε-amine group of Lys residues with activated uric acid metabolites both during normal metabolism and inflammation [24]. Whether such modifications of Lys residues lead to hArg, in analogy to chemical guanidination with *O*-isothiourea or 3,5-dimethylpyrazole-1-cardoxyamidine, remains to be investigated. The effects of guanidination of Lys residues of endogenous peptides, proteins and drugs such as bradykinin, luteinizing hormone, glucagon and vasopressin on biological activity are not predictable and need to be investigated with synthetic and structurally fully characterized analogues [25].

HOMOARGININE IN PREGNANCY AND CHILDHOOD

Previous investigations have shown that two cationic amino acid transport systems in human placental basal plasma membranes are responsible for the transport of Arg and hArg. It took almost two decades until the report that the serum hArg concentration is elevated during normal pregnancy and is related to flow-mediated vasodilatation (FMD) [26]. Thus far, the highest circulating hArg concentrations were measured in pregnancy [26–28]. In nonpregnant women ($n = 61$), the serum hArg concentration was measured by HPLC with fluorescence detection to be 2.7 ± 1.1 μmol/l (Table 1). In healthy pregnant women of the same study, the serum hArg concentration was measured to be 3.1 ± 1.4 μmol/l

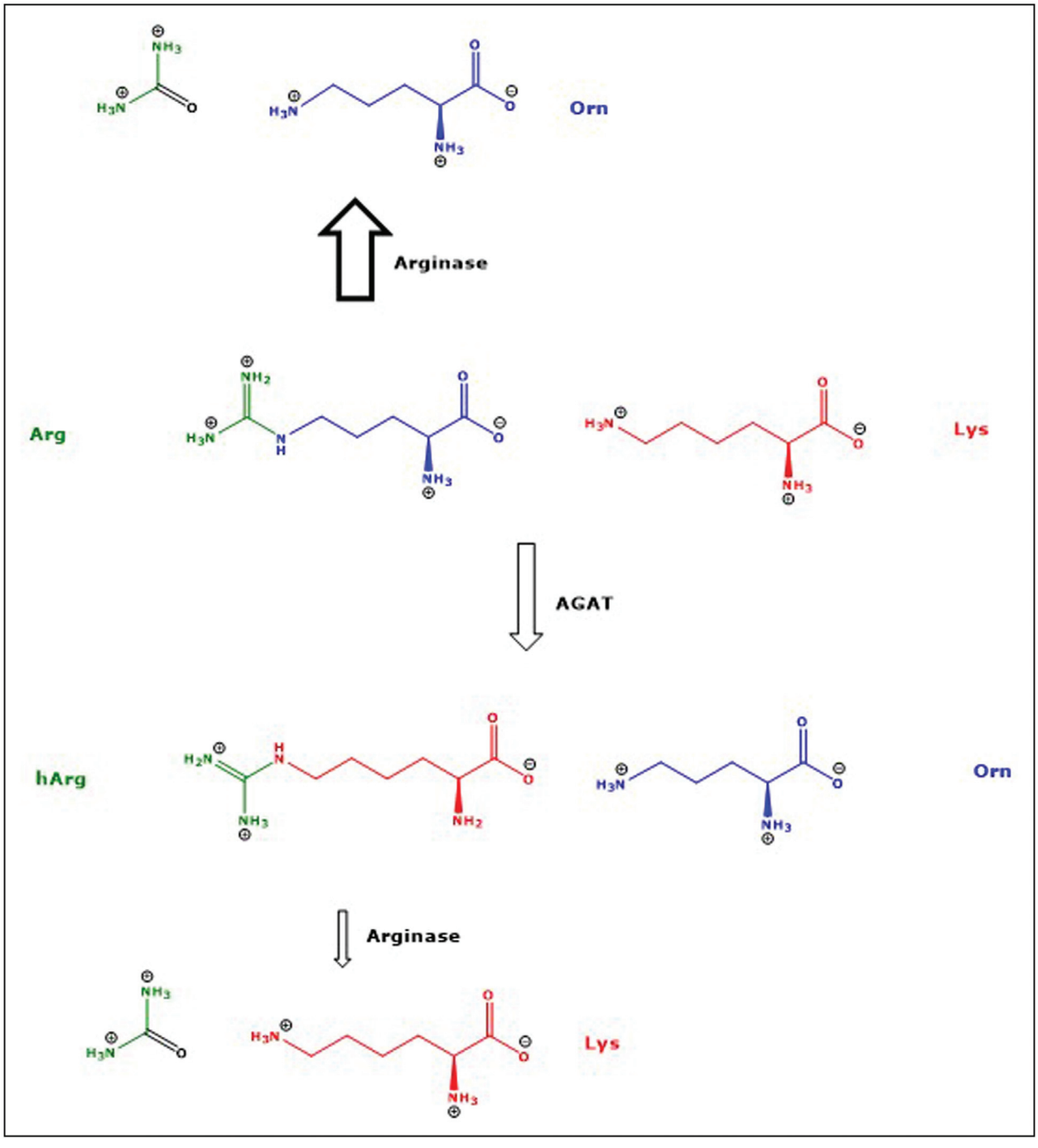

FIGURE 1. Simplified schematic of the arginine:glycine amidinotransferase-catalyzed formation of L-homoarginine from L-arginine (Arg) and L-lysine (Lys). Arginase hydrolyzes Arg to urea and L-ornithine (Orn), and hArg to urea and Lys.

in the first trimester ($n = 13$), 4.8 ± 1.7 μmol/l in the second trimester ($n = 22$) and 5.3 ± 1.5 μmol/l in the third trimester ($n = 23$) [26] (Table 1). In another study, the plasma hArg concentration in the first trimester was measured to be 3.31 (2.56–4.49) μmol/l in healthy pregnant women ($n = 300$), 2.70 (2.30–3.31) μmol/l in women with early preeclampsia ($n = 25$) and 3.53 (2.87–4.74) μmol/l in late preeclamsia ($n = 50$) as measured by GC-MS [27]. These studies indicated that circulating hArg concentration is higher in pregnancy and increases during normal pregnancy. Interestingly, plasma hArg concentrations in ewe's pregnancy were found to be associated with the number of foetuses [29]. In pregnant ewes, hArg plasma concentration varied greatly (44-fold) between the highest and the lowest values, while Arg plasma concentration varied much less as measured by LC-MS/MS [29]. Median hArg

Table 1. Selection of reported homoarginine concentrations (mean or median) in human plasma (P), serum (S) or urine (U)

Homoarginine concentration	Individuals, study, remarks	Methodology	References
2.7 μmol/l (S, women; $n=61$) 3.1 μmol/l (S, women; $n=13$) 4.8 μmol/l (S, women; $n=22$) 5.3 μmol/l (S, women; $n=23$)	nonpregnant, healthy pregnant, healthy; first trimester pregnant, healthy; second trimester pregnant, healthy; third trimester	HPLC-FL	Valtonen *et al.* [26]
3.3 μmol/l (P, women; $n=300$) 2.7 μmol/l (P, women, $n=25$) 3.5 μmol/l (P, women, $n=50$)	pregnant, healthy; first trimester preeclampsia; early preeclampsia; late	GC-MS	Khalil *et al.* [27]
0.47 μmol/l (P, girls; $n=55$) 0.63 μmol/l (P, boys; $n=51$) 0.247 μmol/mmol creatinine (U, girls; $n=34$) 0.269 μmol/mmol creatinine (U, boys; $n=39$)	preterm neonates	GC-MS	Buck *et al.* [32]
0.13 - 0.54 μmol/l (human breast milk)	Neonatal period (first 4 weeks)	GC-MS	Baskal *et al.* [33▪▪]
1.9 μmol/l (P, men and women; $n=100$) 1.5 μmol/l (P, men and women; $n=164$)	healthy individuals rheumatoid arthritis	LC-MS/MS	Erre *et al.* [47]
1.38 μmol/l (P, women; $n=1779$) 1.73 μmol/l (P, men; $n=1552$)	Framingham Offspring Study	LC-MS/MS	Schwedhelm *et al.* [37]
1.25 μmol/l (P, survivors) 0.89 μmol/l (P, nonsurnivors)	Elderly healthy individuals ($n=669$) South African Blacks	LC-MS/MS	Mokaneli *et al.* [38]
1.32 μmol/l (P, $n=166$) 1.42 μmol/l (P, $n=166$)	Elderly healthy individuals South African Blacks	LC-MS/MS	Mokaneli *et al.* [39▪▪]
3–12 μmol/l (S) (minimum to maximum) 3–15 μmol/l (S) (minimum to maximum)	pregnant, healthy; 20–28th weeks preeclampsia; 20–28 weeks	LC-MS/MS	Xiangmei Yuan *et al.* [31▪▪]
1.27 μmol/l (P, young men) 1.68 μmol/l (P, young men)	NaCl supplementation (9 days) $NaNO_3$ supplementation (9 days)	GC-MS	Tsikas *et al.* [60▪▪]

plasma concentrations significantly increased with the number of foetuses independent of mating, feeding, diet and treatment. These observations clearly suggest that hArg is closely associated with pregnancy in mammals, yet the underlying mechanisms are still elusive. One may speculate that hArg is a kind of pregnancy-specific regulatory substance in mammals. In two small groups of pregnant women without and with HELLP (haemolysis, elevated liver enzymes and low platelet count) syndrome, the plasma hArg concentration did not differ between the groups [30].

Preeclampsia is a disorder of pregnancy characterized by the onset of high blood pressure. The condition begins after 20 weeks of pregnancy. Before the 20th pregnancy week and within the 20th - 28th pregnacy week, serum hArg concentration was found to be higher in mild preeclampsia than in pregnant women without preclampsia (about 8 vs. 5 μmol/l) as measured by LC-MS/MS [31▪▪] (Table 1). After the 28th pregnancy week, the serum hArg concentration was found be considerably lower in severe preeclampsia compared with mild preeclampsia [31▪▪]. This study showed that serum hArg and ADMA concentrations were similarly strong predictors of mild preeclampsia.

The Arg/NO pathway in infancy differs from that in adults. High circulating ADMA concentrations are a cardiovascular risk factor in adults, but there is no single report that this also in the case in children. The circulating ADMA concentration is more than twofold higher in the first months of life compared with adults and decreases up to the age of about 18 years to reach adults-levels. In preterm neonates, the plasma ADMA and hArg concentrations were of the order of 900 and 560 nmol/l, respectively [32] (Table 1). ADMA and hArg biosynthesis increased with gestational age without remarkable changes in the hArg/ADMA ratio or nitric oxide biosynthesis. This study suggested that ADMA and hArg are involved in foetal growth presumably independent of nitric oxide. hArg, ADMA, Arg and many other amino acids including their metabolites formed by posttranslational modification (PTM) are present in human breast milk at least in the neonatal period [33▪▪].

Studies on short stature children without and with growth hormone deficiency suggested that hArg and ADMA are involved differently in growth in the childhood. The Arg/NO and hArg pathways were found to be altered in Duchenne muscular dystrophy (DMD) and improved by glucocorticoids.

The Arg/nitric oxide and hArg pathways were also found to be altered in young people with type 1 diabetes mellitus. More recent paediatric studies indicate that the Arg/NO and hArg pathways are altered in cystic fibrosis (CF) [34], ureteropelvic junction obstruction (UPJO) infants requiring surgery [35▪▪] and in paediatric patients with atopic diseases [36▪]. During acute exacerbation of CF, sputum hArg levels dropped significantly after antibiotic treatment [34]. The observation that antibiotic therapy lower hArg concentration in the sputum is striking and warrants further investigations.

HOMOARGININE IN HEALTH AND DISEASE

In the Framingham Offspring study, the median plasma hArg concentrations were determined by LC-MS/MS to be 1.73 μmol/l in men ($n = 1552$) and 1.38 μmol/l in women ($n = 1779$) [37] (Table 1). Plasma ADMA concentrations did not differ between men (0.54 μmol/l) and women (0.53 μmol/l). Lower plasma hArg concentrations were found to be associated with a greater risk of all-cause mortality in the community.

In South African populations of black and white people, hArg and ADMA were investigated in relation to blood pressure, carotid wall thickness, cardiovascular and all-cause mortality [38,39▪▪,40▪▪] (Table 1). In black men and women, carotid intima-media thickness (cIMT) and cross-sectional wall area (CSWA) inversely associated with baseline hArg concentrations. In women, but not in men, CSWA inversely associated with hArg [38]. In a black population of elderly participants ($n = 669$), it was investigated whether plasma hArg is associated with 10-year risk of all-cause and cardiovascular mortality [39▪▪]. Survivors ($n = 526$) had higher plasma hArg levels compared with nonsurvivors ($n = 143$) (1.25 vs. 0.89 μmol/l; $P < 0.001$) as measured by LC-MS/MS. The same group investigated in a black population of elderly participants whether blood pressure after 10 years associates with baseline hArg in participants who remained normotensive or who developed hypertension [40▪▪]. In both groups, median plasma hArg concentrations were similar at baseline (1.32 and 1.42 μmol/l) and 10 years later (1.59 and 1.60 μmol/l). In a group that remained normotensive after 10 years ($n = 166$), baseline hArg correlated positively with follow-up brachial SBP, brachial pulse pressure and central pulse pressure. No significant associations were found in the group that developed hypertension after 10 years ($n = 166$) [40▪▪].

Low hArg serum concentration was reported to be a risk factor for heart disease and a strong risk factor for sudden cardiac disease and death due to heart failure in haemodialysis patients [1–7]. Low hArg plasma concentration was found to be an independent marker of mortality in heart failure. hArg and Arg were found to be antagonistically related to blood pressure in an elderly population. In patients with takotsubo cardiomyopathy (TTC), the hArg plasma concentrations were also lower compared with healthy individuals (1.3 vs. 2.1 μmol/l). The association of plasma hArg and ADMA was investigated in patients with rheumatoid arthritis and has been hypothesized that hArg is cardiovascular corrective in rheumatoid arthritis. Plasma and tissue hArg concentrations were measured in healthy and obese humans. hArg has been rarely measured in urine. High urinary hArg excretion was found to be associated with low rates of all-cause mortality and graft failure in renal transplant recipients. In the same population, low plasma hArg concentration was found to be associated with high rates of all-cause mortality in renal transplant recipients [41]. In contrast to ADMA, low circulating and low excretory concentrations of hArg were reported to be associated with all-cause mortality renal transplant recipients.

The Arg/NO and hArg pathways were investigated in patients with multiple sclerosis and neuromyelitis optica by measuring the metabolites in serum and cerebrospinal fluid (CSF). The hArg concentration was about 2.6 μmol/l in serum and 0.7 μmol/l in the CSF. This study suggested that hArg is not likely to play an important role in multiple sclerosis and neuromyelitis optica. The potential relationship between aortic distensibility or aortic intima-media thickness (aIMT) and circulating hArg was investigated in elderly patients with recent ischemic stroke or transient ischemic attack. The median hArg plasma concentration was lower in the women ($n = 24$) compared with the men ($n = 54$) (1.02 vs. 1.64 μmol/l, $P = 0.013$). In this study, plasma hArg concentration correlated with aIMT but not with aortic distensibility, suggesting that different mechanisms may apply to these two aspects of aortic wall remodelling.

The biomarker and causal roles of hArg in the development of cardiometabolic diseases [42], and cross-sectional associations between hArg, intermediate phenotypes and atrial fibrillation in healthy humans [43] were recently investigated. The relationship between hArg and the methionine-homocysteine balance was investigated in patients with ischemic heart disease [44]. In the patients, low hArg concentrations were associated with impaired metabolism of sulphur-containing amino acids involved in transmethylation reactions. Distinct associations between plasma osteoprotegerin, hArg and ADMA were observed in

chronic kidney disease male patients with coronary artery disease [45]. Given that hArg and guanidinoacetate (GAA) are Arg metabolites in the AGAT pathway (Scheme 1), the relationship between hArg and GAA was investigated in adult renal transplant recipients. High plasma GAA-to-hArg ratios were found to be associated with high all-cause and cardiovascular mortality rate underlying the importance of AGAT for a healthy kidney [46].

In a clinical trial, patients with rheumatoid arthritis had lower plasma hArg concentrations than healthy controls (1.5 ± 0.6 vs. 1.9 ± 0.8 μmol/l), whereas plasma ADMA concentrations were higher in patients than in controls (0.53 ± 0.09 vs. 0.46 ± 0.07 μmol/l) [47] (Table 1). These researchers found in a large cohort that hArg and ADMA independently predict mortality in critically ill patients [48▪▪]: the area under the receiver operator characteristic curve (AUC-ROC) for risk of death score, hArg and ADMA combined for mortality was greater than for risk of death score alone. Another group found that hArg ADMA and SDMA are predictors of long-term outcome in patients presenting with suspicion of venous thromboembolism (VTE) [49▪▪]. The importance of hArg was investigated in patients with arterial hypertension [50] and myocardial ischaemia [51].

In the ZSF1 animal model for heart failure with preserved ejection fraction, hArg in blood, liver and heart, and hArg creatinine-corrected hArg excretion were found to be lower in the obese ZSF1 rats compared with the lean ZSF1 rats ([52,53].

EFFECTS OF DRUGS ON ENDOGENOUS HOMOARGININE SYNTHESIS

Supplementation of Becker muscular dystrophy (BMD) patients with L-citrulline enhanced hArg synthesis, whereas metformin reduced hArg synthesis [54]. The effects of L-citrulline are likely to be due to elevation of Arg in the body and its AGAT-catalyzed conversion to hArg. In contrast, metformin seems to inhibit this activity of AGAT [55].

Statin-induced myopathy affects more than 10 million people worldwide. An *AGAT* gene is associated with statin-induced myopathy in humans. In cerebrovascular patients treated with statin, lower hArg and GAA plasma concentrations were found than in nonstatin patients, indicating suppressed AGAT expression and/or activity in muscle cells [56,57].

During acute exacerbation of CF in children, sputum hArg levels dropped significantly after antibiotic treatment [36▪]. This effect has not been further investigated.

Being the substrate of NOS and precursor of nitric oxide, supplementation of Arg was early considered as a possibility to increase nitric oxide production in conditions associated with diminished nitric oxide synthesis. With respect to pregnancy, L-arginine and L-citrulline supplementation is discussed as a potential strategy to improve birth outcomes in low-resource settings [58]. However, this discussion does not consider the possibility of supplementing hArg itself, presumably because of lack of solid evidence of biological activities.

Inorganic nitrate, which is the major metabolite of nitric oxide, is currently investigated as an ergogenic supplement [59]. Very recently, short-term supplementation of inorganic nitrate at a dose of 0.1 mmol/kg body weight/d for 9 days to young men resulted in elevation of hArg synthesis in the volunteers independent of physical exercise [60▪▪] (Table 1). The increase in plasma hArg concentration was of the same order of the contribution as the synthetic capacity of one kidney in healthy humans [41]. The hArg-induced effect of supplemented inorganic nitrate is unexpected, and the underlying mechanisms remain to be elucidated. They are unlikely to be dependent upon nitric oxide [61]. Very recently, hArg administration was shown to inhibit atherogenesis by modulating T-cell function [62].

EFFECTS OF HOMOARGININE SUPPLEMENTATION

The cognitive performance in healthy humans was investigated with respect to memory, learning and attention following supplementation with placebo or hArg supplementation (125 mg/day) for 4 weeks to healthy humans [63]. As the study did not reveal any effects of hArg on cognition, hArg supplementation is not expected to cause acute neurocognitive or behavioural side effects.

In mice, hArg supplementation for 12 weeks (50 mg/l in drinking water; subcutaneous infusion 0.72 mg/kg/day) was reported to prevent diabetic kidney damage [64]. Oral administration of hArg increased plasma hArg concentration and kidney content on hArg but did not change Arg concentration [64]. The hArg-ameliorated diabetic nephropathy was proposed to be independent of endothelial NOS [65].

CONCLUSION

hArg is the methylene homolog of Arg. hArg is a nonproteinogenic amino acid and occurs in its free form and concentrations in the range 0.5–2.0 μmol/l in the circulation and up to about 5 μmol/l in pregnancy (Table 1). Arg is a proteinogenic amino acid and occurs in its free form and concentrations

in the range 25–100 μmol/l in the circulation and up to several mmol/l-concentrations in total in proteins. In recent years, hArg received a revival of research in multiple disciplines. Scientists were guided by the plethora of knowledge on Arg gained over the past century. We learned a lot about hArg, in part expectable and in part unexpectable novel things. Recent research revealed low circulating and urinary hArg concentrations as an important risk factor for renal and cardiovascular disease in blood and urine in a relatively short period. Yet, hArg keeps many secrets. Is hArg a risk factor or solely a risk marker? What are the biological mechanisms that make hArg so pivotal for humans? Are we on the wrong track? Do we oversee something or did we not learn enough key properties of hArg? Does hArg act on its own or in cooperation with other factors such as ADMA? The physiological occurrence of peptides and proteins that contain hArg is underdetermined. Preparation of antibodies towards peptidic hArg would be useful in this topic. Is supplementation of hArg aiming to reach concentrations found in healthy humans the right strategy? Is inorganic nitrate supplementation risky to increase hArg biosynthesis because of the cancerogenic potential of nitrosating species that derive from nitrate? Are perhaps our expectations too high? Obviously, we need to learn much more about hArg. Homoarginine seems to be much more than the homo-arginine.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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- ■■ of outstanding interest

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