Homoarginine and Cardiovascular Outcome in the Population-Based Dallas Heart Study

Dorothee Atzler, M. Odette Gore, Colby R. Ayers, Chi-un Choe, Rainer H. Böger, James A. de Lemos, Darren K. McGuire, Edzard Schwedhelm

Objective—The nonproteinogenic amino acid homoarginine has been postulated to have antiatherosclerotic effects as a weak substrate of nitric oxide synthase. This investigation in the population-based Dallas Heart Study (DHS) aimed to evaluate the association of homoarginine with clinical and subclinical cardiovascular outcomes.

Approach and Results—Plasma homoarginine was measured in 3514 participants of the DHS using liquid chromatography-tandem mass spectrometry. Associations between homoarginine and major adverse cardiovascular events and all-cause mortality were analyzed using Cox proportional hazard models adjusting for cardiovascular risk factors. Linear regression was used to assess cross-sectional associations between homoarginine and subclinical cardiovascular disease, including coronary artery calcium measured by electron beam-computed tomography, and aortic plaque burden and aortic wall thickness by MRI. Median age was 43 (interquartile range, 36–52) years, with 56% women and 52% black participants. Median follow-up was 9.4 (9.0–9.8) years. Median plasma homoarginine was 2.80 (2.14–3.54) μmol/L. In multivariable models, higher homoarginine was associated with lower rate of major adverse cardiovascular events (hazard ratio, 0.86; 95% confidence interval, 0.75–0.98) and lower all-cause mortality (hazard ratio, 0.82; 0.73–0.92; per 1 log SD increase in homoarginine). Homoarginine was inversely and independently associated with aortic wall thickness (β-estimate, −0.04; P<0.01) but not with aortic plaque burden and coronary artery calcium.

Conclusions—Homoarginine is inversely associated with subclinical vascular disease and with risk for cardiovascular disease events. Additional studies are needed to evaluate whether the regulation of plasma homoarginine could emerge as a novel therapeutic option to improve outcomes in cardiovascular disease. (*Arterioscler Thromb Vasc Biol, 2014;34:00-00.)*

Key Words: atherosclerosis ■ epidemiology ■ nitric oxide

Homoarginine is a naturally occurring nonproteinogenic amino acid structurally related to L-arginine. Homoarginine has been postulated to be an antiatherosclerotic factor because it is a weak substrate for nitric oxide (NO) synthase and can, therefore, be converted to NO.1 Experimental studies have shown that exogenous homoarginine exerts antihypertensive and antiarrhythmic effects.2–5 Recently, clinical data suggested a role of low endogenous homoarginine as a risk marker for cardiovascular and cerebrovascular events.4–6 Importantly, in contrast to other cardiovascular biomarkers, such as N-terminal brain natriuretic peptide or asymmetrical dimethylarginine that are positively associated with cardiovascular outcomes,7,8 plasma concentrations of homoarginine were found to be inversely associated with cardiovascular and all-cause mortality in patients at risk.4,6,9-12 In addition, experimental studies in mice have supported a mechanistic link between low homoarginine and cerebrovascular disease.10 To explore the potential role of homoarginine in the development of cardiovascular disease (CVD) further, we measured plasma homoarginine in the multiethnic, population-based Dallas Heart Study (DHS), to investigate (1) the associations of homoarginine with cardiovascular outcomes in the general population and (2) a potential relationship with subclinical disease measures.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Baseline Characteristics of the DHS Population
Plasma homoarginine and outcomes data were available for 3514 DHS participants. The cohort consisted of 56% women; the median age of the cohort was 43 (interquartile range, 36–52) years. The study comprised 52% blacks, 29% whites, and 17% Hispanics. Median plasma homoarginine was 2.80 (interquartile range, 2.14–3.54) μmol/L. The baseline

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characteristics of the entire cohort are presented in Table 1. In bivariate analyses, men had higher plasma homoarginine (2.92 [2.29–3.62] μmol/L; n=1552) than women (2.70 [2.06–3.46] μmol/L; n=1962; P<0.001); and Hispanics had higher levels (3.10 [2.43–3.95] μmol/L; n=596) than black (2.92 [2.25–3.66] μmol/L; n=1812) and white participants (2.44 [1.86–3.04] μmol/L; n=1032; P<0.001).

Factors Associated With Circulating Homoarginine
Unadjusted correlation analyses for circulating homoarginine are provided in Table I in the online-only Data Supplement. The strongest correlations with homoarginine were seen for L-arginine, cystatin C, and several anthropometric measures (eg, lean mass and body mass index [BMI]). No correlation was found between plasma homoarginine and the methylated arginine derivatives, asymmetrical dimethylarginine and symmetrical dimethylarginine. In addition, a weak association was observed between homoarginine and endothelial cell-selective adhesion molecule but not with vascular cell adhesion molecule 1 or intercellular adhesion molecule 1. In a multivariable linear regression model, log-homoarginine was inversely associated with sex, black race, BMI, estimated glomerular filtration rate, and hypertension and inversely associated with current smoking status, diabetes mellitus, and history of stroke (Table II in the online-only Data Supplement).

Association of Homoarginine With Cardiovascular Outcomes
During a median follow-up of 9.4 (9.0–9.8) years, 184 individuals experienced major adverse cardiovascular events (MACE), including 81 cardiovascular deaths, 51 nonfatal stroke, or 52 nonfatal myocardial infarction. To assess the effect of homoarginine on MACE across the range of circulating homoarginine, restricted cubic spline curves were constructed. Continuous analyses using the homoarginine median as the cut point revealed high hazards at low circulating homoarginine, spaying upward above the homoarginine median (Figure 1). In Cox regression analyses, adjusted for age, sex, and race, an increase of 1 SD, log plasma homoarginine (0.4; antilog=1.49 μmol/L) was associated with a hazard reduction of 16% for the MACE end point (model 1,Table 2). Similar results were obtained in the fully adjusted model (model 4; Table 2).

Relation of Homoarginine to Mortality
Among 3514 individuals with follow-up and plasma homoarginine data available, 218 deaths because of any cause were ascertained. Circulating homoarginine was significantly higher in participants who survived when compared with those who died during the follow-up period (2.81 [2.16–3.55] versus 2.59 [2.01–3.27] μmol/L; P<0.01). In continuous survival analysis, increasing circulating homoarginine was associated with a linear decrease in hazard reduction (Figure 2). In the fully adjusted model of Cox regression analyses, an increase of 1 log SD homoarginine (0.4; antilog=1.49 μmol/L) revealed a significant hazard reduction of 18% for overall mortality (model 4; Table 2).

Association Between Homoarginine and Subclinical CVD Measures
In age, sex, and race-adjusted linear regression analyses, log-homoarginine was inversely associated with aortic wall thickness (AWT; β-estimate [SE], −0.05 [0.01]; P<0.001) and aortic plaque burden (β-estimate [SE], −0.25 [0.09]; P<0.01) but not with coronary artery calcium. AWT remained significantly associated with circulating homoarginine even after further adjustment for traditional cardiovascular risk factors (model 2), as well as after full adjustment (β-estimate [SE], −0.04 [0.01]; P<0.01; model 4; Table 3).

Homoarginine, BMI, and AGAT Single Nucleotide Polymorphism rs1288775
Associations between plasma homoarginine levels and the candidate single nucleotide polymorphism rs1288775 were analyzed. Genotype frequencies at the AGAT 1288775 locus significantly differed between the investigated ethnic groups. A majority of black participants were AA carriers (Table 4). In Hispanic individuals, the majority carried the heterozygous AT genotype. In contrast, only 8% of the white participants are homozygous for the AA genotype. However, regardless of the genotype frequency, in each race/ethnic group, the homoarginine plasma concentration decreased from AA to TT allele carriers (Table 4). In the overall population, as well as in black and Hispanic individuals, the BMI also decreased from AA to TT carriers (Table 4).

Discussion
There are several novel findings from this study. First, homoarginine is inversely associated with MACE. Second, low homoarginine predicts all-cause mortality in the general US population; third, circulating homoarginine shows an inverse association with AWT; and finally, genetic variations within the AGAT gene are linked to homoarginine levels and higher BMI.

Preliminary evidence suggests potential use of the L-arginine derivative homoarginine as a prognostic marker for adverse cardiovascular events in patients at increased cardiovascular risk. Results from several studies showed an independent association between homoarginine levels and markers of bone metabolism,13,14 CVD (ie, sudden cardiac death, heart failure, and myocardial infarction), cerebrovascular outcome, and mortality (both all-cause and cardiovascular) in these at risk patients.4,6,9–12 Interestingly, in contrast to other biomarkers related to the L-arginine pathway, such as asymmetrical dimethylarginine15 and symmetrical dimethylarginine,16 homoarginine was inversely associated with outcome in these
Table 1. Baseline Demographics and Clinical Characteristics of the Entire Cohort and of Participants Who Experienced A MACE or Died During Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>All Participants</th>
<th>MACE (n=184)</th>
<th>P Value*</th>
<th>Participants Who Died</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>43 (36–52)</td>
<td>52 (46–58)</td>
<td>&lt;0.001</td>
<td>52 (44–58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, men (%)</td>
<td>1552 (44)</td>
<td>108 (59)</td>
<td>&lt;0.001</td>
<td>131 (60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race/ethnicity, black (%)</td>
<td>1913 (52)</td>
<td>143 (78)</td>
<td>&lt;0.001</td>
<td>166 (76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoker; n (%)</td>
<td>1022 (29)</td>
<td>82 (45)</td>
<td>&lt;0.001</td>
<td>103 (47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus; n (%)</td>
<td>407 (12)</td>
<td>67 (36)</td>
<td>&lt;0.001</td>
<td>65 (30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>1178 (34)</td>
<td>137 (75)</td>
<td>&lt;0.001</td>
<td>144 (69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous CVD; n (%)</td>
<td>269 (8)</td>
<td>57 (31)</td>
<td>&lt;0.001</td>
<td>61 (28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous stroke; n (%)</td>
<td>87 (3)</td>
<td>17 (9)</td>
<td>&lt;0.001</td>
<td>18 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR&lt;60 mL/min per 1.73 m², n (%)</td>
<td>78 (2)</td>
<td>21 (11)</td>
<td>&lt;0.001</td>
<td>29 (13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blocker, n (%)</td>
<td>192 (6)</td>
<td>35 (19)</td>
<td>&lt;0.001</td>
<td>41 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE-inhibitor, n (%)</td>
<td>339 (10)</td>
<td>58 (32)</td>
<td>&lt;0.001</td>
<td>57 (26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diuretic, n (%)</td>
<td>306 (9)</td>
<td>54 (30)</td>
<td>&lt;0.001</td>
<td>49 (23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>215 (6)</td>
<td>38 (21)</td>
<td>&lt;0.001</td>
<td>33 (15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/cm²</td>
<td>28.4 (24.5–33.2)</td>
<td>29.6 (25.5–33.8)</td>
<td>0.075</td>
<td>26.3 (23.2–34.2)</td>
<td>0.727</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.91 (0.85–0.96)</td>
<td>0.95 (0.90–1.00)</td>
<td>&lt;0.001</td>
<td>0.95 (0.89–1.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83 (71–98)</td>
<td>90 (77–103)</td>
<td>&lt;0.01</td>
<td>84 (70–104)</td>
<td>0.072</td>
</tr>
<tr>
<td>Lean mass, %</td>
<td>55 (46–64)</td>
<td>61 (51–68)</td>
<td>&lt;0.001</td>
<td>56 (50–66)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fat, %</td>
<td>26 (19–34)</td>
<td>26 (19–34)</td>
<td>0.725</td>
<td>26 (16–34)</td>
<td>0.135</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>122 (112–134)</td>
<td>138 (127–156)</td>
<td>&lt;0.001</td>
<td>138 (122–154)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>77 (71–84)</td>
<td>85 (78–93)</td>
<td>&lt;0.001</td>
<td>84 (75–91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>93 (85–102)</td>
<td>100 (88–143)</td>
<td>&lt;0.001</td>
<td>98 (85–123)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>177 (154–203)</td>
<td>179 (153–213)</td>
<td>0.393</td>
<td>171 (143–200)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>104 (82–126)</td>
<td>103 (80–134)</td>
<td>0.756</td>
<td>96 (71–124)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>47 (40–57)</td>
<td>46 (40–57)</td>
<td>0.352</td>
<td>46 (38–56)</td>
<td>0.185</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>96 (67–147)</td>
<td>120 (75–166)</td>
<td>&lt;0.001</td>
<td>104 (69–154)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>L-Arginine, μmol/L</td>
<td>59.3 (44.0–77.1)</td>
<td>60.8 (45.6–76.6)</td>
<td>0.804</td>
<td>60.4 (44.0–78.4)</td>
<td>0.687</td>
</tr>
<tr>
<td>ADMA, μmol/L</td>
<td>0.48 (0.42–0.56)</td>
<td>0.51 (0.44–0.59)</td>
<td>&lt;0.001</td>
<td>0.53 (0.45–0.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDMA, μmol/L</td>
<td>0.41 (0.35–0.47)</td>
<td>0.46 (0.38–0.56)</td>
<td>&lt;0.001</td>
<td>0.46 (0.39–0.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NTproBNP, pg/mL</td>
<td>29 (13–61)</td>
<td>59 (23–168)</td>
<td>&lt;0.001</td>
<td>60 (25–214)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>2.8 (1.2–7.0)</td>
<td>4.3 (1.8–11.4)</td>
<td>&lt;0.001</td>
<td>4.4 (1.6–11.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-cTnT, ng/mL</td>
<td>1.5 (1.5–3.3)</td>
<td>5.1 (1.5–10.8)</td>
<td>&lt;0.001</td>
<td>5.1 (1.5–12.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESAM</td>
<td>34.4 (27.1–43.4)</td>
<td>42.7 (31.9–56.7)</td>
<td>&lt;0.001</td>
<td>43.7 (33.9–59.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>988 (724–1388)</td>
<td>1043 (790–1525)</td>
<td>&lt;0.05</td>
<td>1143 (785–1506)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>606 (455–829)</td>
<td>633 (464–934)</td>
<td>0.070</td>
<td>745 (511–1011)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAC&gt;10; n (%)</td>
<td>579 (21)</td>
<td>93 (60)</td>
<td>&lt;0.001</td>
<td>71 (47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAC</td>
<td>0.5 (0–5.5)</td>
<td>30.1 (1.9–223.0)</td>
<td>&lt;0.001</td>
<td>6.7 (0.5–197.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AWT</td>
<td>1.66 (1.48–1.84)</td>
<td>1.85 (1.70–2.12)</td>
<td>&lt;0.001</td>
<td>1.90 (1.67–2.24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APB</td>
<td>0 (0–26)</td>
<td>2 (0–7)</td>
<td>&lt;0.001</td>
<td>2 (0–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR, mL/min</td>
<td>102 (98–114)</td>
<td>88 (71–108)</td>
<td>&lt;0.001</td>
<td>93 (73–111)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C, mg/L</td>
<td>0.82 (0.74–0.93)</td>
<td>0.92 (0.83–1.11)</td>
<td>&lt;0.001</td>
<td>0.93 (0.80–1.12)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range), or n (%), as appropriate. ACE indicates angiotensin-converting enzyme; ADMA, asymmetrical dimethylarginine; APB, aortic plaque burden; AWT, aortic wall thickness; BP, blood pressure; CAC, coronary artery calcium; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ESAM, endothelial cell-selective adhesion molecule; HDL-C, high density lipoprotein cholesterol; hs-cTnT, high sensitivity-cardiac troponin T; hs-CRP, high sensitivity-C reactive protein; ICAM-1, intercellular adhesion molecule 1; LDL-C, low density lipoprotein cholesterol; MACE, major adverse cardiovascular events; NTproBNP, N-terminal brain natriuretic peptide; SDMA, symmetrical dimethylarginine; and VCAM-1, vascular cell adhesion molecule 1.

*P values provided for MACE vs no MACE, or died vs not died, respectively.
Mice with genetic deletion of AGAT have low plasma homoarginine levels. Supporting the clinical data, we have previously reported an inverse correlation between homoarginine and stroke severity in this mouse model. Of particular note, this phenotype was reversible by homoarginine supplementation, suggesting a causal link between homoarginine and stroke pathogenesis.

Although the underlying mechanism linking homoarginine with cardiovascular risk remains poorly understood, involvement in NO generation is plausible because homoarginine is both a substrate for NO synthase 1 and a weak inhibitor of arginase activity. By inhibiting arginase, homoarginine can increase the availability of the NO molecule 1.4 Although this association was not supported in the present study, we found a higher concentration of the homoarginine and its precursor L-arginine, as well as with NO synthase, suggesting a causal link between homoarginine and cardiovascular risk.

Recent results from genome-wide association studies identified a strong link between plasma homoarginine and the enzyme L-arginine:glycine-amidinotransferase (AGAT).16,17 Mice with genetic deletion of AGAT have low plasma homoarginine levels.10 Supporting the clinical data, we have previously reported an inverse correlation between homoarginine and stroke severity in this mouse model. Of particular note, this phenotype was reversible by homoarginine supplementation, suggesting a causal link between homoarginine and stroke pathogenesis.8 Although the underlying mechanism linking homoarginine with cardiovascular risk remains poorly understood, involvement in NO generation is plausible because homoarginine is both a substrate for NO synthase 1 and a weak inhibitor of arginase activity.9 By inhibiting arginase, homoarginine can increase the availability of the NO molecule 1.4 Although this association was not supported in the present study, we found a higher concentration of the homoarginine and its precursor L-arginine, as well as with NO synthase, suggesting a causal link between homoarginine and cardiovascular risk.

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Data Supplement), presumably as a consequence of low renal function, as indicated by low estimated glomerular filtration rate or high cystatin C, was related to low plasma concentrations of homoarginine in our study (Table I in the online-only Data Supplement). Although an explanation for these apparently contradictory findings is still lacking, they are concordant with recent results obtained from the Hoorn Study, in which, despite a strong positive correlation of circulating homoarginine with systolic and diastolic blood pressure,29 low plasma homoarginine emerged as an independent predictor for all-cause and cardiovascular mortality.27 One possible explanation for these findings is that homoarginine increases in response to risk factors, such as obesity or hypertension but then attenuates, to some extent, their adverse cardiovascular influence. Evidence from animal models also indicates a positive association between homoarginine and metabolic parameters that are traditionally associated with CVD risk. For example, our group has recently published data showing that creatine- and homoarginine-deficient AGAT knockout mice are protected from diet-induced obesity.24 This metabolic phenotype was normalized on creatine supplementation, suggesting a homoarginine-independent effect. In line with this observation, results from the present study revealed an independent and inverse association between circulating homoarginine and AWT, despite greater risk factor burden. At this point, additional studies are required to explore and elucidate this mechanistic link.

Candidate single nucleotide polymorphism analyses in the present study revealed a graded association between plasma homoarginine and the AGAT single nucleotide polymorphism rs1288775, a known missense mutation within the AGAT gene accounting for an A→T (Gln110His) exchange.10,17 Among all ethnic groups, AA carriers had the highest and TT carriers the lowest homoarginine plasma concentrations (Table 4). In line with previous experimental and epidemiological evidence,10 these data suggest that plasma homoarginine is, at least partly, dependent on genetic variants of the AGAT gene, and moreover, irrespective of ethnic background. In addition, in our investigated US population this genotype outcome, we observed positive associations between circulating homoarginine and multiple CVD risk factors, including hypertension, obesity, insulin resistance, and dyslipidemia (Tables I and II in the online-only Data Supplement). Although an explanation for these apparently contradictory findings is still lacking, they are concordant with recent results obtained from the Hoorn Study, in which, despite a strong positive correlation of circulating homoarginine with systolic and diastolic blood pressure,29 low plasma homoarginine emerged as an independent predictor for all-cause and cardiovascular mortality.27 One possible explanation for these findings is that homoarginine increases in response to risk factors, such as obesity or hypertension but then attenuates, to some extent, their adverse cardiovascular influence. Evidence from animal models also indicates a positive association between homoarginine and metabolic parameters that are traditionally associated with CVD risk. For example, our group has recently published data showing that creatine- and homoarginine-deficient AGAT knockout mice are protected from diet-induced obesity.24 This metabolic phenotype was normalized on creatine supplementation, suggesting a homoarginine-independent effect. In line with this observation, results from the present study revealed an independent and inverse association between circulating homoarginine and AWT, despite greater risk factor burden. At this point, additional studies are required to explore and elucidate this mechanistic link.

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<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Genotype</th>
<th>Frequency</th>
<th>HOMOARGININE, μmol/L</th>
<th>P Value</th>
<th>BMI, kg/m²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>AA</td>
<td>0.43</td>
<td>3.07 (2.38–3.87)</td>
<td>...</td>
<td>29.4 (25.2–34.7)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>0.36</td>
<td>2.73 (2.14–3.40)</td>
<td>...</td>
<td>28.2 (24.5–32.5)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.21</td>
<td>2.35 (1.79–2.99)</td>
<td>&lt;0.01</td>
<td>27.6 (24.0–31.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Black</td>
<td>AA</td>
<td>0.64</td>
<td>3.06 (2.37–3.82)</td>
<td>...</td>
<td>29.6 (25.2–35.2)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>0.32</td>
<td>2.72 (2.15–3.37)</td>
<td>...</td>
<td>28.5 (24.6–33.1)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.04</td>
<td>2.41 (1.83–3.07)</td>
<td>&lt;0.01</td>
<td>28.5 (24.4–33.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>White</td>
<td>AA</td>
<td>0.08</td>
<td>2.80 (2.09–3.5)</td>
<td>...</td>
<td>27.8 (23.7–31.6)</td>
<td>...</td>
</tr>
<tr>
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<td>AT</td>
<td>0.38</td>
<td>2.57 (2.01–3.17)</td>
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<td>27.0 (23.7–31.8)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>TT</td>
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<td>2.29 (1.77–2.93)</td>
<td>&lt;0.01</td>
<td>27.5 (24.0–31.6)</td>
<td>0.60</td>
</tr>
<tr>
<td>Hispanic</td>
<td>AA</td>
<td>0.40</td>
<td>3.36 (2.65–4.16)</td>
<td>...</td>
<td>28.4 (25.8–32.9)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>0.44</td>
<td>3.00 (2.48–3.87)</td>
<td>...</td>
<td>28.8 (25.8–32.4)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.16</td>
<td>2.60 (1.90–3.37)</td>
<td>&lt;0.01</td>
<td>27.7 (23.9–31.4)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AGAT indicates L-arginine:glycine-amidinotransferase; BMI, body mass index; and SNP, single nucleotide polymorphism.

*Data presented as median (interquartile range).
seems to associate with not only circulating homoarginine concentrations but also higher BMI. This genetic link may, at least in part, explain the close correlation observed between homoarginine and BMI (Tables I and II in the online-only Data Supplement).

Limitations of this study include a low frequency of events in our population-based sample, the availability of only single measurements of homoarginine and other covariates at baseline, and that previous CVD was self-reported information. The relatively low frequency of clinical events did not allow race-specific genotype—outcome analyses. Because the statistical analyses do not account for over sampling of blacks, the findings may not be generalizable to populations with different race/ethnic composition.

In summary, our findings in a large multiethnic population-based cohort identified an independent inverse association of circulating homoarginine with MACE and with all-cause mortality. In-depth investigations are now needed to prove causality.

Acknowledgments

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

Sources of Funding

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Disclosures

Dr de Lemos has received grant support and consulting income from Roche Diagnostics and Abbott Diagnostics, and consulting income from Diadexus, Inc. The other authors report no conflicts.

References


Significance

Homoarginine is an endogenous compound related to the pathogenesis of atherosclerotic cardiovascular disease. The present study demonstrates robust inverse associations of homoarginine with major adverse cardiovascular events and all cause-mortality, after adjusting for classical cardiovascular risk factors, such as age, hypertension, body mass index, and renal dysfunction. Its predictive value in our large multiethnic US population is furthermore independent of other novel risk predictors (ie, C-reactive protein and symmetrical dimethylarginine). Homoarginine is easily measurable in plasma and potentially adjustable by either supplementation or pharmacological L-arginine:glycine-amidinotransferase modulation. Additional studies are needed to evaluate whether the regulation of plasma homoarginine might emerge as a novel therapeutic option to improve subclinical and clinical atherosclerotic impairment or even helpful to reduce cardiovascular events in the long term.
Homoarginine and Cardiovascular Outcome in the Population-Based Dallas Heart Study
Dorothee Atzler, M. Odette Gore, Colby R. Ayers, Chi-un Choe, Rainer H. Böger, James A. de Lemos, Darren K. McGuire and Edzard Schwedhelm

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Material and Methods:

Dallas Heart Study (DHS) population

The DHS is a population-based sample of adults aged 30-65 years who are residents in Dallas county, including intentional oversampling of Black individuals to comprise approximately 50% of the cohort. The study design, participant selection and phenotypical characterization have been described previously. The study protocol was approved by the University of Texas Southwestern Medical Center Institutional Review Board, and written informed consent was provided by all study participants.

Definition of variables

Race/ethnicity and smoking status were self-reported. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg based on the average of 5 measurements during the first study visit, or current use of antihypertensive drugs. Hypercholesterolemia was defined as fasting low-density lipoprotein (LDL) cholesterol ≥160 mg/dL (4.1 mmol/L) or current use of statins. Diabetes mellitus was defined as glucose ≥126 mg/dL (fasting) or ≥200 mg/dL (non-fasting), or use of glucose-lowering drugs at study entry. Obesity was defined as body mass index (BMI) ≥30 kg/m². Prior cardiovascular disease (CVD) was defined as self-reported history of myocardial infarction (MI), revascularization, congestive heart failure (CHF), or stroke. Glomerular filtration rate (eGFR) was estimated using the CKD-EPI formula. Chronic kidney disease (CKD) was defined as eGFR <60 mL/min or eGFR ≥60 mL/min and microalbuminuria ≥17 mg/L (females) or ≥25 mg/L (males).

Measurement of plasma homoarginine and other laboratory parameters

Venous blood was collected into EDTA tubes and stored at 4°C. After centrifugation, EDTA plasma was stored at <-70°C. Plasma homoarginine was determined with a high throughput mass spectrometric (MS) assay, applying electrospray ionization-liquid chromatography (LC)-MS/MS. Briefly, proteins were precipitated by adding 25 µL of EDTA plasma to 100 µL of internal standard (2.5 µmol/L [13C6]-homoarginine) dissolved in
methanol. The samples were centrifuged, evaporated, and subsequently converted to their butyl ester derivatives using 1 N butanolic hydrochloric acid. After centrifugation, the eluates were dried by heating, and redissolved in 100 µL of methanol:water (25:75) containing 0.1% ammonium formate before measurement. The plates were placed in a CTC PAL autosampler and 20-µL 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 µmol/L. Intra- and interassay coefficients of variation were ≤7.5%. L-Arginine, symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA) were measured applying LC-MS/MS as described in detail elsewhere. Cystatin C, endothelial cell-selective adhesion molecule (ESAM), vascular cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), N-terminal pro-brain-type natriuretic peptide (NTproBNP), high-sensitivity C-reactive protein (hs-CRP), and high-sensitivity cardiac troponin T (hs-cTnT) were measured using dedicated commercial assays as described elsewhere. Genomic DNA was extracted from circulating leukocytes. Genotypes for the AGAT polymorphism rs1288775 (659 A>T) were determined with the TaqMan AD assay (Applied Biosystems).

Measurement of coronary artery calcium, aortic wall thickness and plaque burden

Coronary artery calcium (CAC) was measured using a single electron beam-computed tomography (EBCT) scanner, as described in detail before. An average of two consecutive measurements were used to assess the final CAC score and CAC scores >10 Agatston units were assigned as CAC positive. Aortic wall thickness (AWT) and aortic plaque burden (APB) were determined by magnetic resonance imaging (MRI) using a 1.5-T whole-body MRI system (Intera, Philips Medical Systems). In brief, six transverse slices of the infrarenal abdominal aorta were obtained with a free-breathing, ECG-gated, T2-weighted turbo spin-echo (black-blood) sequence. Images were analyzed with the Magnetic Resonance Analytical Software Systems cardiac analysis software package (version 4.2 beta, Medis Medical Imaging Systems, Leiden, Netherlands). Luminal and adventitial aortic contours were traced manually by observers blinded to participant identifiers. AWT was
calculated by dividing the total vessel wall area by the aortic circumference in each slice, and averaging over the six slices. Total vascular area (TVA) and total plaque area (TPA) over the six slices were assessed to calculate APB by the formula: \( \text{APB} = 100 \times \frac{\text{TPA}}{\text{TVA}} \).

**Mortality and major adverse cardiovascular events assessment**

Death events were ascertained through December 31, 2010 for all subjects in the DHS using the National Death Index. Deaths were classified as CV if they included International Statistical Classification of Diseases, 10th Revision (ICD-10) codes I00-I99. Participants were contacted annually to participate in a detailed health survey regarding interval non-fatal CV events. In addition, subjects who provided consent were tracked quarterly for regional hospital admissions using the Dallas Fort Worth Hospital Council Data Initiative database that includes hospital claims data for 77 hospitals covering 28 counties in North Texas and representing 90% of the healthcare market volume in this region. This database is updated quarterly and consists of over 23 million patient encounters and over 7 million uniquely identified patients starting from 1998. Up to 25 ICD-9 diagnosis codes and 25 procedure codes were provided for each inpatient hospitalization. Greater than 90% of subjects from the initial imaging visit were followed for non-fatal events with these data sources. Primary records were requested for all suspected CV events and these events, including MI, and stroke were each adjudicated separately by two cardiologists blinded to all participant study information. Major adverse CV events (MACE) were classified as CV death, non-fatal stroke or MI.

**Statistical analyses**

Categorical variables are reported as proportions, and continuous variables are reported as medians and interquartile ranges [IQR] or means with standard deviations (SD) where appropriate. Comparison of two groups was performed with \( \chi^2 \) testing for categorical variables and Mann-Whitney-U-test for continuous. Because of the right-skewed nature of homoarginine, log-transformation was implemented in analyses of this marker as a
continuous variable. Univariable associations between log-homoarginine and continuous variables were assessed by using Spearman-rank correlation coefficients. To assess the impact of homoarginine on major cardiovascular events (MACE; comprising cardiovascular death, non-fatal myocardial infarction or stroke) and all-cause mortality across the range of plasma homoarginine concentration in the population, restricted cubic spline curves were constructed. Five knots were used at the 5th, 25th, 50th, 75th, and 95th percentiles of the homoarginine distribution. The median value of 2.8 was chosen as the reference value for the calculation of the HRs. Cox proportional hazards modeling was used to calculate hazard ratios (HR) and 95% confidence limits (CI) continuously for a one standard deviation (1 SD) change in the log-transformed value of homoarginine. A series of multivariable adjustments was performed. In model 1, age, race, and sex were included. Model 2 comprised model 1 with additional adjustment for diabetes mellitus, BMI, hypertension, hypercholesterolemia, current smoking, history of MI, CHF, or stroke, and medication (statin, β-blocker, and ACE-inhibitor). In model 3 log-transformed values of high-sensitivity C-reactive protein were added. And model 4 comprised model 3 plus glomerular filtration rate estimated using the CKD-EPI formula, and symmetric dimethylarginine. Linear regression analyses were performed to study cross-sectional associations between homoarginine and AWT, APB, and ln(CAC+1). Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). All statistical tests reported are two-sided, with significance defined by P-value <0.05.
References:


7. de Lemos JA, McGuire DK, Khera A, Das SR, Murphy SA, Omland T, Drazner MH. Screening the population for left ventricular hypertrophy and left ventricular systolic dysfunction using natriuretic peptides: Results from the dallas heart study. *Am Heart J*. 2009;157:746-753 e742


Supplemental Data

Supplemental Table I. Cross-sectional correlation analyses of log-homoarginine.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rho</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.043</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>0.10</td>
<td>&lt;0.001</td>
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</table>

Anthropometric and metabolic measurements

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<th>Variables</th>
<th>Rho</th>
<th>P-Value</th>
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</thead>
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<td>BMI</td>
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<td>&lt;0.001</td>
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<tr>
<td>Weight</td>
<td>0.183</td>
<td>&lt;0.001</td>
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<tr>
<td>Insulin</td>
<td>0.170</td>
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<tr>
<td>HOMA-IR</td>
<td>0.168</td>
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<tr>
<td>Waist/Hip Ratio</td>
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<tr>
<td>Fat mass</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.085</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.046</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Markers of renal function

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rho</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td>Cystatin C</td>
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</tr>
<tr>
<td>eGFR</td>
<td>0.071</td>
<td>&lt;0.001</td>
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<tr>
<td>Creatinine</td>
<td>0.036</td>
<td>&lt;0.05</td>
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</table>

Blood lipids

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rho</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>0.102</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.055</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>0.048</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL</td>
<td>0.046</td>
<td>&lt;0.01</td>
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Markers of vascular function

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<tr>
<th>Variables</th>
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<th>P-Value</th>
</tr>
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<tbody>
<tr>
<td>ESAM</td>
<td>-0.097</td>
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</tr>
<tr>
<td>VCAM-1</td>
<td>0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>-0.024</td>
<td>0.17</td>
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</table>
**Arginine derivatives**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>0.192</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDMA</td>
<td>-0.029</td>
<td>0.09</td>
</tr>
<tr>
<td>ADMA</td>
<td>-0.008</td>
<td>0.64</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HOMO-IR, homeostatic model assessment to quantify insulin resistance; eGFR, glomerular filtration rate estimated using the CKD EPI formula; HDL, high density lipoprotein; LDL, low density lipoprotein; ESAM, endothelial cell-selective adhesion molecule; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular cell adhesion molecule 1; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.
**Supplemental Table II. Linear regression modeling for log-homoarginine.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>β-estimate (SE)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.001 (0.001)</td>
<td>0.193</td>
</tr>
<tr>
<td>Sex, male</td>
<td>0.11 (0.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race/Ethnicity, Black</td>
<td>0.08 (0.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.04 (0.02)</td>
<td>0.010</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.05 (0.02)</td>
<td>0.015</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-0.14 (0.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low HDL</td>
<td>-0.010 (0.014)</td>
<td>0.495</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0.03 (0.02)</td>
<td>0.145</td>
</tr>
<tr>
<td>BMI</td>
<td>0.008 (0.001)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prior MI</td>
<td>-0.03 (0.04)</td>
<td>0.494</td>
</tr>
<tr>
<td>Prior CHF</td>
<td>-0.03 (0.04)</td>
<td>0.461</td>
</tr>
<tr>
<td>Prior stroke</td>
<td>-0.09 (0.04)</td>
<td>0.037</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.0016 (0.0004)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

For the continuous variables, β-estimates are shown for a 1 unit change in the variable. SE indicates standard error; HDL, high density lipoprotein; BMI, body mass index; MI, myocardial infarction, CHF, congestive heart failure, eGFR, glomerular filtration rate estimated using the CKD-EPI formula.