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Asymmetrical Dimethylarginine Independently Predicts Total and Cardiovascular Mortality in Individuals with Angiographic Coronary Artery Disease (The Ludwigshafen Risk and Cardiovascular Health Study)

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Background: **Asymmetrical dimethylarginine (ADMA) is increased in conditions associated with increased risk of atherosclerosis. We investigated the use of ADMA to predict total and cardiovascular mortality in patients scheduled for coronary angiography.**

Methods: **In 2543 persons with and 695 without coronary artery disease (CAD) identified by angiography we measured ADMA and recorded total and cardiovascular mortality during a median follow-up of 5.45 years.**

Results: **ADMA was correlated positively to age, female sex, diabetes mellitus, former and current smoking, and C-reactive protein and inversely to HDL cholesterol and triglycerides. ADMA was not associated with body mass index, hypertension, LDL cholesterol, or the presence or absence of angiographic CAD. Glomerular filtration rate and homocysteine were the strongest predictors of ADMA. At the 2nd, 3rd and 4th quartile of ADMA, hazard ratios for all-cause mortality adjusted for age, sex, and cardiovascular risk factors were 1.12 [95% confidence interval (CI) 0.83–1.52], 1.35 (95% CI 1.01–1.81), and 1.87 (95% CI 1.43–2.44), respectively, compared with the 1st quartile. Hazard ratios for cardiovascular death were 1.13 (95% CI 0.78–1.63), 1.42 (95% CI 1.00–2.02), and 1.81 (95% CI 1.31–2.51). ADMA in the highest quartile remained predictive of mortality after accounting for medication at baseline. The predictive value of ADMA was similar to that in the entire cohort in persons with CAD, stable or unstable, but was not statistically significant in persons without angiographic CAD.**

Conclusions: **ADMA concentration predicts all-cause and cardiovascular mortality in individuals with CAD independently of established and emerging cardiovascular risk factors.**

Asymmetrical dimethylarginine (ADMA),[6] an endogenous inhibitor of endothelial nitric oxide synthase, is increased in conditions associated with increased risk of atherosclerosis, such as impaired renal function *(1–4)*, hypercholesterolemia *(5)*, hypertriglyceridemia *(6)*, insulin resistance *(7)*, diabetes mellitus *(8)*, hyperhomocysteinemia *(9)*, and hypertension *(10)*. ADMA may contribute to endothelial dysfunction *(1, 11)*.

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[6] Nonstandard abbreviations: ADMA, asymmetrical dimethylarginine; ESRD, end-stage renal disease; CAD, coronary artery disease; LURIC, Ludwigshafen Risk and Cardiovascular Health study; CRP, C-reactive protein; NSTEMI, non–ST-elevation myocardial infarction; MI, myocardial infarction, STEMI, ST-elevation MI; GFR, glomerular filtration rate; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; BMI, body mass index; HR, hazard ratio; DDAH, dimethyl-arginine dimethylaminohydrolase.

A few small studies have addressed the ability of ADMA to predict mortality and have found that high ADMA was related to mortality in critically ill patients *(12, 13)* and to unfavorable pulmonary hemodynamics and mortality *(14)*. ADMA was predictive of cardiovascular events and total mortality in kidney disease *(15)*, end-stage renal disease (ESRD) *(3)*, and coronary artery disease (CAD) *(16–18)*. We investigated the value of ADMA to predict total mortality in patients scheduled for coronary angiography.

Materials and Methods

STUDY DESIGN AND PARTICIPANTS

We studied participants of the Ludwigshafen Risk and Cardiovascular Health (LURIC) study *(19)*. Inclusion criteria were: German ancestry, clinical stability except for acute coronary syndromes, and the availability of a coronary angiogram. The indications for angiography in individuals in clinically stable condition were chest pain and/or noninvasive test results consistent with myocardial ischemia. Individuals suffering from acute illness other than acute coronary syndromes, chronic noncardiac diseases, or malignancy within the 5 past years, and those unable to understand the purpose of the study were excluded. The study was approved by the ethics committee at the "Ärztekammer Rheinland-Pfalz". Informed written consent was obtained from all participants.

CAD was assessed by angiography with maximum luminal narrowing estimated by visual analysis. Clinically relevant CAD was defined as the occurrence of ≥1 stenosis of ≥20% in ≥1 of 15 coronary segments. Individuals with stenoses <20% were considered as not having CAD. The severity of CAD was quantified with the Friesinger score.

Diabetes mellitus was diagnosed if plasma glucose was >1.25 g/L in the fasting state, or >2.00 g/L 2 h after an oral glucose load *(20)*, or if individuals were receiving antidiabetic treatment. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mmHg or if there was a history of hypertension, evident through the use of antihypertensive drugs. Women were categorized into pre-, peri- or postmenopausal according to menstrual bleeding history and the concentrations of follicle-stimulating hormone *(19)*.

Measurements of ADMA, lipoproteins, C-reactive protein (CRP), fibrinogen, creatinine, and homocysteine were complete in 3238 of 3279 individuals with coronary angiograms. Study participants included 928 patients who presented within 7 days after onset of unstable angina, non–ST-elevation myocardial infarction (MI) (NSTEMI, troponin T >0.1 μg/L), or ST-elevation MI (STEMI, troponin T >0.1 μg/L) because the mean (SD) ADMA concentrations in these patients [827 (145) nmol/L] were close to those in the stable CAD patients [829 (150) nmol/L].

Information on vital status was obtained from local registries. No patients were lost to follow-up. Of the 3238 persons studied, 497 deaths (15.3%) occurred during a median follow-up of 5.45 years. Death certificates were missing for 15 decedents (3%) who were included in the total mortality analysis but excluded from the cardiovascular mortality analysis. Cardiovascular death included the following: sudden death, fatal MI, death because of congestive heart failure, death immediately following intervention to treat CAD, fatal stroke, and other causes of death due to CAD.

LABORATORY PROCEDURES

To perform ADMA and all other analyses we collected fasting blood samples before angiography to rule out ADMA alterations attributable to food intake *(21)*. The standard laboratory methods have been described *(19)*. Glomerular filtration rate (GFR) was calculated as GFR $[mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}] = 186 \cdot creatinine^{-1.154} \cdot age^{-0.203}$ and GFR $[mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}] = 138 \cdot creatinine^{-1.154} \cdot age^{-0.203}$ in males and in females, respectively *(22)*.

ADMA was measured in frozen serum (−80 °C) with reversed-phase HPLC *(23)*. Within-day and between-day CVs were 3.1% (620 nmol/L) and 1.0% (2000 nmol/L), and 9% (620 nmol/L) and 1.5% (2000 nmol/L), respectively. CRP was measured with high sensitivity immunonephelometry (Dade Behring), and fibrinogen with the Clauss method (Dade Behring).

STATISTICAL ANALYSIS

Triglycerides and CRP were transformed logarithmically. We established quartiles of continuous variables according to the values in individuals without CAD. Characteristics of individuals with and without CAD are presented as percentages for categorical variables and as mean (SD) or median and 25th and 75th percentiles for continuous variables. Associations of categorical and continuous variables were analyzed by logistic regression and univariate ANOVA, respectively, with covariables as indicated (Table 1). Using ANOVA models in which we included those factors not under examination as covariables, we studied the effects on ADMA of sex, age, CAD, and cardiovascular risk factors (Table 2) and of drugs. We used the Cox proportional hazards model to examine the relationship between ADMA and mortality (Tables 3 and 4). As indicated by log-minus-log diagnostic plots, the proportional hazards assumption was met.

Multivariable adjustment was carried out for sex, age, CAD, cardiovascular risk factors, and drugs (Tables 3 and 4). All statistical tests were 2-sided; $P < 0.05$ was considered significant. The SPSS 11.0 statistical package (SPSS Inc.) was used.

Results

STUDY PARTICIPANTS

Compared with the group without CAD, CAD patients were significantly older, more likely to be current or past smokers, and had higher prevalence of diabetes mellitus,

Table 1. Clinical and biochemical characteristics of study participants at baseline.

	No CAD (n = 695)	CAD (n = 2543)	*P*[a]
Age, years	58 (12)	64 (10)	<0.001
Male sex, %	52	75	<0.001[b]
BMI, kg/m^2	27 (4)	28 (4)	0.376
Diabetes mellitus, %	18	36	<0.001
Systemic hypertension, %	63	75	0.001
Smoking			
Never, %	52	32	
Past, %	30	48	
Current, %	18	20	<0.001
Previous MI, %	–	53	–
Peripheral vascular disease, %	2	12	<0.001
Cerebrovascular disease, %	5	9	<0.019
Systolic blood pressure, mmHg	136 (22)	143 (24)	<0.009[c]
Diastolic blood pressure, mmHg	80 (11)	81 (11)	0.433[c]
Fasting blood glucose, g/L	1.05 (0.28)	1.16 (0.37)	<0.001
LDL-C, g/L	1.20 (0.31)	1.16 (0.35)	<0.001[d]
HDL-C, g/L	0.43 (0.12)	0.38 (0.10)	<0.001[d]
Triglycerides, g/L median (25th and 75th percentile)	1.33 (0.97–1.94)	1.50 (1.13–2.02)	<0.001[d,e]
Fibrinogen, g/L	3.55 (0.83)	4.08 (1.10)	<0.001
CRP, mg/L median (25th and 75th percentile)	2.2 (1.0–6.0)	3.8 (1.5–9.2)	<0.001[d,e]
GFR, mL/min	83 (18)	81 (19)	0.829
Homocysteine, μmol/L	12 (5)	14 (6)	0.006[d]

Values are mean (SD) unless otherwise noted.
[a] Analysis of variance or logistic regression, respectively, adjusted for age and gender.
[b] Logistic regression, adjusted for age only.
[c] Adjusted for use of beta blockers, ACE inhibitors, AT1 receptor antagonists, calcium channel blockers, diuretics, and lipid-lowering agents.
[d] Adjusted for use of lipid-lowering agents.
[e] ANOVA of logarithmically transformed values.

hypertension, and cerebrovascular and peripheral artery disease. A history of MI occurred in 53% of the CAD patients. The CAD patients had higher systolic blood pressure, fasting glucose, fibrinogen, homocysteine, and triglycerides and lower HDL cholesterol (HDL-C). Unadjusted LDL cholesterol (LDL-C) was lower in individuals with CAD than those without CAD ($P = 0.002$). 57% of the CAD patients received lipid-lowering drugs compared with 18% of those without CAD. In accounting for this we found that LDL-C was significantly higher ($P < 0.001$) in CAD patients (adjusted mean: 1.20 g/L) than individuals without CAD (adjusted mean: 1.16 g/L). CRP and fibrinogen were higher in CAD patients, a finding related to the presence of patients with acute coronary syndromes in the CAD group. Body mass index (BMI), diastolic blood pressure, and GFR were similar in both groups (Table 1).

ASSOCIATION OF ADMA WITH CARDIOVASCULAR RISK FACTORS, CAD STATUS, AND MEDICATION

For study participants as a whole, ADMA was significantly higher in women than in men ($P < 0.001$). ADMA in pre- or perimenopausal women was similar to that in men, whereas ADMA was significantly higher in postmenopausal women (Table 2). ADMA was positively related to age, diabetes mellitus, former and current smoking, CRP, and homocysteine. ADMA was increased at the lowest quartile of HDL-C and was decreased at the highest quartile of triglycerides. The GFR was inversely related to ADMA. We found no statistically significant relationships between ADMA and hypertension, LDL-C, or BMI.

Unadjusted ADMA concentrations were higher in patients with CAD than in persons without CAD (828 vs 814 nmol/L, $P = 0.022$), but this difference was not seen on multivariate analysis. When we stratified our cohort based on the severity of angiographic changes (the strata of the Friesinger scores were 0–1, 1.01–4, 4.01–8, and 8.01–15), unadjusted ADMA was higher in the 2nd, 3rd, and 4th strata ($P < 0.001$, $P = 0.031$, $P = 0.003$, respectively) compared with the lowest stratum, but these differences were also insignificant on multivariate analysis ($P = 0.075$, $P = 0.219$, $P = 0.450$, respectively). By multiple linear regression with forward selection, significant predictors of ADMA were selected in the order of GFR > homocysteine > age > CRP > HDL-C > sex > triglycerides > smoking status > diabetes mellitus. The overall R^2 was 0.095, suggesting that <10% of the variation of ADMA was explained by the selected covariates.

ADMA may be increased in morbid obesity *(24)*, but we observed no significant difference when we compared ADMA between study participants with a BMI <25 kg/m^2 (n = 903) and those with a BMI >35 kg/m^2 (n =

Table 2. Association of cardiovascular risk factors and angiographic status with ADMA.

		ADMA, nmol/L[a]	Difference, %[b]	P[c]
Gender and menopausal status				
Men	(n = 2258)	817 (811–823)		
Women, pre- or perimenopausal	(n = 98)	806 (777–836)	−1.3	NS
Women, postmenopausal	(n = 882)	848 (837–858)	+3.7	0.01
Age, years				
<60	(n = 1196)	807 (799–816)		
60–70	(n = 1171)	828 (820–836)	+2.6	0.001
>70	(n = 871)	845 (835–856)	+4.7	<0.001
CAD				
No	(n = 695)	830 (819–841)		
Yes	(n = 2543)	824 (818–829)	−0.7	NS
BMI, kg/m^2				
≤26 or 27[d]	(n = 1518)	827 (820–835)		
≥26 or 27[d]	(n = 1720)	823 (816–830)	−0.5	NS
Diabetes mellitus				
No	(n = 2204)	821 (815–827)		
Yes	(n = 1034)	833 (824–842)	+1.5	0.032
Hypertension				
No	(n = 882)	826 (816–836)		
Yes	(n = 2356)	825 (819–830)	−0.1	NS
Smoking				
Never	(n = 1165)	815 (806–823)		
Former	(n = 1441)	827 (819–834)	+1.5	0.046
Current	(n = 632)	840 (828–852)	+3.1	0.001
LDL-C, g/L				
1st quartile (<1.00)	(n = 1044)	832 (823–840)		
2nd quartile (1.00–1.19)	(n = 774)	828 (818–838)	−0.5	
3rd quartile (1.20–1.40)	(n = 693)	818 (807–828)	−1.7	
4th quartile (≥1.41)	(n = 727)	819 (809–830)	−1.6	NS
HDL-C, g/L				
4th quartile (≥0.50)	(n = 479)	802 (788–815)		
3rd quartile (0.42–0.49)	(n = 644)	820 (808–831)	+2.2	0.200
2nd quartile (0.34–0.41)	(n = 880)	827 (818–837)	+3.1	0.033
1st quartile (<0.34)	(n = 1235)	835 (827–844)	+4.1	0.001
Triglycerides, g/L				
1st quartile (<0.97)	(n = 571)	829 (817–842)		
2nd quartile (0.97–1.32)	(n = 764)	832 (822–842)	0.4	0.718
3rd quartile (1.33–1.94)	(n = 1018)	828 (820–837)	−0.1	0.872
4th quartile (≥1.95)	(n = 885)	812 (803–822)	−2.1	0.038
CRP, mg/L				
<3	(n = 1511)	815 (807–823)		
3–10	(n = 1068)	832 (823–840)	+2.1	0.007
≥10	(n = 659)	836 (823–850)	+2.6	0.019
Fibrinogen, g/L				
1st quartile (<2.98)	(n = 503)	814 (800–828)		
2nd quartile (2.98–3.44)	(n = 674)	824 (813–835)	+1.2	
3rd quartile (3.45–3.99)	(n = 750)	818 (808–828)	+0.5	
4th quartile (≥400)	(n = 1311)	834 (825–843)	+2.5	NS
GFR, mL/min				
≥90	(n = 1098)	816 (807–825)		
60–89	(n = 1785)	819 (813–826)	+0.4	0.529
30–59	(n = 332)	872 (856–888)	+6.9	<0.001
<30	(n = 23)	1030 (971–1089)	+26.2	<0.001
Homocysteine, μmol/L				
1st quartile (<9.2)	(n = 606)	801 (790–813)		
2nd quartile (9.2–11.1)	(n = 648)	810 (799–821)	+1.1	0.285
3rd quartile (11.2–14.1)	(n = 879)	826 (816–835)	+3.1	0.001
4th quartile (≥14.2)	(n = 1105)	847 (838–822)	+5.7	<0.001

[a] Estimated marginal means and 95% confidence intervals obtained in a general linear model (ANOVA) adjusted for each of the other factors, whereby age, BMI, GFR, fibrinogen, C-reactive protein, LDL-C, HDL-C, triglycerides, and homocysteine were included as continuous rather than categorical covariables. NS, not significant.

[b] Compared to the first category of each variable.

[c] Post hoc pairwise comparisons with the first category of each variable, reported if the overall $P < 0.05$.

[d] Thresholds of 26 and 27 kg/m^2 apply to males and females, respectively.

Table 3. HR for death from all causes according to ADMA.

ADMA, nmol/L	Model 1 HR (95% CI)	*P*	Model 2 HR (95% CI)	*P*	Model 3 HR (95% CI)	*P*
All individuals (n = 3238)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.09 (0.81–1.48)	0.572	1.12 (0.83–1.52)	0.460	1.107 (0.79–1.45)	0.660
3rd quartile (801–889)	1.40 (1.05–1.87)	0.022	1.35 (1.01–1.81)	0.042	1.26 (0.94–1.69)	0.115
4th quartile (≥890)	2.04 (1.57–2.66)	<0.001	1.87 (1.43–2.44)	<0.001	1.60 (1.22–2.09)	0.001
per quartile[a]	1.30 (1.19–1.41)	<0.001	1.25 (1.15–1.36)	<0.001	1.18 (1.09–1.29)	<0.001
Angiographic CAD (n = 2543)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.18 (0.85–1.63)	0.326	1.21 (0.88–1.68)[b]	0.240	1.16 (0.84–1.61)[b]	0.359
3rd quartile (801–889)	1.46 (1.07–2.01)	0.018	1.40 (1.02–1.92)[b]	0.038	1.30 (0.95–1.79)[b]	0.103
4th quartile (≥890)	2.15 (1.62–2.87)	<0.001	2.00 (1.50–2.67)[b]	<0.001	1.71 (1.28–2.29)[b]	<0.001
per quartile[a]	1.31 (1.20–1.43)	<0.001	1.27 (1.16–1.38)[b]	<0.001	1.20 (1.10–1.31)[b]	<0.001
Stable CAD (n = 1518)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.10 (0.74–1.64)	0.647	1.15 (0.77–1.72)[b]	0.240	1.06 (0.71–1.58)[b]	0.788
3rd quartile (801–889)	1.34 (0.90–1.99)	0.147	1.30 (0.87–1.95)[b]	0.194	1.25 (0.84–1.87)[b]	0.268
4th quartile (≥890)	2.10 (1.48–2.98)	<0.001	1.78 (1.25–2.53)[b]	0.001	1.55 (1.08–2.22)[b]	0.017
per quartile[a]	1.31 (1.17–1.46)	<0.001	1.22 (1.09–1.36)[b]	<0.001	1.17 (1.05–1.31)[b]	0.005
Unstable CAD (n = 928)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.65 (0.91–3.01)	0.100	1.67 (0.91–3.05)[b]	0.097	1.70 (0.93–3.11)[b]	0.086
3rd quartile (801–889)	1.99 (1.11–3.56)	0.020	1.88 (1.04–4.00)[b]	0.036	1.65 (1.91–2.98)[b]	0.036
4th quartile (≥890)	2.49 (1.42–4.34)	<0.001	2.40 (1.36–4.23)[b]	0.003	2.10 (1.19–3.70)[b]	0.011
per quartile[a]	1.31 (1.12–1.52)	0.001	1.28 (1.10–1.51)[b]	0.002	1.22 (1.04–1.43)[b]	0.016

Model 1: adjusted for age and gender.

Model 2: in addition adjusted for presence or absence of CAD on angiography, BMI, type 2 diabetes, hypertension, smoking status, LDL-C, HDL-C, triglycerides, CRP, fibrinogen, GFR, and homocysteine. All continuous variables were treated as such in the modeling.

Model 3: in addition adjusted for ACE inhibitors, antiplatelet agents, lipid-lowering drugs, and diuretics.

[a] The HRs are the ratios associated with an increase of one quartile in ADMA.

[b] Presence or absence of CAD on angiography not included as a covariable.

137) or 40 kg/m^2 (n = 30), respectively. Systolic blood pressure has also been reported to be related to ADMA *(25)*, but we found no evidence of such a relationship whether or not we adjusted for confounding variables (age, sex, risk factors, and use of antihypertensive drugs).

We investigated whether common medications were associated with ADMA. In users of ACE inhibitors (+1.5%, $P = 0.015$) and diuretics (+3.6%, $P < 0.001$), ADMA was significantly higher than in nonusers, whereas it was lower in users of lipid-lowering (97% statins, −2.1%, $P < 0.001$) and antiplatelet drugs (−2.2%, $P = 0.001$). Administration of β-blockers, AT1 receptor antagonists, calcium channel blockers, and insulin or oral anti-diabetics was not related to ADMA.

ADMA and mortality from all causes

Among the 3238 persons studied, 497 deaths (15.3%) occurred. Compared with patients in the lowest ADMA quartile, the age and sex-adjusted hazard ratios (HR) for death in the 2nd, 3rd and 4th quartiles were 1.09, 1.40, and 2.04, respectively (Table 3, Fig. 1). Inclusion of cardiovascular risk factors as covariables changed these estimates to 1.12, 1.35, and 1.87, respectively (Table 3). Although HRs decreased, ADMA retained prognostic importance after additional adjustment for the use of drugs significantly associated with ADMA (Table 3). Per quartile of ADMA, the HRs of death were 1.29, 1.23, and 1.16, respectively, in the 3 models incrementally adjusted for age and sex, risk factors, and the use of drugs (Table 3).

Among the 2543 patients with CAD, 440 died (17.3%). In this subgroup, HRs for death were slightly higher than those in the entire cohort (Table 3).

Only 57 deaths (8.2%) occurred among the 695 study participants without CAD. Although there was a tendency toward an increased risk of death at high ADMA concentrations in this group (unadjusted HR of 1.88 for the 4th compared with the 1st quartile), the association did not reach statistical significance.

Among the patients with CAD, 1518 were studied at least 14 days past the onset of symptoms of an acute coronary event and were considered as having stable CAD. Nine hundred twenty eight patients underwent angiography within 7 days after the onset of symptoms of acute coronary syndromes (unstable angina, NSTEMI, or STEMI) and were classified as having unstable CAD. In this subgroup of patients, 277 (18.2%) and 150 deaths (16.1%), respectively, occurred in the stable and unstable CAD patients. In both groups, we found consistent and

Table 4. HR for death from cardiovascular causes according to ADMA.

ADMA (nmol/L)	Model 1 HR (95% CI)	P	Model 2 HR (95% CI)	P	Model 3 HR (95% CI)	P
All individuals (n = 3223)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.10 (0.76–1.58)	0.622	1.13 (0.78–1.63)	0.460	1.07 (0.74–1.55)	0.707
3rd quartile (801–889)	1.50 (1.06–2.13)	0.022	1.42 (1.00–2.02)	0.048	1.32 (0.93–1.87)	0.123
4th quartile (≥890)	2.00 (1.45–2.77)	<0.001	1.81 (1.31–2.51)	<0.001	1.52 (1.01–2.12)	0.012
per quartile[a]	1.29 (1.16–1.42)	<0.001	1.23 (1.11–1.36)	<0.001	1.16 (1.05–1.29)	0.004
Angiographic CAD (n = 2528)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.20 (0.81–1.78)	0.326	1.24 (0.84–1.84)[b]	0.280	1.19 (0.80–1.77)[b]	0.391
3rd quartile (801–889)	1.60 (1.09–2.34)	0.015	1.50 (1.03–2.20)[b]	0.036	1.34 (0.95–2.03)[b]	0.092
4th quartile (≥890)	2.16 (1.52–3.07)	<0.001	1.99 (1.34–2.84)[b]	<0.001	1.68 (1.18–2.41)[b]	0.004
per quartile[a]	1.31 (1.18–1.46)	<0.001	1.26 (1.13–1.40)[b]	<0.001	1.19 (1.07–1.33)[b]	0.002
Stable CAD (n = 1510)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	1.13 (0.70–1.80)	0.624	1.16 (0.73–1.86)[b]	0.529	1.07 (0.67–1.72)[b]	0.769
3rd quartile (801–889)	1.36 (0.86–2.17)	0.194	1.30 (0.81–2.08)[b]	0.278	1.24 (0.77–1.98)[b]	0.373
4th quartile (≥890)	2.05 (1.36–3.10)	<0.001	1.72 (1.14–2.62)[b]	0.011	1.52 (0.99–2.32)[b]	0.053
per quartile[a]	1.29 (1.14–1.47)	<0.001	1.20 (1.06–1.37)[b]	0.005	1.16 (1.02–1.32)[b]	0.028
Unstable CAD (n = 922)						
1st quartile (<720)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (720–800)	2.03 (0.90–4.62)	0.090	2.06 (0.90–4.71)[b]	0.087	2.12 (0.92–4.84)[b]	0.076
3rd quartile (801–889)	2.79 (1.27–6.14)	0.011	2.61 (1.17–5.78)[b]	0.019	2.21 (0.99–4.92)[b]	0.053
4th quartile (≥890)	3.21 (1.49–6.92)	<0.003	3.06 (1.40–6.69)[b]	0.005	2.62 (1.20–5.74)[b]	0.016
per quartile[a]	1.38 (1.13–1.68)	0.001	1.35 (1.10–1.66)[b]	0.004	1.27 (1.03–1.56)[b]	0.024

Model 1: adjusted for age and gender.

Model 2: in addition adjusted for presence or absence of CAD on angiography, BMI, type 2 diabetes, hypertension, smoking status, LDL-C, HDL-C, triglycerides, CRP, fibrinogen, GFR, and homocysteine. All continuous variables were treated as such in the modeling.

Model 3: in addition adjusted for the use of ACE inhibitors, antiplatelet agents, lipid-lowering drugs, and diuretics.

[a] The HRs are the ratios associated with an increase of one quartile in ADMA.

[b] Presence or absence of CAD on angiography not included as a covariable.

robust associations of ADMA with mortality from all causes, with slightly higher HRs in patients with unstable CAD (Table 3).

ADMA and mortality from cardiovascular causes

Death certificates were not available from 15 deceased persons, therefore the analysis for cardiovascular mortality included a total of 3223 individuals. Among these, 334 (10.4%) died from cardiovascular causes, 31 (1%) died from fatal infection, 55 (1.7%) died from fatal cancer, and 62 (1.9%) died from miscellaneous causes. HRs for death from cardiovascular causes according to ADMA were similar to those obtained for mortality from all causes in all models and across all subgroups examined, whereby the HRs for mortality from cardiovascular causes were slightly higher than the HRs for all-cause mortality in patients presenting with unstable CAD (Table 4, Fig. 1). Compared with the lowest quartile, there were also increased HRs for death from noncardiovascular causes in the highest quartile of ADMA (HR 2.18, adjusted for age and sex).

Discussion

We demonstrated that ADMA is associated with all-cause and cardiovascular mortality in stable und unstable CAD, regardless of established and emerging cardiovascular risk factors. We noticed a tendency toward an increased risk of death at high ADMA concentrations in individuals without CAD as well, but this did not reach statistical significance.

To identify confounding variables, we examined the relationship between ADMA and conditions involved in the development of endothelial dysfunction or CAD. Multiple linear regression analysis revealed GFR as the strongest predictor of ADMA, which supports previous findings of increased ADMA in ESRD *(1–3)*. Two mechanisms may account for the relationship between ADMA and renal function. First, ADMA itself is eliminated by renal excretion. Second, the enzyme dimethyl-arginine dimethylaminohydrolase (DDAH), which converts ADMA to citrulline and dimethylamine, is highly expressed in the kidney, so that a deterioration of renal function may go along with a decrease in DDAH *(26)*. We emphasize, however, that the association of ADMA with

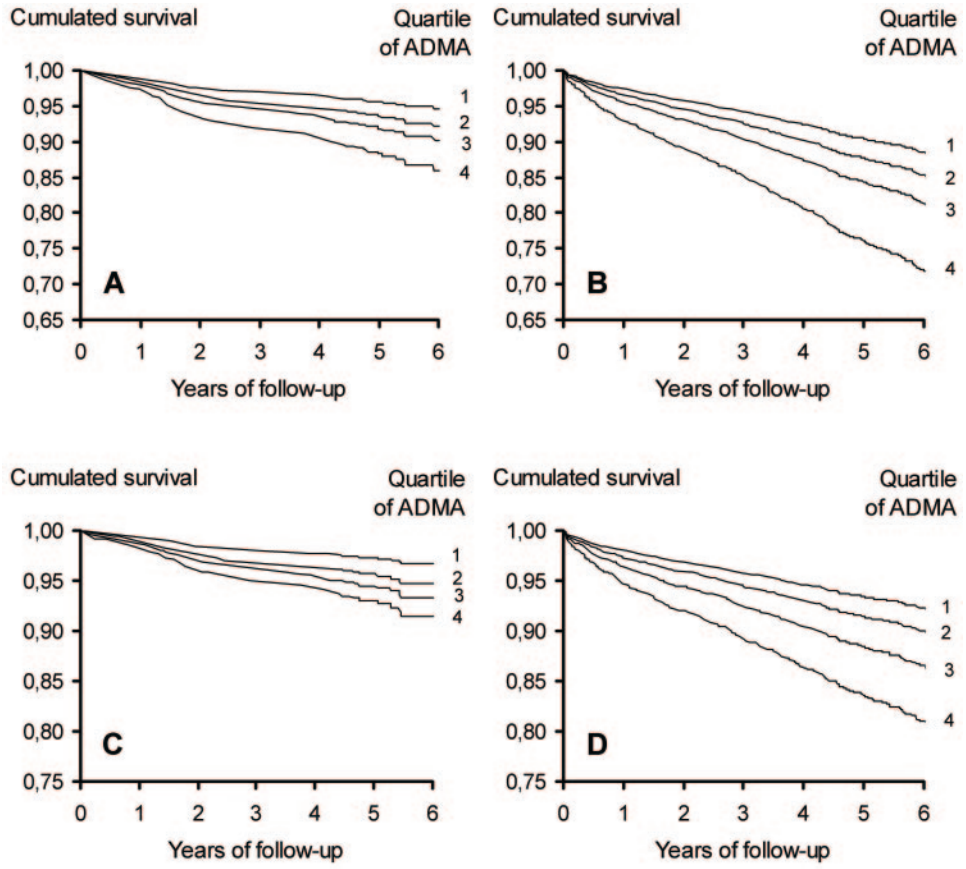

Fig. 1. Cumulated survival functions according to quartiles of ADMA in individuals with (*panels B* and *D*) or without (*panels A* and *C*) angiographic CAD.

Panels A and B: total mortality. *Panels C and D*: cardiovascular mortality. For HRs and CIs cf. Tables 3 and 4.

mortality from all causes (HR per quartile 1.27, 95% CI 1.17–1.39) and cardiovascular causes (HR per quartile 1.23, 95% CI 1.10–1.37) also occurred among individuals with GFRs >60 mL/min and was thus independent of renal function.

The 2nd most important predictor of ADMA was plasma homocysteine. ADMA has been suggested to link hyperhomocysteinemia and endothelial dysfunction *(9)*. Although not shown consistently *(27, 28)*, experimental hyperhomocysteinemia may raise ADMA *(29, 30)*. Three mechanisms may underlie the correlation between homocysteine and ADMA. First, because the formation of homocysteine and ADMA requires L-methionine as a methyl group donor, expansion of the L-methionine pool may fuel both pathways at an increased rate *(31)*. Second, homocysteine may inhibit the degradation of ADMA *(31)*. Third, as homocysteine is eliminated by the kidney, both ADMA and homocysteine may concurrently accumulate once the GFR declines. Partial correlation analysis suggests that the latter mechanism might indeed be operative: When we controlled for the GFR, the correlation coefficient between homocysteine and ADMA decreased from 0.212 (n = 3238, $P < 0.001$) to 0.140 (n = 3238, $P < 0.001$), which also reflects the strong and well-known *(32)* association between homocysteine and GFR ($r = -0.390$, n = 3238, $P < 0.001$).

In women ADMA was higher than in men. This difference obviously originates from the fact that ADMA increases after menopause *(33)*, and that the vast majority of the women participating in the LURIC study were postmenopausal.

The finding that ADMA was higher in smokers than in nonsmokers is in line with recent findings *(34)*, but contrasts with a study in 563 elderly men in which smokers had lower ADMA than nonsmokers *(35)*. However, the difference was no longer significant after adjustment for multiple confounding factors.

The relationship between LDL-C and ADMA has remained elusive. In their study of 49 hypercholesterolemic adults, Boger et al. *(5)* found an association between ADMA and LDL-C. This observation has not been confirmed by others *(7, 21, 36)* or by the current study.

Intravenous administration of ADMA increased systemic vessel resistance and arterial blood pressure *(37)*, and systolic blood pressure has been found to be related to ADMA *(25, 38)*. These findings, however, have not been confirmed by all studies. Interestingly, acute infusion of ADMA in healthy men, which led to an increase in mean (SD) plasma ADMA from 950 (270) to 22 950 (4910) nmol/L, increased mean arterial pressure from 83.9 (1.1) mmHg to only 88.3 (1.2) mmHg, *(39)*, and mice with low ADMA as a consequence of DDAH overexpression ex-

hibit a modest decrease in blood pressure *(40)*. In the current study, we did not detect increased ADMA in hypertensive patients nor were we able to prove a relationship between ADMA and blood pressure. Taken together, the effect of ADMA on blood pressure may thus be minor.

Increased ADMA has been shown to coincide with other features of the metabolic syndrome *(6, 8, 10)*. Krzyzanowksa and colleagues *(24)* found ADMA to be significantly increased in women with morbid obesity [n = 34, mean (SD) BMI 49 (1) kg/m^2]. We did not detect any such association, however, and therefore suggest that variations in BMI across the commonly observed range would not appreciably affect ADMA.

ADMA has been found to be increased in persons with normotensive insulin-resistance *(7)* and diabetes mellitus *(8)*. Consistently, ADMA was higher in diabetic than in nondiabetic individuals and inversely related to HDL-C. Finally, our results showed a significant correlation of ADMA with CRP and a tendency toward high ADMA at high fibrinogen. These findings are in line with reports that ADMA is increased in low-grade systemic inflammation *(24)*.

Our attempt to identify confounding nonrandomized medications revealed higher ADMA in users of ACE inhibitors, a result that contrasts with a report suggesting that ACE inhibitors lower ADMA *(41)*. Patients receiving diuretics had higher ADMA than those not on diuretics. Confirming some *(42)* but not all *(36, 43–46)* pertinent earlier work, individuals treated with lipid-lowering drugs (97% statins) had lower ADMA than those untreated. The use of antiplatelet drugs was associated with lower ADMA. Insulin and oral antidiabetics were not related to ADMA, as would have been expected from earlier research *(7, 13, 47)*. We stress, however, that these associations are cross-sectional in nature and do not prove any pharmacological effect of the drugs on ADMA.

So far, few small prospective studies have addressed the predictive value of ADMA for total mortality and cardiovascular mortality. In patients with mild to advanced chronic kidney disease, ADMA was a strong and independent risk marker for progression to ESRD and death *(15)*. Zoccali et al. *(3)* examined 225 hemodialysis patients with a mean ADMA of 2520 nmol/L (interquartile range 1580–3850 nmol/L). Compared with the 1st and 2nd quartile, HRs for death at ADMA concentrations in the 3rd and 4th quartile were 1.72 and 3.11, respectively. An even higher mean ADMA concentration (3060 nmol/L) and corresponding associations with all-cause mortality and cardiovascular mortality were seen in a subsequent study of the same group *(48)*. The overall risk of death and cardiovascular events is excessive in ESRD. In the 2 ESRD studies *(3, 48)*, the annual mortality rate was nearly 5-fold that in the current study. The high cardiovascular mortality of dialyzed patients is not solely explained by classical risk factors. Uremia-specific risk factors may be important. Findings in ESRD, including interpretation of ADMA concentrations, are therefore difficult to extrapolate to other situations. Although we found relative increments of risk by quartiles of ADMA similar to those in ESRD *(3, 48)*, our median and 75th percentile values of ADMA were one fourth to one fifth of the values in ESRD *(3, 48)*. Thus, our work extends the information on ADMA and all-cause mortality in ESRD to a lower range of ADMA.

In a small nested case-control study of middle-aged nonsmoking men from Eastern Finland with (n = 70) or with no (n = 80) CAD, Valkonen et al. *(16)* observed a 3.9-fold increase in the risk of cardiovascular events in the highest quartile of ADMA compared with the remaining quartiles. The association of ADMA and acute coronary events was not significant in men with no history of CAD, nor was it significant in smokers. The relationship of ADMA and all-cause mortality was not reported. To compare our results with those by Valkonen et al. *(16)*, we separately considered men and women, individuals who did not have a history of STEMI, and current or previous smokers. ADMA attained virtually identical prognostic importance in women and men. Furthermore, the predictive value of ADMA in former or current smokers was equivalent to that in the entire sample. ADMA also stayed predictive of total mortality in individuals with no previous STEMI (data not shown). Thus, by demonstrating that ADMA predicts all-cause and cardiovascular death in persons at intermediate risk regardless of sex, smoking status, or previous MI, our results add to those of Valkonen et al. *(16)*.

Lu et al. *(17)* studied ADMA in 153 Chinese stable CAD patients undergoing percutaneous coronary intervention. Consistent with the current study, Lu et al. found ADMA to be associated with future death or nonfatal MI *(17)*, but our study extends their observations by showing an association of high ADMA with adverse outcomes in patients with acute coronary syndromes. Schnabel et al. *(18)* reported that ADMA was predictive of death from cardiovascular causes or nonfatal MI in patients with CAD. Although their findings are basically consistent with ours, Schnabel et al. did not report on total mortality, nor did they differentiate between individuals presenting in a stable clinical condition or with acute coronary syndromes.

In our cohort total mortality was evidently driven by mortality from cardiovascular causes, and we also observed an association of ADMA with noncardiovascular mortality. Because the number of deaths from noncardiovascular causes was low and because this study was not designed to study diseases other than cardiovascular, this finding should not be considered definite.

The pooled analysis of patients with stable and unstable CAD is a possible limitation of our study. Another study of 48 patients with acute coronary syndromes and 48 healthy volunteers indicated that acute coronary syndromes might increase ADMA *(49)*. Because that study did not include patients with stable CAD, it does not

refute our finding of similar ADMA in stable and unstable CAD. Therefore a combined analysis of these groups appears justified and is supported by the fact that the separate analyses of stable and unstable patients in our study yielded very similar HRs.

The observation that ADMA predicts total and cardiovascular mortality in patients with CAD does not prove causality. We identified several biochemical markers of cardiovascular risk correlated with ADMA that may by themselves explain the current findings. Among the confounding factors, GFR may deserve particular attention because impaired renal function represents a cardiovascular risk factor by itself and high ADMA may merely indicate renal dysfunction. Nevertheless, after we controlled for confounding variables in multivariable models and for the use of drugs, ADMA retained predictive power. Other confounders that may be related to ADMA may have been disregarded in this study, however.

Our study population consisted of selectively enrolled middle-aged to elderly Caucasians; therefore the results cannot be generalized to younger persons or other races or ethnicities. That coronary angiography was indicated in each study participant may have produced referral bias. The definition of the coronary artery status, however, may be a strength of the study. The prevalence of clinically asymptomatic coronary atherosclerosis has been reported to be high at ≥50 years of age. Hence, angiography-based recruitment prevents inadvertent allocation of individuals with significant, clinically unapparent CAD to the control group. Surprisingly, among the study participants classified as having no CAD the major cardiovascular risk factors occurred at a frequency similar to that of the general population. For example, the prevalence of hypertension is close to that found in the German population *(50)*. Diabetes mellitus in our group first appeared to be 2 to 3 times more frequent than in the German population, but this is likely due to the fact that we measured fasting glucose and performed an oral glucose challenge in individuals not known to have diabetes. Based on clinical history or fasting glucose measurements, the National Health and Nutrition Examination surveys 1999–2000 reports prevalences of diabetes mellitus of 9.2% and 19.3% in adults 40–59 or >60 years of age, respectively *(51)*. In the current study, 12.1% of the persons without CAD had diabetes mellitus according to this criterion, and another 5.6% were detected by increased postchallenge glucose.

In this study, the largest to examine ADMA as a predictor of mortality, ADMA was a surprisingly robust predictor of mortality from all causes and from cardiovascular causes. The predictive value of ADMA was similar in patients with stable CAD or acute coronary syndromes and in those with or without history of STEMI. Because the association of ADMA with mortality did not reach statistical significance in persons with no CAD, investigation in asymptomatic persons is warranted.

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References

1. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992;339:572–5.
2. Anderstam B, Katzarski K, Bergstrom J. Serum levels of NG, NG-dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. J Am Soc Nephrol 1997;8:1437–42.
3. Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001;358:2113–7.
4. Kielstein JT, Zoccali C. Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age. Am J Kidney Dis 2005;46:186–202.
5. Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842–7.
6. Lundman P, Eriksson MJ, Stuhlinger M, Cooke JP, Hamsten A, Tornvall P. Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine. J Am Coll Cardiol 2001;38:111–6.
7. Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, et al. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. JAMA 2002;287:1420–6.
8. Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM, et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. Am J Cardiol 2001;88:1201–3.
9. Stuhlinger MC, Stanger O. Asymmetric dimethyl-L-arginine (ADMA): a possible link between homocyst(e)ine and endothelial dysfunction. Curr Drug Metab 2005;6:3–14.
10. Surdacki A, Nowicki M, Sandmann J, Tsikas D, Boeger RH, Bode-Boeger SM, et al. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999;33:652–8.
11. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? Circulation 2004;109:1813–8.
12. Nijveldt RJ, Teerlink T, Van Der Hoven B, Siroen MP, Kuik DJ, Rauwerda JA, et al. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. Clin Nutr 2003;22:23–30.
13. Siroen MP, van Leeuwen PA, Nijveldt RJ, Teerlink T, Wouters PJ, Van den Berghe G. Modulation of asymmetric dimethylarginine in critically ill patients receiving intensive insulin treatment: a possible explanation of reduced morbidity and mortality? Crit Care Med 2005;33:504–10.
14. Kielstein JT, Bode-Boger SM, Hesse G, Martens-Lobenhoffer J, Takacs A, Fliser D, et al. Asymmetrical dimethylarginine in idio-

pathic pulmonary arterial hypertension. Arterioscler Thromb Vasc Biol 2005;25:1414–8.
15. Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. J Am Soc Nephrol 2005;16:2449–55.
16. Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 2001;358:2127–8.
17. Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. Eur Heart J 2003;24: 1912–9.
18. Schnabel R, Blankenberg S, Lubos E, Lackner KJ, Rupprecht HJ, Espinola-Klein C, et al. Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: results from the AtheroGene Study. Circ Res 2005;97: e53–9.
19. Winkelmann BR, März W, Boehm BO, Zotz R, Hager J, Hellstern P, et al. Rationale and design of the LURIC study–a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. Pharmacogenomics 2001;2:S1–73.
20. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2006;29(Suppl 1):S43–8.
21. Fard A, Tuck CH, Donis JA, Sciacca R, Di Tullio MR, Wu HD, et al. Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. Arterioscler Thromb Vasc Biol 2000;20:2039–44.
22. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461–70.
23. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. Anal Biochem 2002;303:131–7.
24. Krzyzanowska K, Mittermayer F, Kopp HP, Wolzt M, Schernthaner G. Weight loss reduces circulating asymmetrical dimethylarginine concentrations in morbidly obese women. J Clin Endocrinol Metab 2004;89:6277–81.
25. Curgunlu A, Uzun H, Bavunoglu I, Karter Y, Genc H, Vehid S. Increased circulating concentrations of asymmetric dimethylarginine (ADMA) in white coat hypertension. J Hum Hypertens 2005; 19:629–33.
26. Zoccali C, Kielstein JT. Asymmetric dimethylarginine: a new player in the pathogenesis of renal disease? Curr Opin Nephrol Hypertens 2006;15:314–20.
27. Wanby P, Brattstrom L, Brudin L, Hultberg B, Teerlink T. Asymmetric dimethylarginine and total homocysteine in plasma after oral methionine loading. Scand J Clin Lab Invest 2003;63:347–53.
28. Doshi S, McDowell I, Goodfellow J, Stabler S, Boger R, Allen R, et al. Relationship between S-adenosylmethionine, S-adenosylhomocysteine, asymmetric dimethylarginine, and endothelial function in healthy human subjects during experimental hyper- and hypohomocysteinemia. Metabolism 2005;54:351–60.
29. Boger RH, Lentz SR, Bode-Boger SM, Knapp HR, Haynes WG. Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inaemia in humans. Clin Sci (Lond) 2001;100:161–7.
30. Stuhlinger MC, Oka RK, Graf EE, Schmolzer I, Upson BM, Kapoor O, et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. Circulation 2003;108: 933–8.
31. Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 2001;104:2569–75.
32. Kielstein JT, Boger RH, Bode-Boger SM, Frolich JC, Haller H, Ritz E, et al. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol 2002;13:170–6.
33. Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn E, Boger RH. Determination of a reference value for N(G), N(G)-dimethyl-L-arginine in 500 subjects. Eur J Clin Invest 2005;35:622–6.
34. Zhang WZ, Venardos K, Chin-Dusting J, Kaye DM. Adverse effects of cigarette smoke on NO bioavailability: role of arginine metabolism and oxidative stress. Hypertension 2006;48:278–85.
35. Eid HM, Arnesen H, Hjerkinn EM, Lyberg T, Seljeflot I. Relationship between obesity, smoking, and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. Metabolism 2004;53:1574–9.
36. Paiva H, Laakso J, Lehtimaki T, Isomustajarvi M, Ruokonen I, Laaksonen R. Effect of high-dose statin treatment on plasma concentrations of endogenous nitric oxide synthase inhibitors. J Cardiovasc Pharmacol 2003;41:219–22.
37. Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 2003;23:1455–9.
38. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation 1999;99:1141–6.
39. Kielstein JT, Impraim B, Simmel S, Bode-Boger SM, Tsikas D, Frolich JC, et al. Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. Circulation 2004;109:172–7.
40. Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuehlinger MC, Wang BY, et al. Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: genetic and physiological evidence. Circulation 2003;108:3042–7.
41. Chen JW, Hsu NW, Wu TC, Lin SJ, Chang MS. Long-term angiotensin-converting enzyme inhibition reduces plasma asymmetric dimethylarginine and improves endothelial nitric oxide bioavailability and coronary microvascular function in patients with syndrome X. Am J Cardiol 2002;90:974–82.
42. Lu TM, Ding YA, Leu HB, Yin WH, Sheu WH, Chu KM. Effect of rosuvastatin on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia. Am J Cardiol 2004;94:157–61.
43. Sasaki S, Kuwahara N, Kunitomo K, Harada S, Yamada T, Azuma A, et al. Effects of atorvastatin on oxidized low-density lipoprotein, low-density lipoprotein subfraction distribution, and remnant lipoprotein in patients with mixed hyperlipoproteinemia. Am J Cardiol 2002;89:386–9.
44. Eid HM, Eritsland J, Larsen J, Arnesen H, Seljeflot I. Increased levels of asymmetric dimethylarginine in populations at risk for atherosclerotic disease. Effects of pravastatin. Atherosclerosis 2003;166:279–84.
45. Janatuinen T, Laakso J, Laaksonen R, Vesalainen R, Nuutila P, Lehtimaki T, et al. Plasma asymmetric dimethylarginine modifies the effect of pravastatin on myocardial blood flow in young adults. Vasc Med 2003;8:185–9.
46. Maas R, Quitzau K, Schwedhelm E, Spieker L, Rafflenbeul W, Steenpass A, et al. Asymmetrical dimethylarginine (ADMA) and coronary endothelial function in patients with coronary artery disease and mild hypercholesterolemia. *Atherosclerosis*. 2006 Jul 6; [Epub ahead of print].

47. Asagami T, Abbasi F, Stuelinger M, Lamendola C, McLaughlin T, Cooke JP, et al. Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. Metabolism 2002;51:843–6.
48. Mallamaci F, Tripepi G, Cutrupi S, Malatino LS, Zoccali C. Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction, and myocardiopathy in patients with ESRD. Kidney Int 2005;67:2330–7.
49. Bae SW, Stuhlinger MC, Yoo HS, Yu KH, Park HK, Choi BY, et al. Plasma asymmetric dimethylarginine concentrations in newly diagnosed patients with acute myocardial infarction or unstable angina pectoris during two weeks of medical treatment. Am J Cardiol 2005;95:729–33.
50. Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense HW, Joffres M, et al. Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. JAMA 2003;289:2363–9.
51. Prevalence of diabetes and impaired fasting glucose in adults–United States, 1999–2000. MMWR Morb Mortal Wkly Rep 2003; 52:833–7.