Homoarginine, Cardiovascular Risk, and Mortality

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Background—Homoarginine is an amino acid derivative that may increase nitric oxide availability and enhance endothelial function. The effect of the level of homoarginine on cardiovascular outcome and mortality is unknown.

Methods and Results—We assessed cardiovascular and all-cause mortality according to homoarginine levels in a cohort of 3305 subjects referred for coronary angiography from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study. After investigating the relation of homoarginine with kidney function and markers of endothelial dysfunction, we explored its effects on adverse outcomes in a second high-risk cohort of 1244 patients with type 2 diabetes mellitus receiving maintenance hemodialysis (4D study [Die Deutsche Diabetes Dialyse Studie]). In the LURIC study, mean serum homoarginine levels were 2.6±1.1 μmol/L. During a median follow-up of 7.7 years, 766 patients died. After adjustments for age and sex, patients in the lowest quartile (<1.85 μmol/L) had a >4-fold higher rate of dying of cardiovascular disease (hazard ratio 4.1, 95% confidence interval 3.0 to 5.7) than patients in the highest quartile (>3.1 μmol/L). Lower homoarginine levels were associated with lower estimated glomerular filtration rate and higher levels of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. Hemodialysed patients had lower mean homoarginine levels of 1.2±0.5 μmol/L and experienced a 5-fold increased mortality rate compared with LURIC patients (608 deaths during a median follow-up of 4 years). Homoarginine consistently affected mortality, which was 2-fold higher in 4D study patients in the lowest quartile (<0.87 μmol/L) than in patients in the highest quartile (>1.4 μmol/L).

Conclusions—Homoarginine levels are independently associated with cardiovascular and all-cause mortality in patients referred for coronary angiography and in patients undergoing hemodialysis. Future studies are needed to elucidate the underlying pathomechanisms. **(*Circulation*. 2010;122:967-975.)**

Key Words: amino acids ■ coronary disease ■ kidney ■ renal dialysis ■ survival

Because of advances in prevention and treatment, mortality due to cardiovascular disease declined substantially in westernized countries during the past 5 decades.[1] Despite highly effective measures to control conventional risk factors, many cardiovascular events still occur, which indicates that important pathways relevant to the development or progression of cardiovascular disease have not yet been recognized.[2] We investigated the hypothesis that homoarginine is involved in the generation of the key vasodilator nitric oxide (NO) and therefore may be directly related to vascular disease outcome.

Clinical Perspective on p 975

Homoarginine is a cationic amino acid that is derived from lysine. Homoarginine may increase the availability of NO[3,4] in 2 ways: First, homoarginine itself serves as a precursor of NO. Second, it potentially increases the intracellular concentration of L-arginine, the main substrate for NO synthase, by inhibiting the enzyme arginase, which competes with NO synthase for the key substrate L-arginine.[5–8] The significance of homoarginine to NO metabolism is not fully understood, but recent evidence suggests that homoarginine is positively related to endothelial function.[7] Homoarginine may exert further actions that are relevant to cardiovascular health, including inhibition of platelet aggregation and stimulation of insulin secretion.[9–11]

In view of these findings that link homoarginine to cardiovascular health, we examined whether the concentration of homoarginine in the plasma is associated with cardiovascular risk factors and mortality. We investigated individuals from 2 follow-up cohorts with intermediate to high global risk of

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adverse cardiovascular outcomes, namely, more than 3000 patients referred for coronary angiography[12,13] and more than 1200 high-risk patients with type 2 diabetes mellitus undergoing maintenance hemodialysis.[14,15] Our results establish for the first time an important role of homoarginine as an independent risk marker of adverse cardiovascular outcomes.

Methods

Study Design and Participants

The LUdwigshafen RIsk and Cardiovascular Health (LURIC) study is a prospective cohort study designed to investigate environmental and genetic risk factors for cardiovascular diseases.[13] Patients of German ancestry who were clinically stable except for acute coronary syndromes and who were referred for coronary angiography were enrolled between 1997 and 2000. Individuals with acute illness other than acute coronary syndromes, chronic noncardiac diseases, or malignancy within the previous 5 years were excluded. During a continuous follow-up, information on vital status was obtained from local registries. Death certificates and medical records of local hospitals and autopsy data were reviewed independently by 3 experienced clinicians who were blinded to patient characteristics and who classified the causes of death.

The 4D study (Die Deutsche Diabetes Dialyse Studie) was a prospective randomized controlled trial that included 1255 patients with type 2 diabetes mellitus, 18 to 80 years of age, who had been treated by hemodialysis for less than 2 years.[14] Between March 1998 and October 2002, patients were recruited in 178 dialysis centers in Germany. Patients were randomly assigned to double-blinded treatment with either 20 mg of atorvastatin (n=619) or placebo (n=636) once daily and were followed up until the date of death, censoring, or end of the study in March 2004. The primary end point of the 4D study was defined as a composite of death due to cardiac causes, stroke, and myocardial infarction, whichever occurred first. 4D study end points were centrally adjudicated by 3 members of the endpoints committee blinded to study treatment and according to predefined criteria.[15] In both studies, cardiovascular deaths included sudden cardiac death, fatal myocardial infarction, death due to congestive heart failure, death after intervention to treat coronary artery disease, other deaths due to cardiac causes, and fatal stroke. The studies were approved by the medical ethics committees, and written informed consent was obtained from all participants.

Data Collection

Information on age, sex, and smoking status was obtained through patient interviews. Smoking status was classified as never or former versus current. Comorbidities were reported by the patients' treating physicians. Blood pressure was measured with the patient seated. Hypertension was diagnosed if the systolic or diastolic blood pressure exceeded 140 or 90 mm Hg, respectively, or if a history of hypertension was evidenced by the use of antihypertensive drugs.

Blood samples were taken with patients in the fasting state before coronary angiography in LURIC patients and before the start of dialysis sessions in 4D patients. In both studies, homoarginine was uniformly measured in serum stored at −80°C by use of a reverse-phase high-performance liquid chromatography method.[16,17] Intraday coefficients of variation (CVs) at different concentrations (mean levels) were 4.7% (1.21 μmol/L) and 2.2% (3.53 μmol/L), and between-day CVs were 7.9% (1.25 μmol/L) and 6.8% (3.66 μmol/L), respectively. C-reactive protein was measured with high-sensitivity immunonephelometry (Dade Behring, Marburg, Germany), with an intra-assay and interassay CV of 2.3% to 4.4% and 2.6% to 5.7%, respectively. Further biomarkers evaluated in the LURIC study included serum lysine, arginine, ornithine, and asymmetrical dimethylarginine (ADMA; all measured by high-performance liquid chromatography) and fibrinogen (Clauss method; Dade Behring). Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were measured by enzyme immunoassays (R&D Systems, Wiesbaden, Germany). Intra-assay and interassay CVs were 3.3% to 4.8% and 6.0% to 10.1%, respectively, for intercellular adhesion molecule-1 and 4.3% to 5.9% and 8.5% to 10.2%, respectively, for vascular cell adhesion molecule-1. For ADMA measurements, intraday CVs were 1.0% to 3.1%, and between-day CVs were 1.5% to 9%. Fibrinogen measurements had intra-assay CVs <5%.

Statistical Analysis

Continuous variables were expressed as mean with SD (for normally distributed variables) or median with interquartile range (for skewed variables). Variables that deviated significantly from a normal distribution were $\log_{10}$ transformed before use in parametric procedures. Categorical variables were expressed as percentages.

We explored associations of homoarginine with kidney function, biomarkers of endothelial dysfunction (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), fibrinogen, and D-dimers, because these hypothetically might be related to NO metabolism. Similarly, we examined the relationship of homoarginine with alkaline phosphatase, which was previously shown to be inhibited by homoarginine. In addition, we performed correlation analyses of homoarginine with parameters of arginine metabolism including arginine, ADMA, and the arginine-to-ornithine ratio. Pearson correlation coefficients were assessed in the entire LURIC cohort. Furthermore, we performed linear regression analyses that were adjusted for common cardiovascular risk factors, L-arginine, and ADMA. These latter analyses were stratified by sex to account for potential effect modification.

We assessed the association of homoarginine with cardiovascular and all-cause death in the LURIC study using homoarginine as a continuous variable (logarithmically transformed) and as a categorical variable according to quartiles. We calculated absolute (incidence) rates and hazard ratios (HRs) derived from Cox regression analyses. Multivariable adjustments were made for confounders including age and sex (model 1), as well as for these confounders plus smoking status, high-density lipoprotein and low-density lipoprotein cholesterol, hemoglobin, and kidney function (model 2, main analyses). Additional adjustments were made for coronary artery disease, congestive heart failure, glycohemoglobin A1c, systolic blood pressure, body mass index, albumin, and C-reactive protein, which may also represent intermediate variables (model 3). We did not adjust for other amino acids or ADMA because these factors either may lie in the causal pathway of homoarginine deficiency or are only modestly associated with homoarginine levels.

We investigated the association of homoarginine with cardiovascular and all-cause mortality in 4D participants, applying the same methods and outcome criteria as were used for the LURIC population. Finally, we evaluated and compared the absolute incidence rates of mortality (deaths per 100 person-years) in both cohorts according to homoarginine status. Frequencies of patients stratified by homoarginine levels (starting from those with lowest levels <0.5 μmol/L, with stepwise increases by 0.5 μmol/L) were assessed together with the respective mortality rates in each group. All *P* values are reported as 2-sided. Analyses were performed with SPSS version 16.0 (SPSS Inc, Chicago, Ill) and MedCalc version 11.3.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Homoarginine Status in Persons Undergoing Coronary Angiography (LURIC Study)

The characteristics of the 3305 study participants in whom homoarginine levels at baseline were available (99.7% of the entire study cohort) are shown in Table 1. The mean homoarginine level was 2.6±1.1 μmol/L and was significantly higher in men (2.7±1.1 μmol/L) than in women (2.3±1.0 μmol/L, $P<0.001$ by Student *t* test). When patients were divided into quartiles according to homoarginine levels, those with the lowest levels (first quartile) were older and had lower levels of albumin, lower body mass index, a lower estimated glomerular filtration rate, and a higher percentage

Table 1. Baseline Characteristics of Individuals Participating in the LURIC Study (Patients Referred for Coronary Angiography) and the 4D Study (Type 2 Diabetics on Hemodialysis)

Characteristic	Cardiac-Risk Patients (LURIC, n=3305)	Hemodialysis Patients (4D, n=1244)
Age, y	62.7 (10.6)	65.7 (8.3)
Sex, % male	70	54
Arterial hypertension, %	73	89
Systolic BP, mm Hg	141 (24)	146 (22)
Diastolic BP, mm Hg	81 (11)	76 (11)
Smoker/ex-smoker, %	64	41
BMI, kg/m^2	27.5 (4.1)	27.5 (4.8)
Diabetes mellitus, %	32	100
Coronary artery disease, %	78	29
Congestive heart failure,* %	48	35
Laboratory parameters		
Homoarginine, μmol/L	2.6 (1.1)	1.2 (0.5)
LDL cholesterol, mmol/L	3.0 (0.9)	3.3 (0.8)
HDL cholesterol, mmol/L	1.0 (0.3)	0.9 (0.3)
Albumin, g/L	44 (5)	38 (3)
Hemoglobin, mmol/L	8.6 (0.9)	6.8 (0.8)
HbA1c, %	6.3 (1.2)	6.7 (1.3)
C-reactive protein, mg/L	4.0 (1.0–10.1)	5.0 (2.3–12.4)
ADMA, μmol/L	0.8 (0.7–0.9)	0.9 (0.8–1.0)
L-Arginine, μmol/L	81 (20)	N/A
Ornithine, μmol/L	58 (17)	N/A
L-Arginine/ornithine ratio	1.47 (0.42)	N/A
L-Lysine	204 (42)	N/A
eGFR, mL · min^{-1} · 1.73 m^{-2}	81 (19)	†

BP indicates blood pressure; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, glycohemoglobin A1c; N/A, data not available; and eGFR, estimated glomerular filtration rate according to the MDRD (modification of diet in renal disease) study formula to estimate GFR.

Values are presented as mean (SD), median (interquartile range), or percentage.

*Congestive heart failure as defined by New York Heart Association class II to IV.

†Glomerular filtration rate <15 mL/min as defined by the need for maintenance dialysis.

of congestive heart failure than patients in the highest homoarginine quartile.

Associations of Homoarginine With Kidney Function, Biomarkers of Vascular Damage, and Arginine Metabolism (LURIC Study)

By correlation analyses, homoarginine levels were significantly associated with estimated glomerular filtration rate (r=0.23, P<0.001) and inversely with fibrinogen (r=−0.25, P<0.001), D-dimers (r=−0.28, P<0.001), and alkaline phosphatase (r=−0.22, P<0.001). Furthermore, homoarginine levels showed significant negative associations with the endothelial adhesion molecules intercellular adhesion molecule-1 (r=−0.16, P<0.001) and vascular cell adhesion molecule-1 (r=−0.23, P<0.001; values available in 1945 study subjects). In addition, a positive association was seen between serum homoarginine and lysine concentrations (r=0.34, P<0.001; values available in 2236 study subjects). There was a significant correlation between homoarginine and arginine levels (r=0.325, P<0.001). The ratio of arginine to ornithine, which is considered an indirect measure of arginase activity and relative arginine bioavailability,[18] was significantly correlated with homoarginine concentrations (r=0.326, P<0.001). Serum levels of ADMA, an inhibitor of NO synthase that is associated with increased cardiovascular risk, were not significantly correlated with homoarginine concentrations (r=−0.031, P=0.077). By additional linear regression analyses, the strong univariate associations persisted after adjustment for common cardiovascular risk factors and stratification by sex (Table 2).

Table 2. Sex-Stratified Regression Analyses of Homoarginine With Baseline Characteristics of the LURIC Cohort

	Men		Women	
Parameter	Unadjusted*	Adjusted†	Unadjusted*	Adjusted†
Age, y	−0.14‡	−0.01	−0.23‡	−0.17‡
Systolic BP, mm Hg	0.06§	0.07	0.05	0.06
Diastolic BP, mm Hg	0.10‡	−0.03	0.10§	−0.04
BMI, kg/m^2	0.19‡	0.19‡	0.13‡	0.18‡
LDL cholesterol, mmol/L	0.04	−0.01	0.01	−0.05
HDL cholesterol, mmol/L	0.07§	−0.04	0.14‡	0.07
Albumin, g/L	0.14‡	0.00	0.11§	−0.03
Hemoglobin, mmol/L	0.27‡	0.16‡	0.21‡	0.17‡
HbA1c, %	−0.04	−0.03	−0.05	−0.03
C-reactive protein, mg/L	−0.27‡	−0.20‡	−0.15‡	−0.06
eGFR, mL · min^{-1} · 1.73 m^{-2}	0.15‡	0.11‡	0.24‡	0.12§
Arginine, μmol/L	0.33‡	0.31‡	0.24‡	0.30‡
ADMA, μmol/L	0.04	0.01	−0.13‡	−0.15§

BP indicates blood pressure; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, glycohemoglobin A1c; and eGFR, estimated glomerular filtration rate according to the MDRD (modification of diet in renal disease) study formula to estimate GFR.

β-Coefficients for regression analyses with homoarginine as the outcome variable and all other parameters of this table as explanatory variables; adjusted R^2 for the entire model was 0.25 for males and 0.23 for females.

Homoarginine, hemoglobin A1c, C-reactive protein, and ADMA were logarithmically transformed (log 10) for regression analyses.

*Unadjusted model.

†Model adjusted for age and sex.

‡P<0.001; §P<0.01; all other P>0.05.

Homoarginine Levels and Mortality in Persons Undergoing Angiography (LURIC Study)

After a median follow-up of 7.7 years, 766 patients had died, including 482 deaths due to cardiovascular causes. The absolute incidence rates were 3.3/100 person-years and 2.1/100 person-years, respectively (Table 3). For 24 deceased individuals, we did not obtain sufficient data to classify their causes of death, and these individuals were excluded from analyses for cardiovascular mortality but were included in the analyses for all-cause mortality.

Table 3. Absolute Rates of All-Cause Mortality and Cardiovascular Mortality in Participants in the LURIC and 4D Studies, Overall and Stratified by Quartiles of Homoarginine at Baseline

Characteristic	Cardiac-Risk Patients (LURIC; n=3305)	Hemodialysis Patients (4D; n=1244)
All-cause mortality		
Events	766	608
Person-years	23 348	3558
Incidence rate/100 person-years (95% CI)	3.3 (3.1–3.5)	17.3 (15.8–18.5)
Homoarginine quartile 1	5.9 (5.3–6.6)	23.4 (20.9–26.1)
Homoarginine quartile 2	3.5 (3.0–4.0)	20.8 (17.8–24.1)
Homoarginine quartile 3	2.3 (1.9–2.7)	14.3 (11.9–17.0)
Homoarginine quartile 4	1.8 (1.5–2.2)	11.4 (9.4–13.7)
Cardiovascular mortality*		
Events	482	307
Person-years	23 348	3558
Incidence rate/100 person-years (95% CI)	2.1 (1.9–2.3)	8.6 (7.7–9.7)
Homoarginine quartile 1	3.9 (3.4–4.5)	11.2 (9.0–13.7)
Homoarginine quartile 2	2.3 (1.9–2.7)	11.4 (9.2–13.9)
Homoarginine quartile 3	1.4 (1.1–1.7)	7.5 (5.8–9.5)
Homoarginine quartile 4	0.9 (0.7–1.2)	5.3 (4.0–7.0)

*Cardiovascular mortality included sudden cardiac death, fatal myocardial infarction, death due to congestive heart failure, death after intervention to treat coronary artery disease, fatal stroke, and other deaths due to cardiac causes.

By Cox regression analyses, decreasing homoarginine levels were associated with an increased rate of cardiovascular death (HR 3.1 per unit decrease in log-transformed homoarginine values, 95% confidence interval [CI] 2.3 to 4.1) and all-cause death (HR 2.6, 95% CI 2.1 to 3.1). Patients in the lowest homoarginine quartile (<1.85 μmol/L) had a >4-fold higher rate of cardiovascular death (HR 4.5, 95% CI 3.2 to 6.2) and a >3-fold increased rate of death due to any cause (HR 3.3, 95% CI 2.6 to 4.2) than patients in the highest quartile (>3.1 μmol/L; Table 4; Figure 1). The associations remained significant after adjustments for age and sex (HR for cardiovascular death 4.1, 95% CI 3.0 to 5.7; HR for death due to any cause 3.0, 95% CI 2.3 to 3.8, respectively; model 1) and further potential confounders (HR for cardiovascular death 3.6, 95% CI 2.6 to 5.1; HR for death due to any cause 2.7, 95% CI 2.1 to 3.4, respectively; model 2, main analyses). The additional adjustment for potential intermediate variables did not materially decrease the effects, which suggests the presence of additional pathways by which homoarginine increases the risk of adverse outcome. For all analyses, the results were not materially different when men and women were analyzed separately or when cystatin C was used instead of estimated glomerular filtration rate.

Homoarginine Levels and Mortality in Hemodialysis Patients (4D Study)

We extended the findings in the LURIC study with investigations of homoarginine in 1244 patients with type 2 diabetes mellitus who were undergoing maintenance hemodialysis (99.1% of the 4D study cohort). The baseline characteristics of these patients are shown in Table 1. As expected, homoarginine levels were lower in the 4D study population, with a mean of 1.2±0.5 μmol/L. Homoarginine levels were higher in men than in women and were lower with increasing age. Low homoarginine levels correlated with low albumin, low body mass index, low levels of low-density lipoprotein cholesterol, presence of congestive heart failure, and longer duration of type 2 diabetes mellitus. During a median follow-up of 4 years, 608 patients died, of whom 307 died of cardiovascular causes. Compared with subjects in the LURIC study, patients in the 4D study had a 5-fold higher mortality rate (17.3 per 100 person-years; Table 3; Figure 1). Low homoarginine levels were strongly associated with increased mortality: The relative rates of cardiovascular and all-cause mortality were more than doubled (120% increase) per unit decrease in log-transformed homoarginine (HR for cardiovascular death 2.2, 95% CI 1.7 to 3.0; HR for death due to all causes 2.2, 95% CI 1.8 to 2.7). Accordingly, in patients in the lowest homoarginine quartile (<0.87 μmol/L), mortality was >2 fold higher than in patients in the highest quartile (>1.4 μmol/L). Such strong associations persisted after multivariable adjustments (Table 4). To strengthen our results, we eliminated any potential influence by atorvastatin treatment and repeated all analyses stratified by intervention. The results were similar in the placebo and atorvastatin groups, which indicates no interaction and supports the use of the complete data.

Finally, when assessing the absolute mortality rates in more detailed ranges of homoarginine, we found an incrementally increasing risk with lower homoarginine levels in both cohorts, with only a relatively small gap relative to the different clinical background of the 2 populations (Figure 2). Linearity assumptions for all Cox regression analyses were tested by log-minus-log survival plots and partial (Schoenfeld) residuals versus survival time plots and were found to be valid.

Discussion

This prospective study investigated the effect of homoarginine on cardiovascular outcome and mortality in 2 separate large cohorts of 3305 subjects referred for coronary angiography (LURIC study) and 1244 diabetic patients undergoing maintenance hemodialysis (4D study). In persons undergoing angiography, low homoarginine levels were associated with 3.6-fold higher cardiovascular mortality and 2.7-fold higher all-cause mortality after adjustment for potential confounders. Homoarginine levels were decreased markedly in patients with low estimated glomerular filtration rate and were inversely related to adhesion molecules, which potentially reflects the magnitude of endothelial dysfunction. The strong associations of low homoarginine with adverse outcomes were independently confirmed in the patients undergoing hemodialysis.

Homoarginine levels were significantly lower in hemodialysis patients (4D study), who overall had a 5-fold higher mortality rate than patients referred for coronary angiography (LURIC study). In the dialysis patients, a similar association

Table 4. HRs (95% CIs) for All-Cause and Cardiovascular Mortality According to Quartiles of Serum Homoarginine Concentrations at Baseline in Participants in the LURIC and 4D Studies

		Cardiac-Risk Patients (LURIC; n=3305)		Hemodialysis Patients (4D; n=1244)	
Model	Quartile*	HR (95% CI)	*P*	HR (95% CI)	*P*
All-cause mortality					
Crude	1	3.3 (2.6–4.2)	<0.001	2.1 (1.7–2.7)	<0.001
	2	1.9 (1.4–2.4)	<0.001	1.9 (1.5–2.4)	<0.001
	3	1.2 (0.9–1.6)	0.13	1.3 (1.0–1.6)	0.07
	4	1	…	1	…
Adjusted†	1	3.0 (2.3–3.8)	<0.001	2.0 (1.6–2.6)	<0.001
	2	1.7 (1.3–2.2)	<0.001	1.8 (1.4–2.3)	<0.001
	3	1.2 (0.9–1.6)	0.19	1.2 (1.0–1.6)	0.11
	4	1	…	1	…
Adjusted‡	1	2.7 (2.1–3.4)	<0.001	1.9 (1.5–2.4)	<0.001
	2	1.7 (1.3–2.1)	<0.001	1.8 (1.4–2.2)	<0.001
	3	1.2 (0.9–1.6)	0.22	1.2 (0.9–1.6)	0.16
	4	1		1	
Adjusted§	1	2.4 (1.9–3.1)	<0.001	1.6 (1.2–2.0)	<0.001
	2	1.5 (1.2–2.0)	<0.001	1.6 (1.3–2.1)	<0.001
	3	1.2 (0.9–1.6)	0.20	1.2 (0.9–1.6)	0.15
	4	1	…	1	…
Cardiovascular mortality‖					
Crude	1	4.5 (3.2–6.2)	<0.001	2.2 (1.5–3.0)	<0.001
	2	2.4 (1.7–3.4)	<0.001	2.2 (1.5–3.0)	<0.001
	3	1.6 (1.1–2.3)	0.01	1.4 (1.0–2.1)	0.05
	4	1	…	1	…
Adjusted†	1	4.1 (3.0–5.7)	<0.001	2.0 (1.4–2.9)	<0.001
	2	2.2 (1.6–3.2)	<0.001	2.1 (1.5–3.0)	<0.001
	3	1.6 (1.1–2.2)	0.02	1.4 (1.0–2.0)	0.08
	4	1	…	1	…
Adjusted‡	1	3.6 (2.6–5.1)	<0.001	1.9 (1.3–2.8)	<0.001
	2	2.2 (1.6–3.1)	<0.001	2.1 (1.5–2.9)	<0.001
	3	1.5 (1.1–2.2)	0.02	1.4 (0.9–2.0)	0.10
	4	1	…	1	…
Adjusted§	1	3.3 (2.4–4.7)	<0.001	1.7 (1.1–2.4)	0.007
	2	2.1 (1.5–2.9)	<0.001	1.9 (1.4–2.8)	<0.001
	3	1.6 (1.1–2.3)	0.02	1.4 (1.0–2.0)	0.08
	4	1	…	1	…

*Quartiles of homoarginine levels at baseline were <1.85 (quartile 1), 1.85–2.42 (quartile 2), 2.43–3.10 (quartile 3), and >3.10 (quartile 4) in the LURIC study and <0.87 (quartile 1), 0.87–1.10 (quartile 2), 1.10–1.40 (quartile 3), and >1.40 (quartile 4) in the 4D study.

†Adjustments were made for age and sex.

‡Additional adjustments apart from age and sex were made for further potential confounders: Smoking status, low-density lipoprotein and high-density lipoprotein cholesterol, hemoglobin, and status of kidney function (eGFR in the LURIC cohort and duration of hemodialysis in the 4D cohort).

§Adjustments were made for all variables of the adjusted model 2 and additionally for factors that may represent intermediate variables: Coronary artery disease, congestive heart failure, glycohemoglobin A1c, systolic blood pressure, body mass index, albumin, and C-reactive protein.

‖Cardiovascular mortality included sudden cardiac death, fatal myocardial infarction, death due to congestive heart failure, death after intervention to treat coronary artery disease, fatal stroke and other deaths due to cardiac causes.

The numbers of patients at risk and the numbers of events according to quartiles of homoarginine were as follows: in the LURIC study, quartile 1—828 patients at risk, 309 deaths (207 of which were cardiovascular); quartile 2—839 patients at risk, 204 deaths (133 of which were cardiovascular); quartile 3—819 patients at risk, 140 deaths (87 of which were cardiovascular); and quartile 4—819 patients at risk, 113 deaths (55 of which were cardiovascular); in the 4D study, quartile 1—326 patients at risk, 195 deaths (93 of which were cardiovascular); quartile 2—308 patients at risk, 174 deaths (95 of which were cardiovascular); quartile 3—304 patients at risk, 129 deaths (68 of which were cardiovascular); and quartile 4—306 patients at risk, 110 deaths (51 of which were cardiovascular).

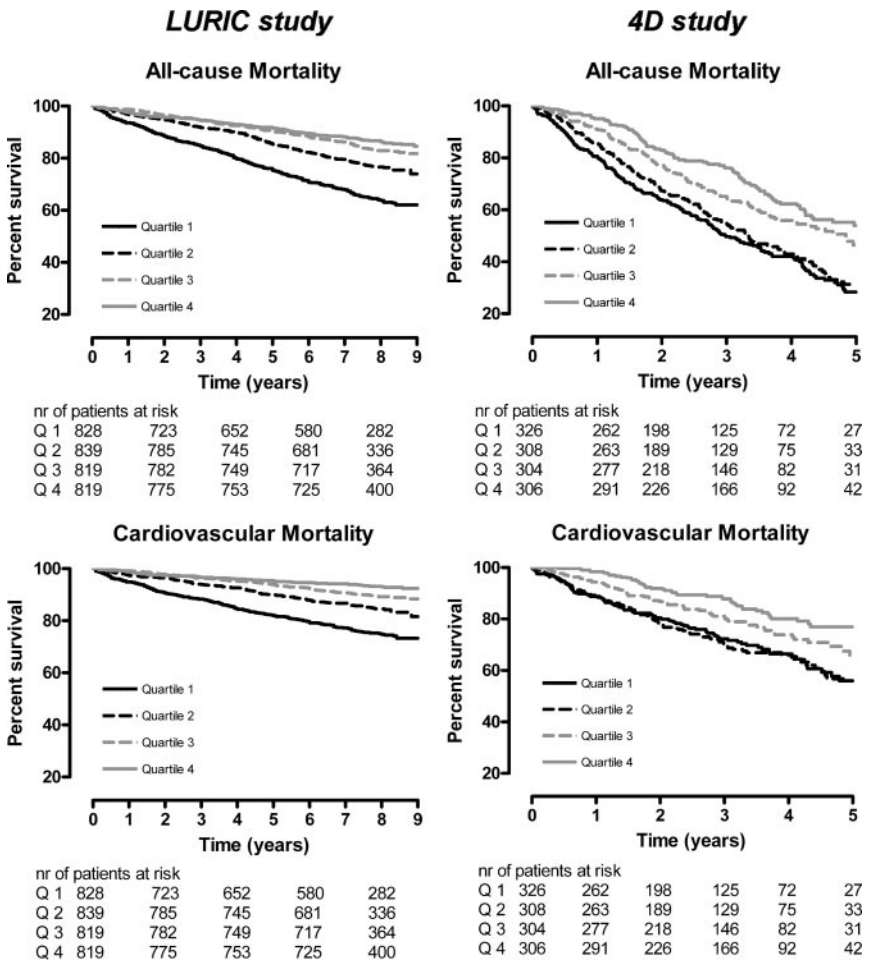

Figure 1. Kaplan–Meier curves for all-cause mortality and cardiovascular mortality according to homoarginine quartiles in the LURIC Study and the 4D study. Q1 indicates quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; and nr, number.

between homoarginine quartiles and mortality was seen, which confirms the ability of homoarginine to indicate the risk of adverse outcomes across a broad range of concentrations (Figure 2). Intriguingly, the graded relationship between decreasing homoarginine and the incidence rates of death extends from the highest concentrations in persons undergoing angiography to the lowest concentrations in hemodialysis patients, with only a small gap accounting for the different clinical background of the 2 cohorts.

Identification of novel cardiovascular risk factors is crucial to further improve diagnosis and treatment of patients who have cardiovascular diseases.[2] The present results, which indicate a strong adjusted association of homoarginine with cardiovascular outcome in 2 independent patient cohorts, suggest homoarginine as a potential new risk factor, with various pathomechanisms being of particular interest.

Evidence suggests that homoarginine may increase NO availability, the lack of which is associated with endothelial and myocardial dysfunction.[5–8] We found an inverse association between homoarginine and markers of impaired endothelial function (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), and we observed lower levels of homoarginine in patients with heart failure than in those without heart failure. In this context, it is of interest that the highest serum levels of homoarginine are found in states of enhanced vasodilation and increased NO production, such as in pregnant women (4.8 ± 1.7 μmol/L in the second trimester and 5.3 ± 1.5 μmol/L in the third trimester).[7] The concentration of L-homoarginine was found to be correlated significantly with gestational age, brachial artery diameter, and endothelium-dependent brachial artery flow-mediated dilation.[7]

We further observed homoarginine to be inversely related to fibrinogen and D-dimers, which is in line with previous studies that found that homoarginine inhibited platelet aggregation. These results support the hypothesis that homoarginine may exert antithrombotic effects and thereby enhance NO availability.[11,19]

We found a significant correlation of homoarginine with lysine, which is consistent with the notion that homoarginine is formed from lysine. Lysine is an inhibitor of arginase, which competes with NO synthase for the substrate L-arginine.[20] Given the structural similarities between lysine and homoarginine, it is likely that homoarginine inhibits arginase, thus increasing the availability of L-arginine for NO production by NO synthase (Figure 3).[5,20] This latter hypothesis appears to be supported by the observed correlation of homoarginine with an increased arginine-to-ornithine ratio, which is considered an indicator of low arginase activity.[18] However, homoarginine is a less potent inhibitor of arginase than ornithine. In further exploratory analyses, arginine was not strikingly associated with mortality (age- and sex-

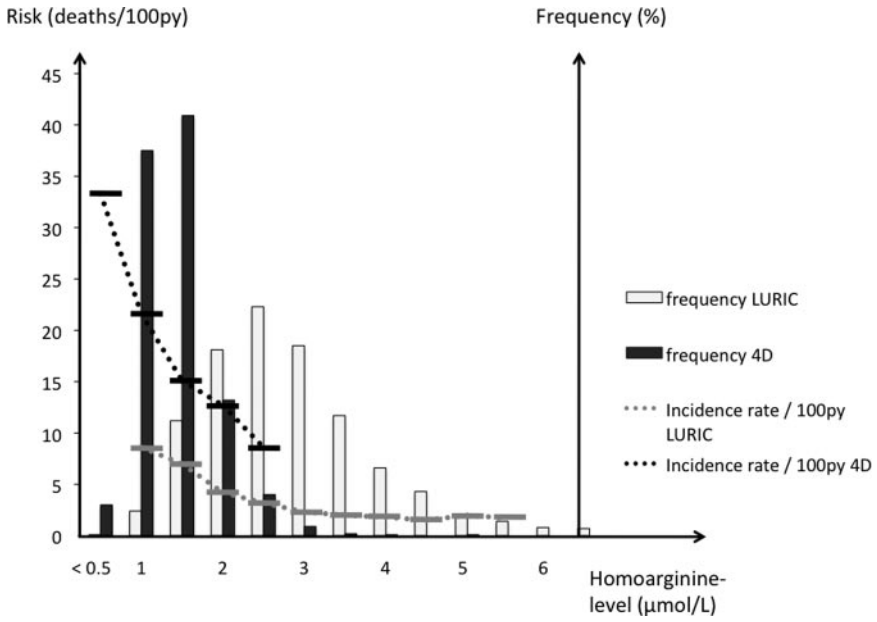

Figure 2. Relationship between the incidence rate of death and homoarginine. py Indicates person-years.

adjusted HR for death due to all causes in the first versus the fourth arginine quartile 1.3, 95% CI 1.0 to 1.6), which suggests that the homoarginine effect is not driven mainly by its potential impact on arginine. Because homoarginine itself is a less efficient substrate for NO synthase than arginine, further in-depth studies are warranted to elucidate the role of homoarginine in adverse outcomes in the context of regulations in arginine metabolism and NO synthesis.

Even though homoarginine is excreted by the kidney, we found lower homoarginine levels in persons with impaired kidney function. This may be due to the fact that the kidney is one of the sites for the transaminidation of L-lysine to homoarginine.[21,22] In the LURIC study, homoarginine consistently was found to be low in patients with a low estimated glomerular filtration rate,[23] and hemodialysis patients (4D study) had even lower concentrations. Because homoarginine is synthesized from the amino acid lysine and is suggested to indicate intestinal absorption of exogenous amino acids,[24] it is likely that homoarginine plays a role in the nutritional status of patients. It is thus of particular interest that wasting, a

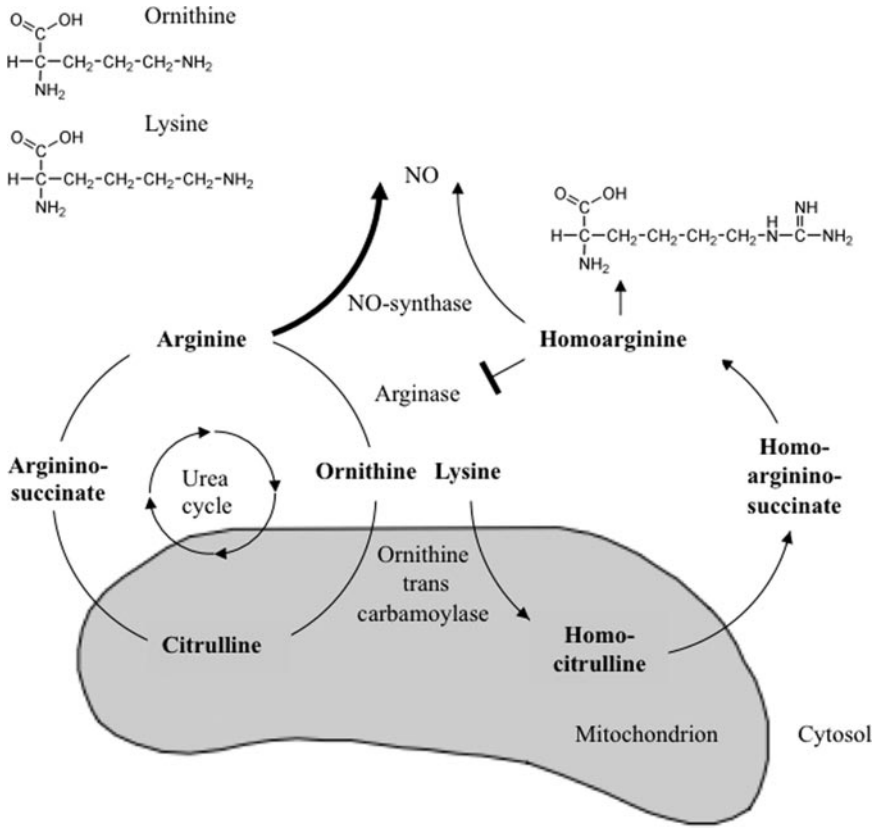

Figure 3. Putative metabolic pathways and effects of homoarginine. Homoarginine is formed from lysine through reactions homologous to those of the urea cycle, in which ornithine is replaced by lysine. The key enzyme for homoarginine synthesis is ornithine-transcarbamoylase. This enzyme has a higher affinity to ornithine but due to low substrate selectivity, it also catalyzes the transaminidation reaction of lysine, thereby initiating homoarginine production. Homoarginine itself serves as a substrate for NO synthase. Additionally, homoarginine may inhibit arginase, which catalyzes the reaction of L-arginine and H_2O to ornithine and urea. Inhibition of arginase by homoarginine may thus increase the availability of L-arginine for NO production by NO synthase.

complex process involving muscle loss, poor food intake, and inflammation, frequently develops in patients with chronic heart and kidney disease.[25,26] It is conceivable that the low homoarginine levels seen in patients with impaired renal function may contribute to malnutrition and wasting. Indeed, indicators of malnutrition and inflammation, including body mass index, albumin, and C-reactive protein, were associated with low homoarginine in both patient groups, which parallels previous observations made with regard to arginine.[27] The association between low homoarginine and adverse outcomes, however, was not changed materially by multivariate adjustments that included markers of nutrition and inflammation. This suggests the existence of further mechanisms that would explain the increased mortality of persons with low homoarginine levels.

Homoarginine may affect blood pressure and insulin secretion. Previous studies found that administration of L-homoarginine increased urinary excretion of nitrate, the degradation product of NO, and reduced blood pressure in salt-sensitive hypertensive rats.[4] Although in the present study, the prevalence of hypertension was similar across homoarginine quartiles, this may have been the result of the use of antihypertensive medication. Furthermore, homoarginine was found to stimulate insulin secretion,[9,10] and despite similar hemoglobin A1c levels across homoarginine quartiles, an inverse correlation was seen between the duration of diabetes mellitus and homoarginine concentrations in patients in the 4D study. Finally, previous experiments showed that homoarginine is an organ-specific noncompetitive inhibitor of human liver and bone alkaline phosphohydrolases.[28,29] Whether these effects of homoarginine are physiologically relevant for bone metabolism, for example, remains to be further elucidated.

The results of the present study also have implications for laboratory methodology for ADMA measurements, for which homoarginine has previously been used as an internal standard.[16] The present finding that homoarginine levels are related to mortality and cardiovascular risk cautions against the use of homoarginine as an internal standard.[16]

The present study has potential limitations. Despite the adjustments that were made in the associations of homoarginine with adverse outcomes, residual confounding (eg, by impaired renal function) cannot be completely ruled out; however, because the known important confounders were considered, the effect of potential residual confounding is likely to be small. The present study included patients with chronic diseases; the results, therefore, may not be generalizable. Although we found a consistent relationship of homoarginine with death even in persons without significant angiographic coronary artery disease (LURIC, data not shown), it would be interesting to reproduce our findings in as-yet asymptomatic individuals. Despite these promising findings from the LURIC and 4D studies, fundamental and challenging questions remain for mitochondrial metabolism, aging, protein energy regulation, and further pathophysiological effects of homoarginine.

The major strengths of the present study are the uniform protocols and the validation of homoarginine in 2 large patient cohorts by the application of identical methods for all measurements and analyses. Both patient cohorts had long-term follow-up; the sample sizes were adequate; and the end points were centrally adjudicated. The 2 cohorts, covering patients of unequal disease states, enabled us to demonstrate a consistent, strong impact of homoarginine on mortality within 2 entirely different ranges of concentration.

In conclusion, the serum level of homoarginine is a strong risk factor for cardiovascular and all-cause mortality in patients undergoing coronary angiography and in patients undergoing maintenance hemodialysis. Further studies are needed to elucidate the underlying mechanisms.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Evidence suggests that homoarginine, a cationic acid formed from lysine, may impact nitric oxide metabolism, endothelial function, and insulin secretion, all of which are relevant for the maintenance of cardiovascular health. In the present study, the association of circulating homoarginine concentrations with cardiovascular outcomes and with mortality was assessed in 2 separate large cohorts: 3305 subjects referred for coronary angiography (LUdwigshafen RIsk and Cardiovascular Health [LURIC] study) and 1244 diabetic patients undergoing maintenance hemodialysis (Die Deutsche Diabetes Dialyse Studie [4D] study). Low blood homoarginine concentration was strongly associated with death in both cohorts. In the LURIC population, persons with values of blood homoarginine in the lowest quartile had a more than 3-fold higher cardiovascular mortality than those with levels in the highest quartile after adjustment for other known vascular risk factors. Circulating homoarginine levels were markedly lower in hemodialysis patients (4D population), who overall had a 5-fold greater mortality than patients referred for coronary angiography. In the dialysis patients, a similar association between lower homoarginine quartiles and greater mortality was seen. With the 2 independent cohorts, which evaluated a diverse spectrum of patients, the present study demonstrates a consistent, strong association of homoarginine with cardiovascular and all-cause mortality and suggests important new avenues for future research.

Homoarginine, Cardiovascular Risk, and Mortality

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