

## Low Homoarginine Levels in the Prognosis of Patients With Acute Chest Pain

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**Background**—The endogenous amino acid homoarginine predicts mortality in cerebro- and cardiovascular disease. The objective was to explore whether homoarginine is associated with atrial fibrillation (AF) and outcome in patients with acute chest pain.

**Methods and Results**—One thousand six hundred forty-nine patients with acute chest pain were consecutively enrolled in this study, of whom 589 were diagnosed acute coronary syndrome (ACS). On admission, plasma concentrations of homoarginine as well as brain natriuretic peptide (BNP), and high-sensitivity assayed troponin I (hsTnI) were determined along with electrocardiography (ECG) variables. During a median follow-up of 183 days, 60 major adverse cardiovascular events (MACEs; 3.8%), including all-cause death, myocardial infarction, or stroke, were registered in the overall study population and 43 MACEs (7.5%) in the ACS subgroup. Adjusted multivariable Cox regression analyses revealed that an increase of 1 SD of plasma log-transformed homoarginine (0.37) was associated with a hazard reduction of 26% (hazard ratio [HR], 0.74; 95% CI, 0.57–0.96) for incident MACE and likewise of 35% (HR, 0.65; 95% CI, 0.49–0.88) in ACS patients. In Kaplan–Meier survival curves, homoarginine was predictive for patients with high-sensitivity assayed troponin I (hsTnI) above 27 ng/L ( $P<0.05$ ). Last, homoarginine was inversely associated with QTc duration ( $P<0.001$ ) and prevalent AF (OR, 0.83; 95% CI, 0.71–0.95).

**Conclusion**—Low plasma homoarginine was identified as a risk marker for incident MACEs in patients with acute chest pain, in particular, in those with elevated hsTnI. Impaired homoarginine was associated with prevalent AF. Further studies are needed to investigate the link to AF and evaluate homoarginine as a therapeutic option for these patients. (*J Am Heart Assoc.* 2016;5:e002565 doi: 10.1161/JAHA.115.002565)

**Key Words:** acute coronary syndrome • atrial fibrillation • homoarginine • L-arginine:glycine amidinotransferase

During the last 5 years, several studies indicated that low concentrations of the naturally occurring amino acid homoarginine predict a risk of adverse cardio- and cerebrovascular outcome and of mortality (reviewed in previous works<sup>1,2</sup>). Analyses of the associated clinical phenotypes, as well as experimental studies in mice, have proven a mechanistic link of impaired homoarginine and cerebrovascular

disease.<sup>3</sup> Synthesis of homoarginine in humans is catalyzed by the enzyme, L-arginine:glycine amidinotransferase (AGAT, EC 2.1.4.1), which is also known as the first and rate-limiting enzyme of creatine synthesis.<sup>1</sup> Because of a structural similarity with L-arginine, homoarginine is supposed to interfere with the nitric oxide (NO) pathway. Homoarginine is a weak alternative NO synthase substrate, competes with L-

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Accompanying Table S1 and Figure S1 are available at <http://jaha.ahajournals.org/content/5/4/e002565/DC1/embed/inline-supplementary-material-1.pdf>

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Received August 18, 2015; accepted March 1, 2016.

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arginine for cellular influx and efflux transporters, and improves L-arginine availability, inhibiting its metabolism by arginases.<sup>4</sup> In line with these mechanistic insights, low homoarginine concentrations have been linked to phenotypes of (sub)clinical atherosclerosis, that is, brachial intima-media and aortic wall thickness.<sup>5,6</sup> In patients with stable coronary heart disease, endothelial dysfunction and vessel obliteration are the clinical features of a general atherosclerotic phenotype with a subsequent coronary manifestation. Onset of an acute coronary syndrome (ACS) is related to plaque instability and subsequent inflammation, coronary thrombosis, and/or vasospasms. The link between endothelial dysfunction and ACS is thus not direct, but peripheral endothelial function testing predicts cardiovascular outcome in ACS, and subsequent normalization of endothelial function in these patients lowers event rates.<sup>7,8</sup>

Atrial fibrillation (AF) is independently associated with an increased risk of incident myocardial infarction (MI).<sup>9</sup> Symptoms of AF and ACS as dyspnea and chest discomfort are similar and may thus obscure the presence of acute myocardial ischemia.<sup>10</sup> The concomitance of AF in ACS was shown to complicate MI, given that conduction of rapid arrhythmias causes additional impairment of coronary perfusion and left ventricular function.<sup>11</sup> Unsurprisingly, patients with ACS and coexisting AF were shown to have worse outcomes.<sup>12</sup>

To explore whether homoarginine levels are associated with AF and outcome in patients with acute chest pain, plasma homoarginine was measured in 1649 consecutive patients presenting at 3 German chest pain unit centers. Specifically, this study aimed to investigate (1) the associations of homoarginine with cardiovascular outcome and (2) a potential relationship with additional biomarkers and cardiovascular phenotypes.

## Methods

### Study Protocol

A total of 1818 patients ages 18 to 85 with suspected ACS were enrolled in an observational multicenter cohort at 3 German tertiary care centers between January 2007 and December 2008 (University Medical Center of the Johannes-Gutenberg University Mainz, University Heart Center Hamburg-Eppendorf, and Federal Armed Forces Hospital Koblenz). Eligible patients were between 18 and 85 years of age. Exclusion criteria were major surgery or trauma within the preceding 4 weeks, pregnancy, intravenous drug misuse, and anemia with hemoglobin level below 10 g/dL. Subjects provided blood samples on admission for biomarker determination in parallel with routine blood draws.<sup>13,14</sup> At the same time point, a 12-lead electrocardiogram (ECG) was recorded. Medical history was taken, including time of chest pain onset,

a physical examination was performed, and questionnaire data on cardiovascular disease risk factors and symptoms were collected based on self-reported information and medical charts. All patients provided written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the institution's human research committees in Rhineland-Palatinate and Hamburg. Of 1818 patients initially enrolled in the study, 1649 (66% men) were included in the present analysis based on available follow-up (n=1785) and homoarginine data (n=1680; overlap exists), of whom 589 (36%) were diagnosed with ACS (n=221 unstable angina pectoris [UAP], n=111 ST-elevation myocardial infarction [STEMI], and n=257 non-ST-elevation myocardial infarction [NSTEMI]).

### Definition of Variables

Individuals treated with antihypertensive medication or previously diagnosed as having hypertension were considered to have hypertension. Individuals with total cholesterol >200 mg/dL at admission or with a previous hyperlipidemia diagnosis were regarded as hyperlipidemic. Patients on medication or dietary treatment for diabetes were considered to have diabetes mellitus. Obesity was defined as body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>. Estimated glomerular filtration rate (eGFR) was computed using the CKD-EPI formula.<sup>15</sup> Previous cardiovascular disease (CVD) and chronic kidney disease (CKD) were self-reported in a standardized interview. Prevalent AF was diagnosed based upon evaluation of the 12-lead ECG recordings at admission. QRS and QTc duration were assessed using baseline (at admission) ECG recordings. For QRS analysis, participants were excluded for prevalent AF on ECG; pacemaker in place; ventricular pre-excitation; second- or third-degree heart block; history of MI or heart failure; and QRS  $\geq 120$  ms. For QT analysis, participants were excluded for prevalent AF on ECG; pacemaker in place; QRS  $\geq 120$  ms.

### Measurement of Plasma Homoarginine and Additional Biomarkers and Laboratory Parameters

Venous blood was collected into EDTA tubes. After centrifugation, EDTA plasma aliquots were stored at  $-80^{\circ}\text{C}$  in 2 central laboratories (University Medical Center of the Johannes-Gutenberg University Mainz and University Heart Center Hamburg-Eppendorf). For each analyte, all samples were analysed in 1 batch in one of the central laboratories. Plasma homoarginine was determined from frozen EDTA plasma samples with a high throughput mass spectrometric (MS) assay, applying electrospray ionization/liquid chromatography (LC)-MS/MS.<sup>16</sup> Briefly, proteins were precipitated by adding 25  $\mu\text{L}$  of EDTA plasma to 100  $\mu\text{L}$  of internal standard (2.5  $\mu\text{mol/L}$  [<sup>13</sup>C<sub>6</sub>]-homoarginine) dissolved in methanol.

Samples were centrifuged, evaporated, and subsequently converted to their butyl ester derivatives using 1 N of butanolic hydrochloric acid. After centrifugation, eluates were dried by heating and redissolved in 100  $\mu$ L of methanol/water (25:75), containing 0.1% ammonium formate, before measurement. Plates were placed in a CTC PAL autosampler, and 20- $\mu$ L aliquots were subjected to further analysis in the MS system (Varian 1200 MS; Agilent Technologies, Santa Clara, CA). The lower limit of quantification for homoarginine was determined to be 0.01  $\mu$ mol/L. Intra- and interassay coefficients of variation were  $\leq 7.5\%$ . L-arginine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) were measured applying LC-MS/MS,<sup>17</sup> as described in detail elsewhere.<sup>18</sup> Brain natriuretic peptide (BNP), high-sensitivity C-reactive protein (hsCRP), high-sensitivity assayed troponin I (hsTnI), midregional proadrenomedullin (MR-proADM), midregional proatrial natriuretic peptide (MR-proANP), cystatin C, high-density lipoprotein (HDL) cholesterol, triglycerides, and creatine kinase were measured using dedicated commercial assays, as described elsewhere.<sup>14,19</sup>

## Mortality and Nonfatal Cardiovascular Event Assessment

Six-month follow-up was performed by standardized telephone interview, letter, and review of medical charts. If patients could not be contacted by telephone interview or letter, the general practitioner was contacted. Medical charts were obtained from hospitals and general practitioners. All self-reported nonfatal strokes and MIs were verified by medical charts. Major adverse cardiovascular events (MACEs), including nonfatal strokes and MIs, fatal strokes and MIs, and all-cause mortality, were adjudicated by at least 2 cardiologists blinded for homoarginine measurements. Follow-up information was missing in 2% of enrolled patients.

## Statistical Analyses

Participants were divided into 2 groups based on diagnosis of ACS and noncardiac chest pain (NCCP). Categorical variables are reported as proportions, and continuous variables are reported as medians and interquartile ranges [IQRs]. Comparison of 2 groups was performed with Fisher's exact test for binary variables and the Mann-Whitney *U* test for continuous variables. Three group comparisons of quantitative variables were done with the help of the Kruskal-Wallis test.

Spearman correlations were used to calculate univariate associations between homoarginine and continuous variables. A linear regression with log-transformed homoarginine as the dependent variable and cardiovascular risk factors as independent variables was performed. The association of homoarginine to QRS and QTc duration was examined linearly

regressing log-homoarginine on each of these ECG variables using different multivariable adjustments. A combined endpoint comprising all-cause mortality, nonfatal stroke, and nonfatal MI (MACE) was used in all survival analyses. A homoarginine cutoff for 6-month prediction of MACE was derived by maximizing the sum of 6-month sensitivity and specificity for MACE. Sensitivity and specificity were computed using the Kaplan-Meier approach described in Heagerty et al.<sup>20</sup> Kaplan-Meier survival curves at the median value of homoarginine as well as at the evaluated cutoff for homoarginine were constructed in the overall sample and the subgroup of ACS patients. Statistical comparisons were made using the log-rank test. Cox proportional hazards modeling was used to calculate hazard ratios (HRs) and 95% CIs using a series of multivariable adjustments. For these analyses, homoarginine was used after being log-transformed. In model 1, age and sex were included. Model 2 comprised model 1 with additional adjustment for other risk factors similar to the GRACE model applied previously in this cohort.<sup>19</sup> Model 3 comprised model 2 with additional adjustment for in-hospital treatment (percutaneous coronary intervention [PCI] or coronary artery bypass grafting [CABG]). HRs are given for a 1-SD increase in log-homoarginine. The proportional hazards assumption was tested and visually assessed using the methods described by Grambsch and Therneau.<sup>21</sup> No deviations from this assumption were found. Patients were divided into 2 BNP groups using a cut-off value of 80 pg/mL as a predictor of the presence of congestive heart failure,<sup>22</sup> and the homoarginine cutoff was used to produce survival curves on each BNP group. The log-rank test was used to test for differences. This was repeated, exchanging BNP by hsTnI, a previously identified biomarker for MI, with a cutoff of 27 ng/L based on the 99th percentile in 5000 individuals of the Gutenberg Health Study.<sup>23</sup>

The association of AF and homoarginine, which was used log-transformed, was examined using logistic regression. Three models were considered, the first one was adjusted for age and sex; the second for variables in model 1 plus common cardiovascular risk factors. Model 3 comprised model 2 including adjustment for in-hospital treatment (PCI or CABG). Statistical analyses were performed using R software (version 3.2.2; R Core Team 2013; R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria: URL <http://www.R-project.org/>). All statistical tests reported are 2-sided, with significance defined by  $P < 0.05$ .

## Results

### Description of the Study Sample

The present study included 1649 patients (66% males) with a mean age of  $62 \pm 13$  years. Among all patients included, 589

were diagnosed with ACS (368 acute myocardial infarctions [AMIs] and 221 UAPs) and 1060 with NCCPs. Bivariate analyses among these groups revealed higher prevalence of

traditional cardiovascular risk factors (specifically, current smoker, diabetes mellitus, hypertension, or history of CVD) in the ACS subsample (Table 1). In patients diagnosed with ACS,

**Table 1.** Baseline Demographics and Clinical Characteristics of All Patients and for Acute Coronary Syndrome and Noncardiac Chest Pain Subsamples

	All Patients n=1649	Acute Coronary Syndrome n=589	Noncardiac Chest Pain n=1060	P Value
Age, y	64 [53, 72]	66 [56, 74]	61 [50, 70]	<0.001
Sex, men (%)	1088 (66)	428 (73)	660 (62)	<0.001
Body mass index, kg/cm <sup>2</sup>	27.2 [24.8, 30.4]	27.5 [24.8, 30.4]	27 [24.7, 30.4]	0.23
Systolic BP, mm Hg	140 [129, 160]	145 [130, 160]	140 [127, 158]	0.06
Diastolic BP, mm Hg	80 [70, 85]	80 [70, 86]	78 [70, 85]	0.08
Current smoker, n (%)	384 (23)	157 (27)	227 (22)	0.01
Diabetes, n (%)	253 (16)	127 (22)	126 (12)	<0.001
Hypertension, n (%)	1223 (74)	473 (80)	750 (71)	<0.001
Previous MI, n (%)	370 (23)	163 (28)	207 (20)	<0.001
Cerebrovascular disease of stroke, n (%)	92 (5.7)	46 (7.9)	46 (4.4)	<0.01
Atrial fibrillation, n (%)	288 (18)	95 (16)	193 (18)	0.28
Congestive heart failure, n (%)	68 (4.3)	27 (4.9)	41 (4.0)	0.44
<b>Medication</b>				
β-Blocker, n (%)	654 (40)	256 (44)	398 (38)	0.02
ACE inhibitor, n (%)	574 (35)	232 (39)	342 (32)	<0.01
Diuretics, n (%)	473 (29)	183 (31)	290 (27)	0.11
Statin, n (%)	517 (31)	211 (36)	306 (30)	<0.01
Antiplatelet drug, n (%)	653 (40)	275 (47)	378 (36)	<0.001
QRS duration, ms	98 [92, 108]	100 [92, 110]	98 [92, 108]	0.10
QTc duration, ms	433 [414, 454]	436 [416, 458]	433 [413, 452]	0.03
Time of chest pain onset, h	4.3 [2.0, 13.2]	4.5 [2.0, 15.4]	4.2 [2.0, 11.8]	0.09
PCI or CABG, n (%)	453 (28)	453 (77)	0 (0)	<0.001
Total cholesterol, mg/dL	195 [165, 227]	198 [167, 233]	194 [164, 224]	0.05
LDL, mg/dL	116 [89, 145]	123 [94, 152]	113 [87, 142]	<0.001
HDL, mg/dL	48 [40, 59]	46 [38, 56]	49 [40, 61]	<0.001
Triglycerides, mg/dL	119 [76, 188]	123 [79, 189]	117 [75, 187]	0.29
Homoarginine, μmol/L	2.38 [1.86, 3.01]	2.37 [1.85, 2.95]	2.40 [1.86, 3.05]	0.40
L-Arginine, μmol/L	117 [92, 145]	117 [87, 144]	118 [94, 146]	0.07
ADMA, μmol/L	0.60 [0.52, 0.69]	0.60 [0.53, 0.69]	0.60 [0.52, 0.68]	0.65
SDMA, μmol/L	0.50 [0.42, 0.60]	0.50 [0.42, 0.60]	0.50 [0.42, 0.60]	0.45
BNP, pg/mL	34.3 [12.2, 10.3]	61.3 [21.0, 182]	26.8 [5.0, 69.0]	<0.001
hsCRP, mg/L	2.5 [1.3, 5.7]	2.9 [1.5, 6.6]	2.3 [1.1, 5.3]	<0.001
hsTnI, ng/L	7.9 [3.7, 40.1]	76.8 [13.3, 637]	4.9 [3.0, 10.5]	<0.001
eGFR, mL/min for 1.73 m <sup>2</sup>	84.3 [69.1, 95.4]	81.9 [65.8, 93.6]	85.4 [71.3, 96.9]	<0.001
Cystatin C, mg/L	0.67 [0.58, 0.79]	0.70 [0.60, 0.83]	0.65 [0.56, 0.77]	<0.001

Data are presented as median [interquartile range] or n (%), as appropriate. ADMA indicates asymmetric dimethylarginine; BP, blood pressure; BNP, brain natriuretic peptide; CABG, coronary artery bypass grafting; eGFR, estimated glomerular filtration rate computed using the CKD-EPI formula; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; hsTnI, high-sensitivity assayed troponin I; LDL, low-density lipoprotein; MI, myocardial infarction; PCI, percutaneous coronary intervention; SDMA, symmetric dimethylarginine.

**Table 2.** Linear Regression Modeling for Homoarginine

Variable	$\beta$ -Estimate (SE)	P Value
Age, y	-0.004 (0.001)	<0.001
Sex, male	0.122 (0.022)	<0.001
BMI, kg/m <sup>2</sup>	0.013 (0.002)	<0.001
Systolic BP, mm Hg	0.001 (0.000)	0.004
Current smoking	-0.104 (0.025)	<0.001
Diabetes mellitus	-0.001 (0.029)	0.98
Congestive HF	-0.159 (0.049)	0.001
Previous MI	-0.029 (0.025)	0.24
HDL cholesterol, mg/dL	-0.001 (0.001)	0.09
eGFR, mL/min for 1.73 m <sup>2</sup>	0.002 (0.001)	<0.01

For the continuous variables,  $\beta$ -estimates are shown for a 1-unit change in the variable. Homoarginine was log-transformed. BMI indicates body mass index; BP, blood pressure; eGFR, glomerular filtration rate estimated using the CKD-EPI formula; HDL, high-density lipoprotein; HF, heart failure; MI, myocardial infarction.

the cardiac biomarkers, BNP, hsTnI, and hsCRP, were significantly increased. Median [IQR] plasma homoarginine was 2.38 [1.86–3.01]  $\mu\text{mol/L}$ , with moderately lower levels in patients with AMI (2.34 [1.80–2.92]  $\mu\text{mol/L}$ ;  $n=368$ ) compared to patients with UAP (2.40 [1.95–3.05]  $\mu\text{mol/L}$ ;  $n=221$ ) or NCCP (2.40 [1.86–3.05]  $\mu\text{mol/L}$ ;  $n=1060$ ;  $P=0.11$  for Kruskal–Wallis test). Crude Spearman correlation analyses for circulating homoarginine are summarized in Table S1. Inverse correlations with homoarginine were noticed for age, QTc duration, cystatin C, and HDL cholesterol. Positive correlations were observed with BMI, triglycerides, and circulating L-arginine concentrations.

In a multivariable linear regression model, log-homoarginine was independently and positively associated with male sex, BMI, systolic blood pressure (BP), and eGFR. Negative associations were observed with congestive heart failure, current smoking, and age. No significant association was observed with HDL cholesterol, diabetes mellitus, and previous MI (Table 2).

### Homoarginine and Major Adverse Outcomes in the Study Sample

During a median follow-up of 183 days, a total of 60 MACEs (3.8%), including 19 deaths, 10 nonfatal strokes, and 31 nonfatal MIs, were observed. Circulating homoarginine concentrations were significantly higher in participants without incident MACE as compared with those who experienced an event during the follow-up period (2.39 [1.87–3.01] vs 2.09 [1.48–2.77]  $\mu\text{mol/L}$ ;  $P=0.008$ ). In the overall study population, Kaplan–Meier analysis revealed that cumulative incidence of MACE was significantly higher in individuals with homoarginine at or below the median

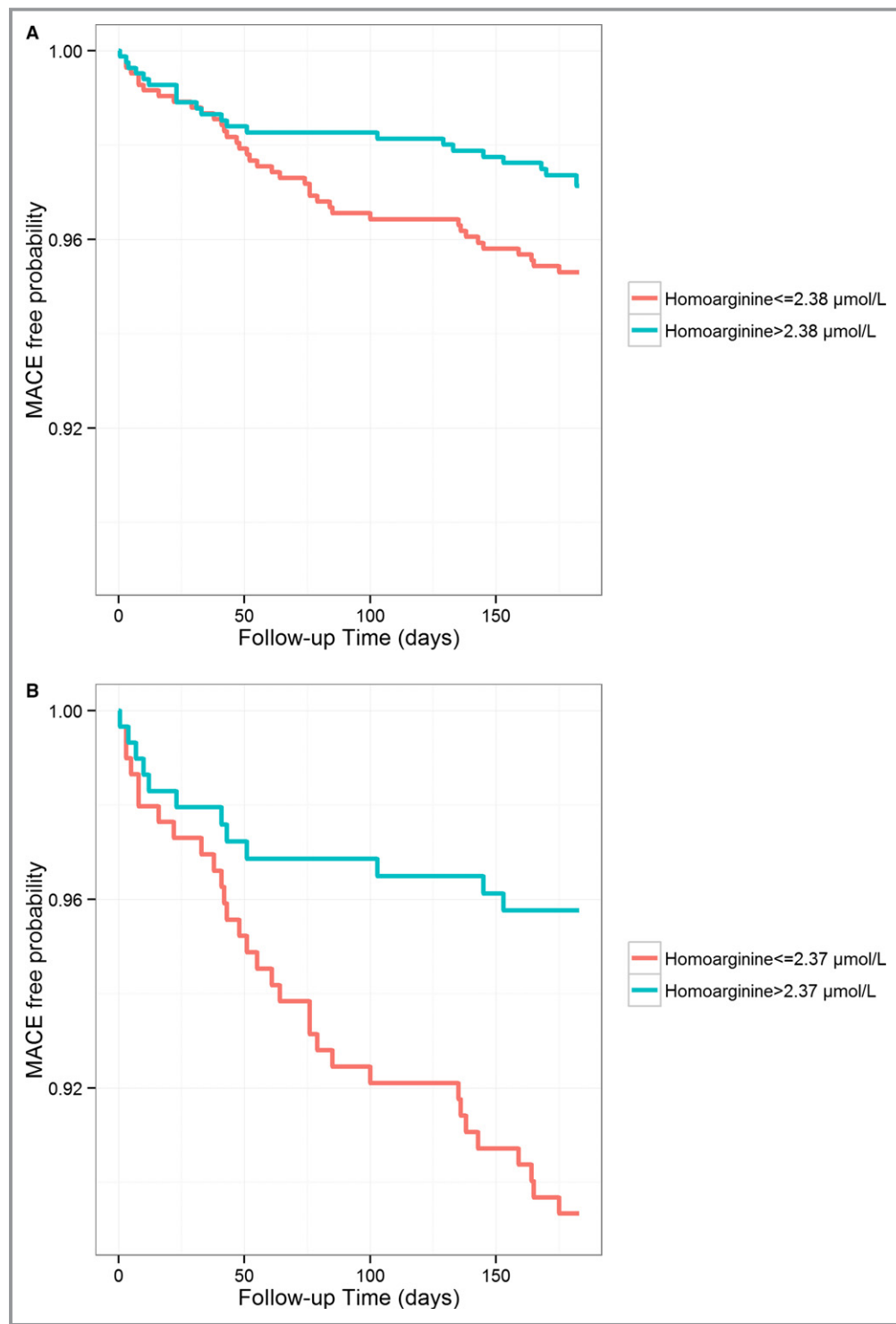
(2.38  $\mu\text{mol/L}$ ; 4.7%) compared with homoarginine above the median (2.9%;  $P<0.05$ ; Figure 1A). A similar association was observed in the subgroup of diagnosed ACS (43 MACE [7.5%], including 13 deaths, 4 nonfatal strokes, and 26 nonfatal MIs;  $P<0.005$ ; Figure 1B). In adjusted Cox regression analyses, an increase of 1 SD log-homoarginine was associated with a hazard reduction of 26% for incident MACE (model 3; Table 3). Similar results were observed in the subgroup of ACS patients; hazard reduction related to an increase of homoarginine was 35% in the adjusted model (model 3; Table 3). The predictive cut-off value for homoarginine for MACE in this study population was determined to be 1.41 (95% CI, 1.25, 2.77)  $\mu\text{mol/L}$ , with a sensitivity of 25% and a selectivity of 91%. Using this cutoff, Kaplan–Meier analysis showed a higher cumulative incidence of MACE in individuals with homoarginine at or below the cutoff (1.41  $\mu\text{mol/L}$ ; 10.7%) compared with homoarginine above the cutoff (3.1%;  $P<0.001$ , log-rank test; Figure S1A). Similar results were obtained in the ACS subgroup (23% vs 6.0%;  $P<0.001$ , log-rank test; Figure S1B). Further subgroup analyses revealed that homoarginine below the cutoff predicted MACE in patients with low and high BNP equally ( $P<0.05$ ; Figure 2A). Of note, homoarginine was only predictive in patients with elevated hsTnI (hsTnI >27 ng/L;  $P<0.01$ ; Figure 2B).

### Homoarginine and Cardiovascular Phenotypes

The strongest correlations of circulating homoarginine with cardiac biomarkers were observed with MR-proADM, BNP, and MR-proANP. Further correlations were observed with creatine kinase and hsTnI (Table 4). In age- and sex-adjusted linear regression analyses, log-homoarginine was inversely associated with QTc duration ( $P<0.001$ ; model 1; Table 5). QTc duration remained significantly associated with circulating homoarginine after further adjustment for BMI, current smoking, diabetes mellitus, hypertension, hyperlipidemia, and in-hospital treatment ( $P<0.001$ ; model 3; Table 5). In 288 patients suffering from AF, significantly more patients had circulating homoarginine concentrations below/at the median compared to above homoarginine median ( $n=168$  vs 120;  $P=0.0023$ , Fisher's exact test; all participants). Similar associations were observed in the ACS subgroup. Adjusted logistic regression analyses showed for an increase of 1 SD log-homoarginine an odds ratio (OR) reduction for prevalent AF by 17% (model 3; Table 6).

### Discussion

There are several novel findings from this study. First, low homoarginine concentrations predict cardiovascular outcome



**Figure 1.** Unadjusted failure curves for incidence of the combined endpoint comprising all-cause mortality, nonfatal stroke, and nonfatal myocardial infarction according to homoarginine median (A) in the overall study population (2.38  $\mu\text{mol/L}$ ;  $n=1649$ ;  $P=0.047$ , log-rank test) and (B) in the ACS subsample (2.37  $\mu\text{mol/L}$ ;  $n=589$ ;  $P<0.005$ , log-rank test). ACS indicates acute coronary syndrome; MACE, major adverse cardiovascular event.

in patients with acute chest pain, in particular in those with elevated hsTnI. Second, homoarginine levels are correlated to biomarkers representing cardiovascular function (MR-

proADM, BNP, and MR-proANP). Last, homoarginine is inversely associated with QTc duration as well as prevalent AF.

**Table 3.** Multivariable-Adjusted HRs for Homoarginine for Major Adverse Events

Model*	HR [95% CI]	Events (n)
All participants		
1 (n=1649)	0.77 [0.60–0.98]	60
2 (n=1471)	0.75 [0.58–0.97]	56
3 (n=1471)	0.74 [0.57–0.96]	56
Acute coronary syndrome		
1 (n=589)	0.61 [0.46–0.82]	43
2 (n=533)	0.66 [0.50–0.88]	41
3 (n=533)	0.65 [0.49–0.88]	41

Data are presented for 1-SD increase in log-homoarginine (0.37). CABG indicates coronary artery bypass grafting; EPV, event per variable; HR, hazard ratio; PCI, percutaneous coronary intervention.

\*Model 1 adjusted for age and sex; model 2 adjusted for variables in model 1 plus body mass index, current smoking, diabetes mellitus, hypertension, and hyperlipidemia; model 3 adjusted for variables in model 2 plus in hospital treatment (PCI or CABG).

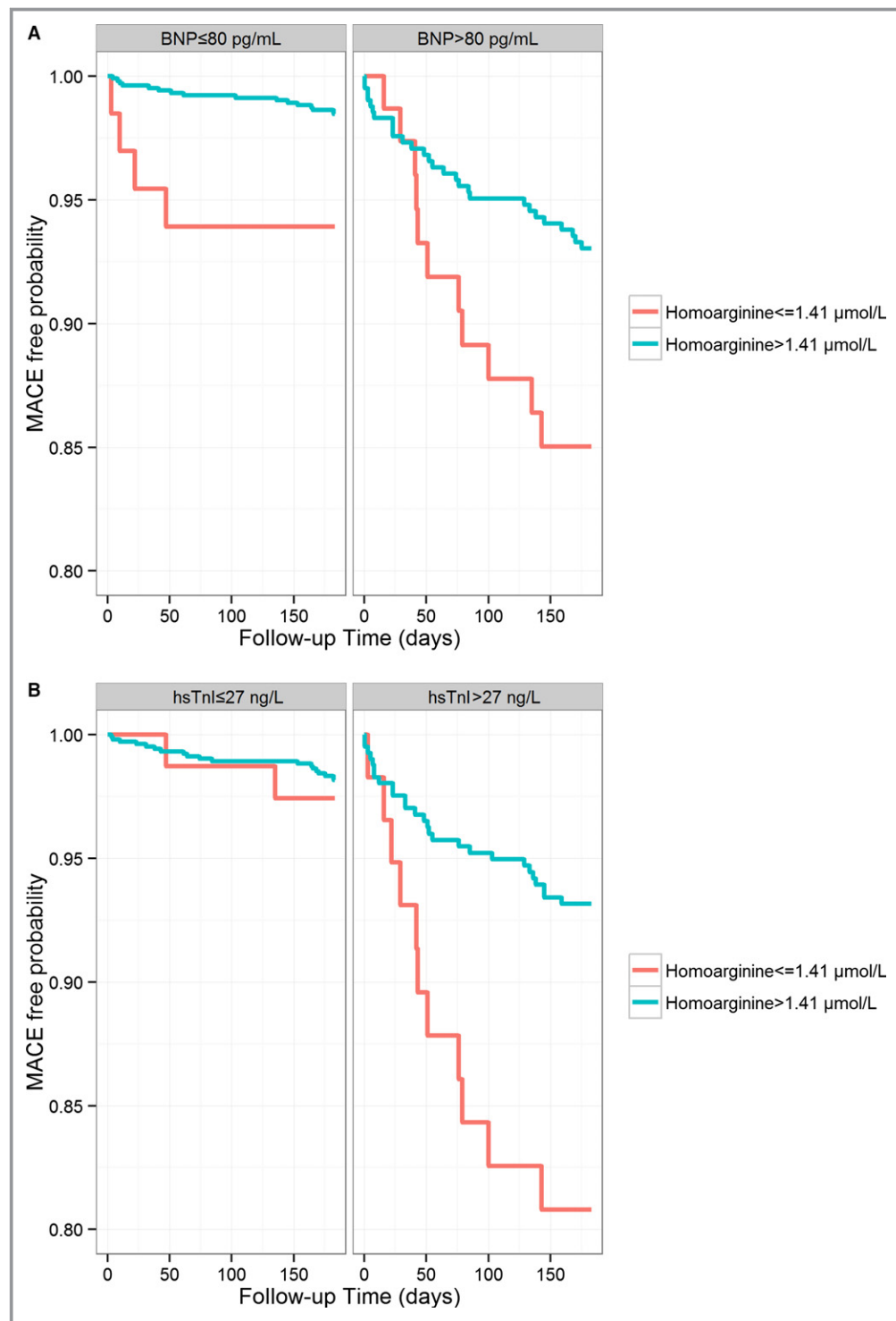
Low circulating concentrations of the endogenous amino acid homoarginine have been reported to be a prognostic marker for mortality and cardiovascular events in a variety of diseases, among them heart failure, stroke, and CKD.<sup>1,2</sup> In the present study sample of patients with acute chest pain, median plasma homoarginine was 2.38 [1.86–3.01]  $\mu\text{mol/L}$ . Although median homoarginine concentrations were not significantly different between ACS and NCCP patients, the predictive value of low homoarginine on incident MACE—in the overall study population as well as in the subsample of ACS patients—was evident. It is generally accepted that atherosclerosis plays a pivotal role in pathogenesis of ACS.<sup>7,8</sup> In line with this, Valtonen et al. found a positive correlation between homoarginine and endothelium-dependent brachial artery flow mediated dilation.<sup>24</sup> Supporting the association between homoarginine and endothelial function, low circulating homoarginine has recently been linked to phenotypes of (sub)clinical atherosclerosis, that is, brachial intima-media and aortic wall thickness.<sup>5,6</sup> Vascular atherosclerosis triggers myocardial stress and may, by this end, directly activate the cardiac neurohormonal system.<sup>25</sup> We and others found a strong association of low homoarginine concentrations and increased levels of BNP.<sup>26–29</sup> In our study, homoarginine was not only correlated with parameters of myocardial stress (MR-proADM, BNP, and MR-proANP), but also with markers of coronary ischemia (creatinine kinase, hsTnI). New onset of an acute event occurs upon vessel obliteration and subsequent myocardial necrosis. Of note, the study population investigated here has already been used to identify the predictive value of hsTnI, MR-proADM, and MR-proANP for cardiovascular outcomes.<sup>13,19,23</sup> We therefore determined a cut-off value for homoarginine for prediction of MACE and evaluated this

cutoff in the context of the predefined cutoffs for those already-established biomarkers, BNP and hsTnI. Only in those patients with elevated hsTnI (cutoff 27 ng/L)<sup>23</sup>, low homoarginine predicted future cardiovascular events in our study population (Figure 2B). This observation may underline the relevance of homoarginine for patients at higher risk for secondary events. Compared to hsTnI, a valuable marker in the acute phase of coronary ischemia, homoarginine may provide additional prognostic information for short- and long-term outcome. Whereas the present study focused on patients with acute chest pain that were followed up for 183 days, a study including patients routinely referred to coronary angiography and followed for 9.9 years identified circulating homoarginine as a predictor for all-cause and cardiovascular mortality.<sup>30</sup>

Of note, homoarginine showed an inverse association with QTc duration. Patients with QTc prolongation are known to experience more likely an ACS.<sup>31</sup> In the MERLIN-TIMI 36 study, an abnormal QTc interval was associated with a 2-fold increased risk of sudden cardiac death in patients with non-ST elevation ACS.<sup>32</sup> So far, nothing is known about homoarginine in AF. In the present study, a statistically significant association of homoarginine with prevalence of AF was detected. Patients suffering from AF were shown to have significantly lower homoarginine concentrations. AF is an important comorbidity in ACS. Most recent studies have demonstrated that AF is associated with a higher risk of subsequent cardiovascular events<sup>33</sup> and mortality<sup>12</sup> in ACS. Thus, both ECG variables (ie, QTc prolongation) and AF are predictors of adverse outcomes in patients with ACS. Our data showed an association of homoarginine with QTc duration and prevalence of AF. Whether homoarginine is lower because of prevalence of the disease and ECG abnormalities or precedes these electrocardiographic changes cannot be answered from the current analyses. Our data should be viewed as hypothesis generating and thus be interpreted with caution. At this point, further experimental studies are necessary to elucidate a potential causal link between homoarginine and electrocardiographic changes. Whether targeting the homoarginine/AGAT pathway in patients with prevalent AF or QTc prolongation may improve outcome, as shown, for example, for ranolazine,<sup>34</sup> needs further investigation.

### Study Limitations

As a qualitative hypothesis-generating study, the hypothesis is based on interview data of the research participants. Even though our data might not be representative for other patients with acute chest pain, the validity and generalizability of the study has been previously shown for the diagnostic value of hsTnI and the prognostic value of MR-proADM and MR-



**Figure 2.** Unadjusted failure curves for incidence of the combined endpoint comprising all-cause mortality, nonfatal stroke, and nonfatal myocardial infarction according to homoarginine cutoff (1.41  $\mu\text{mol/L}$ ) and stratified by (A) BNP (cutoff, 80  $\text{pg/mL}$ ;  $n=1127$  to 488;  $P<0.01$  and  $<0.05$ , log-rank test) or (B) hsTnI (cutoff, 27  $\text{ng/L}$ ;  $n=1131$  to 465;  $P=0.64$  and  $<0.01$ , log-rank test). BNP indicates brain natriuretic peptide; hsTnI, high-sensitivity assayed troponin I; MACE, major adverse cardiovascular event.

proANP.<sup>13,19</sup> Furthermore, the cut-off value evaluated here for homoarginine (1.41  $\mu\text{mol/L}$ ) shows low sensitivity (25%) and might therefore not be generally applicable to other collec-

tives. Further investigations in larger study samples are needed to formulate a cut-off value with higher sensitivity and selectivity values. Another limitation of our study is that



**Table 4.** Spearman Correlations Between Homoarginine and Cardiac and Noncardiac Biomarkers in Patients With Acute Chest Pain

Variables	$\rho$	P Value
BNP	-0.23	<0.001
hsTnl	-0.11	<0.001
MR-proANP	-0.23	<0.001
MR-proADM	-0.29	<0.001
Myoglobin	-0.05	0.068
Creatine kinase	0.13	<0.001
Creatine kinase-MB	0.03	0.26

BNP indicates brain natriuretic peptide; hsTnl, high-sensitivity assayed troponin I; MR-proADM, midregional proadrenomedullin; MR-proANP, midregional proatrial natriuretic peptide.

coronary angiography data are not available for the entire study population. Furthermore, the overall event rate is relatively low and no incident AF was documented.

### Clinical Implications

Homoarginine is an endogenous compound related to QTc duration and AF, implicating a potential role in risk assessment of supraventricular and ventricular arrhythmias. In addition, homoarginine is associated with parameters of cardiac function and coronary ischemia. It is directly related to incidence of MACE and may thus prospectively play a role in the prognosis of ACS. In an advantage to other markers (eg, hsTnl), homoarginine is targetable by oral supplementation.

**Table 5.** Homoarginine and Cardiac Phenotypes in Patients With Acute Chest Pain

Model*	$\beta$ -Estimate (SE)	P Value
QRS duration		
1 (n=1635)	-0.0008 (0.0004)	0.07
2 (n=1462)	-0.0012 (0.0004)	<0.01
3 (n=1462)	-0.0012 (0.0004)	<0.01
QTc duration		
1 (n=1635)	-0.0012 (0.0003)	<0.001
2 (n=1462)	-0.0012 (0.0003)	<0.001
3 (n=1462)	-0.0012 (0.0003)	<0.001

$\beta$ -estimates are shown for a 1-unit change in the variable. CABG indicates coronary artery bypass grafting; PCI, percutaneous coronary intervention.\*Model 1 adjusted for age and sex; model 2 adjusted for variables in model 1 plus body mass index, current smoking, diabetes mellitus, hypertension, and hyperlipidemia; model 3 adjusted for variables in model 2 plus in hospital treatment (PCI or CABG). Homoarginine was log-transformed.

**Table 6.** Multivariable-Adjusted ORs for Atrial Fibrillation in Patients With Acute Chest Pain

Model*	OR [95% CI]	Events (n)
All participants		
1 (n=1635)	0.85 [0.75–0.98]	288
2 (n=1462)	0.83 [0.71–0.95]	262
3 (n=1462)	0.83 [0.71–0.95]	262

Data are presented for 1-SD increase in log-homoarginine (0.37). CABG indicates coronary artery bypass grafting; OR, odds ratio; PCI, percutaneous coronary intervention.\*Model 1 adjusted for age and sex; model 2 adjusted for variables in model 1 plus body mass index, current smoking, diabetes mellitus, hypertension, and hyperlipidemia; model 3 adjusted for variables in model 2 plus in hospital treatment (PCI or CABG).

### Conclusions

In summary, the current findings identified homoarginine as a biomarker for MACE in patients with acute chest pain, in particular in those with elevated hsTnl and as a novel correlate for prevalent AF. Low homoarginine is therefore not only a predictor for adverse outcome in patients with acute chest pain, but might also be a new target in these patients, that is, by homoarginine supplementation or AGAT activation.

### Acknowledgments

We gratefully thank Mariola Kastner and Anna Steenpaß for their excellent technical assistance.

### Sources of Funding

The study was supported by a research grant provided by the Fachbereich Medizin der Universität Hamburg (Forschungsförderungsfond NWF 13/02) to Dr Atzler, the Deutsche Stiftung für Herzforschung (German Heart Research Foundation F/12/08) to Dr Schwedhelm, and an Else Kröner Memorial Stipendium from the Else Kröner-Fresenius-Stiftung to Dr Choe. Dr Atzler acknowledges the support of the European Union under a Marie Curie Intra-European Fellowship for Career Development. Dr Baum is supported by the Clinical Scientist program of the University Medical Center Hamburg-Eppendorf.

### Disclosures

None.

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## SUPPLEMENTAL MATERIAL

**Table S1. Cross-sectional correlation analyses of homoarginine.**

<b>Variables</b>	<b>Rho</b>	<b>P-Value</b>
Age	-0.2	<0.001
Systolic Blood Pressure	0.03	0.31
Diastolic Blood Pressure	0.11	<0.001
<b>Anthropometric and metabolic measurements</b>		
BMI	0.19	<0.001
<b>Markers of renal function</b>		
Cystatin C	-0.16	<0.001
eGFR	0.14	<0.001
Creatinine	0.03	0.31
<b>Blood lipids</b>		
Triglycerides	0.17	<0.001
HDL	-0.14	<0.001
Total Cholesterol	0.02	0.45
LDL	0.01	0.66
<b>ECG variables</b>		
QRS duration	-0.01	0.75
QTc duration	-0.18	<0.001

### Arginine derivatives

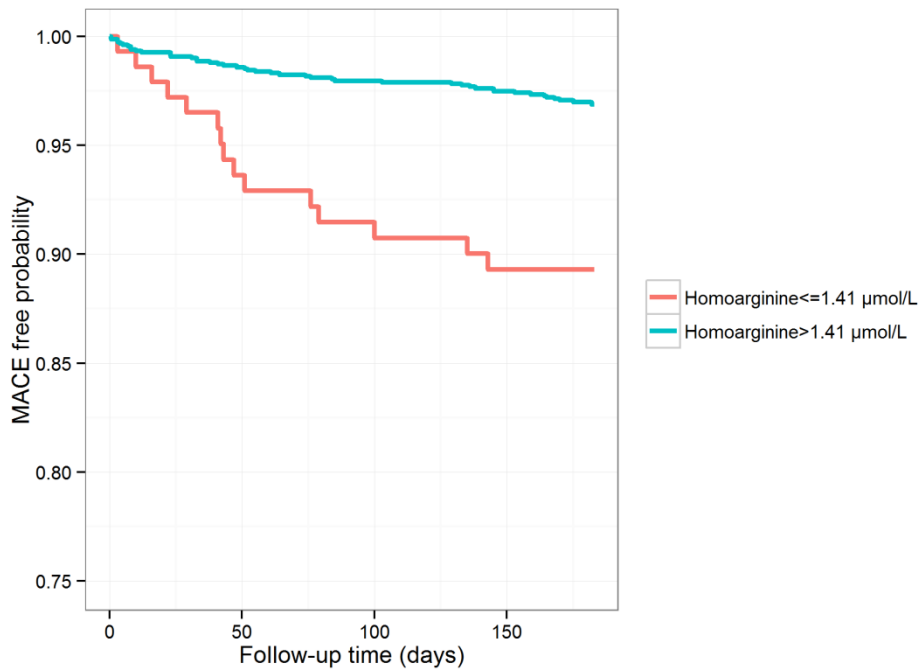
L-Arginine	0.17	<0.001
SDMA	-0.01	0.75
ADMA	-0.13	<0.001

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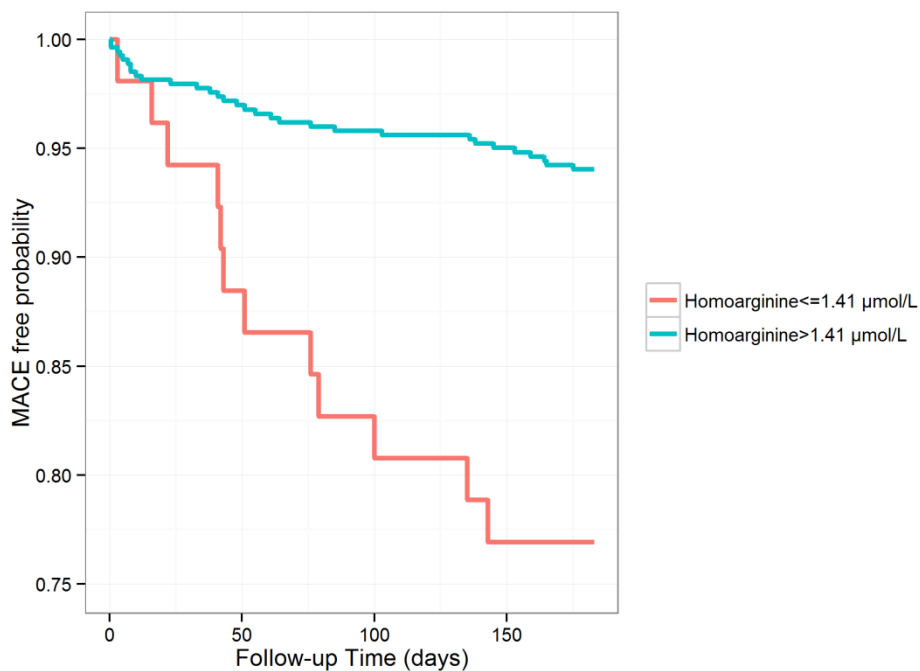
ADMA indicates asymmetric dimethylarginine; BMI, body mass index; eGFR, glomerular filtration rate computed using the CKD-EPI formula; HDL, high density lipoprotein; LDL, low density lipoprotein; SDMA, symmetric dimethylarginine. Homoarginine was log-transformed.

**Figure S1.** Unadjusted failure curves for the incidence of the combined endpoint comprising all-cause mortality, non-fatal stroke, and non-fatal myocardial infarction according to homoarginine cut-off (1.41  $\mu\text{mol/L}$ ) **(a)** in the overall study population ( $n=1,649$ ;  $P<0.001$  log-rank test) and **(b)** in the ACS subsample ( $n=589$ ;  $P<0.001$  log-rank test).

**Figure S1 A**



**Figure S1 B**





## Low Homoarginine Levels in the Prognosis of Patients With Acute Chest Pain

Dorothee Atzler, Christina Baum, Francisco Ojeda, Till Keller, Kathrin Cordts, Renate B. Schnabel, Chi-un Choe, Karl J. Lackner, Thomas Münzel, Rainer H. Böger, Stefan Blankenberg, Edzard Schwedhelm and Tanja Zeller

*J Am Heart Assoc.* 2016;5:e002565; originally published April 13, 2016;

doi: 10.1161/JAHA.115.002565

The *Journal of the American Heart Association* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231  
Online ISSN: 2047-9980

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