Plasma Asymmetric Dimethylarginine and Incidence of Cardiovascular Disease and Death in the Community

Rainer H. Böger, MD*; Lisa M. Sullivan, PhD; Edzard Schwedhelm, PhD; Thomas J. Wang, MD; Renke Maas, MD; Emelia J. Benjamin, MD; Friedrich Schulze, PhD; Vanessa Xanthakis, MS; Ralf A. Benndorf, MD; Ramachandran S. Vasan, MD*

Background—Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, induces endothelial dysfunction. Although elevated ADMA has been associated with an increased risk of cardiovascular disease events and death in referral samples, the prognostic significance of ADMA in the community has not been adequately evaluated.

Methods and Results—We related plasma ADMA, L-arginine (Arg), and the ratio of Arg to ADMA to the incidence of cardiovascular disease (fatal or nonfatal myocardial infarction, coronary insufficiency, angina pectoris, stroke or transient ischemic attack, intermittent claudication, or heart failure) and death in 3320 Framingham Offspring Study participants (1769 women; mean age, 59 years). Over a follow-up period of 10.9 years, 281 individuals of 2956 free of cardiovascular disease at baseline developed incident cardiovascular disease, and 285 participants died. In multivariable models adjusting for established risk factors and other biomarkers (B-type natriuretic peptide, renin, homocysteine, urine albumin excretion, and C-reactive protein), ADMA and the ratio of Arg to ADMA were significantly associated with all-cause mortality (adjusted-hazard ratio [HR] per 1-SD increment, 1.21; 95% CI, 1.07 to 1.37; and HR per 1-SD increment, 0.80; 95% CI, 0.69 to 0.93, respectively), whereas Arg was not (HR per 1-SD increment, 0.89; 95% CI, 0.77 to 1.02). We noted effect modification by diabetes status; ADMA was associated with death in individuals without diabetes (adjusted HR per 1-SD increment, 1.30; 95% CI, 1.13 to 1.50) but not in individuals with diabetes (adjusted HR per 1-SD increment, 0.85; 95% CI, 0.62 to 1.16). ADMA, Arg, and the ratio of Arg to ADMA were not associated with cardiovascular disease incidence ($P>0.10$).

Conclusions—In our large community-based sample, ADMA was significantly associated with all-cause mortality, particularly in nondiabetic individuals. (***Circulation*. 2009;119:1592-1600.**)

Key Words: cardiovascular diseases ■ epidemiology ■ nitric oxide ■ population ■ risk factors

The endothelium plays a major role in regulating vascular tone, mainly by secreting the potent vasodilator nitric oxide (NO), which is antiatherogenic.[1] NO is synthesized from its precursor, L-arginine (Arg), by endothelial NO synthase (NOS).[2] NOS is competitively inhibited by asymmetric dimethylarginine (ADMA), an endogenous compound that is elevated in renal failure, cardiovascular disease (CVD), and diabetes mellitus.[3,4] A low ratio of Arg to ADMA (Arg/ADMA ratio) is also a marker of endothelial dysfunction. Prospective investigations of ADMA have highlighted its role as a predictor of CVD events or death in patients with established coronary artery disease,[5–7] advanced renal failure,[8] or other high-risk conditions.[9]

Clinical Perspective p 1600

Only limited information is available from comparatively small studies regarding whether higher ADMA or a diminished Arg/ADMA ratio is associated with risk of death and CVD events in the general population. Two recent studies[10,11] suggested that elevated ADMA is associated with a 2-fold risk of death in nonsmoking healthy men. Given the lack of data on the prognostic significance of ADMA in the community, we related ADMA, Arg, and the Arg/ADMA ratio to the incidence of CVD and death in a large ambulatory community-based cohort, adjusting for traditional risk factors, including newer biomarkers.[12]

Received August 30, 2008; accepted January 26, 2009.

From the Clinical Pharmacology Unit, Institute of Experimental and Clinical Pharmacology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany (R.H.B., E.S., R.M., F.S., R.A.B.); Framingham Heart Study, Framingham, Mass (L.M.S., T.J.W., E.J.B., V.X., R.S.V.); Department of Biostatistics (L.M.S., V.X.) and Cardiology Division and Preventive Medicine (E.J.B., R.S.V.), Boston University School of Public Health, Boston, Mass; and Cardiology Division, Massachusetts General Hospital, Boston (T.J.W.).

*The first and last authors contributed equally to this work.

Guest Editor for this article was William S. Weintraub, MD.

The online Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.108.838268/DC1.

Reprint requests to Ramachandran S. Vasan, MD, Framingham Heart Study, 73 Mount Wayte Ave, Framingham, MA 01702-5803 (e-mail vasan@bu.edu); or Rainer H. Böger, MD, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany (e-mail boeger@uke.uni-hamburg.de).

Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.108.838268**

Methods

Study Sample

The design of the Framingham Offspring Study has been described previously.[13] The 3532 attendees at the sixth examination (1995 to 1998) were eligible for the present investigation. We excluded 212 individuals for the following reasons: serum creatinine >2.0 mg/dL (n=15), unavailable ADMA or Arg (n=79), and missing covariates (n=118). After exclusions, 3320 participants remained eligible. At each heart study examination, participants undergo standardized measurements of blood pressure (BP), anthropometry, medical history, physical examination, and laboratory assessment of risk factors.[14] The study protocol was approved by the Institutional Review Board of the Boston University Medical Center and the Ethics Committee of the Hamburg Board of Physicians. All participants provided written informed consent.

Assays for ADMA, Arg, and Other Biomarkers

Laboratory assessment of several biomarkers was conducted on fasting samples drawn at the sixth examination cycle (details are given in the Appendix in the online Data Supplement). Plasma samples that were stored for ≈8 years at −80°C without freeze-thaw cycles were used for mass spectrometric determination of ADMA and Arg using a validated high-throughput liquid chromatographic–tandem mass spectrometric assay.[15,16] The following biomarkers were used as covariates because of prior association with CVD/death in our cohort[12]: high-sensitivity C-reactive protein (CRP), plasma B-type natriuretic peptide (BNP), plasma renin concentration, plasma homocysteine, and the urinary albumin-to-creatinine ratio (UACR) assessed on a spot urine specimen.

Outcomes

The outcomes of interest were incidence of a first CVD event and all-cause mortality during follow-up from the baseline examination through December 2006. Major CVD events included fatal or nonfatal coronary heart disease (myocardial infarction, coronary insufficiency, and angina pectoris), stroke or transient ischemic attack, intermittent claudication, or heart failure. Criteria for these events have been described elsewhere (see the Appendix in the Data Supplement).[14]

Statistical Analysis

We used multivariable linear regression to relate ADMA and Arg to the following variables: age, sex, systolic and diastolic BPs, hypertension treatment, ratio of total to high-density lipoprotein (HDL) cholesterol, diabetes, body mass index (BMI), glomerular filtration rate (GFR), smoking status, and alcohol consumption. The Modification of Diet in Renal Disease equation[17] was used to calculate an estimated GFR (eGFR).

We used Cox regression[18] to relate ADMA, Arg, and the Arg/ADMA ratio to the incidence of a first CVD event and death (separate analyses for each biomarker and for each outcome) after confirming the assumption of proportionality of hazards. Individuals with prior CVD were excluded from analyses of incident CVD (leaving 2956 eligible participants) but were eligible for death analyses. We performed pooled sex analyses to maximize statistical power because tests of interaction for sex with these biomarkers were not statistically significant. The biomarkers were normally distributed and analyzed as continuous variables and as quartiles.

Multivariable analyses adjusted for age, sex, diabetes, systolic and diastolic BPs, treatment for hypertension, smoking, ratio of total to HDL cholesterol, and serum creatinine. For CVD analyses, we additionally adjusted for BNP and UACR because these 2 biomarkers have been associated with CVD in our sample.[12] For death analyses, we also adjusted for prevalent CVD, BNP, UACR, homocysteine, CRP, and renin (given associations of the last 4 with death in our sample).[12] We evaluated the contributions of ADMA, Arg, and the Arg/ADMA ratio to the prediction of CVD and death by evaluating the increment in C statistic (to models incorporating established risk factors and other biomarkers).[12]

Table 1. Baseline Characteristics of Study Participants

Characteristic	Men (n=1551)	Women (n=1769)
Age, y	59±10	59±10
Smoking, %	14	16
Serum cholesterol, mg/dL		
Total	197±35	211±38
HDL	44±12	58±16
Total/HDL ratio	4.79±1.37	3.91±1.32
BMI, kg/m^2	28.5±4.4	27.4±5.7
BP, mm Hg		
Systolic	130±17	127±20
Diastolic	77±9	74±9
Hypertension, %	35	28
Use of antihypertensive agents, %	31	25
Blood glucose, mg/dL	107±27	100±25
Diabetes mellitus, %	14	9
Serum creatinine, mg/dL	1.10±0.17	0.95±0.15
eGFR, mL/min	87±24	86±26
Prevalent CVD, %	15	7
Median biomarker levels (25th–75th percentile)		
ADMA, μmol/L	0.54 (0.47–0.62)	0.53 (0.46–0.61)
Arg, μmol/L	78.0 (66.8–91.0)	75.7 (64.1–88.6)
Arg/ADMA	146.0 (120.0–174.6)	144.0 (120.0–172.1)
CRP, mg/dL	1.8 (0.9–3.8)	2.3 (1.0–5.7)
BNP, pg/mL	6.7 (4.0–17.2)	10.0 (4.0–20.3)
Renin, mU/L	14.0 (8.0–25.0)	11.0 (6.0–19.0)
Homocysteine, μmol/L	9.8 (8.3–11.8)	8.4 (7.0–10.3)
UACR, mg/g	4.9 (2.2–10.9)	8.6 (3.6–17.5)

Values are mean±SD when appropriate.

We examined whether the relations of ADMA, Arg, and the Arg/ADMA ratio to CVD/death varied according to age, obesity (BMI ≥30 kg/m^2), hypertension, diabetes, smoking, and low eGFR (<60 m/min). A 2-sided value of $P<0.05$ was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

The baseline characteristics of our sample are shown in Table 1. ADMA was positively correlated with Arg (age-, sex-adjusted $r=0.30$, $P<0.0001$).

Cross-Sectional Correlates of ADMA

ADMA was associated positively with age, BMI, and smoking but inversely related to diastolic BP (Table 2). Importantly, ADMA was not related to renal function. Arg was positively related to age, male sex, smoking, and eGFR but inversely associated with BMI, diabetes, and alcohol consumption. The Arg/ADMA ratio was positively related to male sex and inversely associated with age, BMI, diabetes, and alcohol consumption. Clinical variables explained only ≈3.5% of interindividual variation in ADMA. All 3 biomar-

Table 2. Cross-Sectional Correlates (Multivariable Linear Regression) of ADMA, Arg, and the Arg/ADMA Ratio

Variable	Unit of Increase/Categories	Regression Coefficient	*P*
ADMA			
Age	10 y	0.02	<0.0001
BMI	1 unit	0.002	<0.0001
Diastolic BP	10 mm Hg	−0.007	0.0044
Smoking	Smoker vs non-smoker	0.015	0.0137
Arg			
Age	10 y	1.113	0.0054
Sex	Male vs female	2.946	<0.0001
BMI	1 unit	−0.170	0.0166
Diabetes	Present vs absent	−4.933	<0.0001
Smoking	Smoker vs nonsmoker	3.848	0.0002
Alcohol consumption	Drinks/wk	−0.260	0.0117
eGFR	1 unit	0.037	0.0180
Arg/ADMA ratio			
Age	10 y	−4.921	<0.0001
Sex	Male vs female	3.606	0.0242
BMI	1 unit	−0.845	<0.0001
Diabetes	Present vs absent	−5.205	0.0387
Alcohol consumption	Drinks/wk	−0.689	0.0019

Independent variables reported are those that remained in the model after stepwise backward elimination analysis and were statistically significant in the final model ($P<0.05$). Independent variables were chosen on the basis of significant univariate associations and pathophysiological mechanisms. R^2 of the final model was 0.0347 for ADMA, 0.0179 for Arg, and 0.0247 for the Arg/ADMA ratio.

kers studied were only weakly associated with other novel biomarkers included in multivariable models (all pairwise $r<0.10$, except ADMA and homocysteine, $r=0.13$).

Relations of ADMA to CVD Incidence

Over a follow-up period of 10.9 years, there were 281 incident CVD events (119 in women) among 2956 individuals at risk. Neither biomarker nor their ratio was associated with CVD incidence (Table 3). These results were similar to multivariable analyses that did not adjust for novel biomarkers (data not shown). There was no effect modification by age, sex, hypertension, obesity, diabetes, smoking, or eGFR. With our sample size and at $\alpha=0.05$, we had good power (82% and 96%) to detect hazard ratios (HRs) per 1-SD increment of 1.5 and 1.75, respectively. We had limited power to detect HRs <1.5.

Relations of ADMA to All-Cause Mortality

On follow-up, there were 285 deaths among the 3320 at-risk participants. Death rates rose across ADMA quartiles but declined with increasing Arg/ADMA ratio (Table 4). Although ADMA was positively associated with death, Arg was not (Table 4). The Arg/ADMA ratio was inversely associated with death, which was especially evident for the fourth quartile. In analyses adjusting for all covariates other than the novel biomarkers, results were similar to those shown in Table 4 (data not shown). Figure 1 shows the relations of ADMA, Arg, and Arg/ADMA ratio with death using regression splines.

Neither ADMA nor the Arg/ADMA ratio improved the model C statistic incrementally over traditional risk factors (C for models without and with ADMA=0.772 and 0.768, respectively; C for models without and with Arg/ADMA=0.772 and 0.775, respectively) or when other novel biomarkers were added (C for models without and with ADMA=0.805 and 0.801, respectively; C for models without and with Arg/ADMA=0.805 and 0.804, respectively).

Because the pathogenetic mechanism relates to a molecular interaction between ADMA and Arg, we modeled these biomarkers together as quartiles (instead of as a ratio) for display purposes (Figure 2). The increase in risk of death with increasing ADMA was consistent across Arg quartiles. However, this trend was less clear for Arg; the highest risk of death was associated with high ADMA and low Arg, whereas the lowest risk of death was associated with low ADMA and high Arg.

In secondary analyses, we evaluated the associations of the 3 biomarkers to CVD death versus non-CVD death (Tables I and II of the online Data Supplement). We observed strong and statistically significant associations of ADMA (positive) and the Arg/ADMA ratio (inverse) to non-CVD death but did not find a significant association with CVD death. Of note, the lower proportion of deaths resulting from CVD in these analyses is the result of an emphasis on specificity in the adjudication process in the Framingham study, which may have limited our statistical power.

Interaction Between ADMA and Diabetes for Risk of Death

Despite the fact that people with and without diabetes had similar mean ADMA levels (0.553 μmol/L [SD, 0.13 μmol/L] versus 0.546 μmol/L [SD, 0.126 μmol/L], respectively; *P*=NS), we observed significant interactions between ADMA (and Arg/ADMA ratio) and diabetes for death risk ($P=0.03$ for both); no such interaction was evident for Arg. Therefore, the analyses of death rates for ADMA and the Arg/ADMA ratio were stratified by diabetes status (Tables 5 and 6). Of 285 deaths, 216 occurred in subjects without diabetes (n=2948), and 69 deaths occurred among individuals with diabetes (n=372). In multivariable analyses, ADMA and the Arg/ADMA ratio were associated with death in participants without diabetes but not in participants with diabetes.

Discussion

Principal Findings

First, higher ADMA and a lower Arg/ADMA ratio were associated with death in our community-based sample. Regression splines confirmed these linear relations. The strength of the association (increase in risk of 21% per 1-SD increase [0.13 μmol/L] in ADMA) is comparable to that for a 4.2-year (0.4 SD) increase in age in our sample. However, we observed no incremental contribution of ADMA to the prediction of risk of death. Prior observations indicate modest

Table 3. Biomarkers and CVD Risk

	No. of Events/No. at Risk (%)	Unadjusted HR (95% CI)	*P*	Multivariable-Adjusted HR (95% CI)*	*P*
ADMA (per 1-SD increase)		1.00 (0.90–1.12)	0.94	0.92 (0.82–1.05)	0.21
Quartile 1	54/754 (7.2)	Referent	...	Referent	...
Quartile 2	63/746 (8.5)	1.19 (0.83–1.71)	0.35	1.05 (0.70–1.55)	0.83
Quartile 3	87/744 (11.7)	1.34 (0.95–1.88)	0.10	1.17 (0.80–1.70)	0.42
Quartile 4	77/712 (10.8)	1.00 (0.71–1.42)	0.998	0.85 (0.58–1.25)	0.41
Trend		1.00 (0.90–1.11)	0.97	0.96 (0.85–1.08)	0.47
Arg (per 1-SD increase)		1.01 (0.91–1.12)	0.85	0.99 (0.87–1.11)	0.83
Quartile 1	57/727 (7.8)	Referent	...	Referent	...
Quartile 2	65/757 (8.6)	1.26 (0.88–1.79)	0.21	1.25 (0.85–1.83)	0.26
Quartile 3	82/753 (10.9)	1.17 (0.84–1.65)	0.35	1.04 (0.72–1.51)	0.84
Quartile 4	77/719 (10.7)	1.12 (0.80–1.58)	0.52	0.95 (0.65–1.38)	0.77
Trend		1.02 (0.92–1.14)	0.66	0.97 (0.86–1.09)	0.56
Arg/ADMA (per 1-SD increase)		0.98 (0.88–1.09)	0.70	1.01 (0.91–1.14)	0.80
Quartile 1	74/725 (10.2)	Referent		Referent	
Quartile 2	63/730 (8.6)	0.94 (0.67–1.32)	0.72	0.94 (0.64–1.37)	0.74
Quartile 3	77/752 (10.2)	1.07 (0.78–1.48)	0.66	1.10 (0.77–1.56)	0.60
Quartile 4	67/749 (9.0)	0.99 (0.71–1.38)	0.96	1.17 (0.81–1.69)	0.40
Trend		1.01 (0.91–1.12)	0.84	1.06 (0.95–1.20)	0.30

*Adjusted for age, sex, systolic and diastolic BPs, hypertension treatment, smoking, diabetes, total/HDL cholesterol, creatinine, logBNP, and log UACR. n=2544 for the multivariable-adjusted model. Mean was 0.54 μmol/L (SD, 0.13 μmol/L) for ADMA, 78.8 μmol/L (SD, 20.8 μmol/L) for Arg, and 149.8 (SD, 44.6) for the Arg/ADMA ratio.

increments in the C-statistic with the addition of biomarkers.[12] Accordingly, we do not propose ADMA as a predictive marker for death but as an important pathophysiological mechanism. Second, the association of ADMA with death was strongest in participants without diabetes, whereas there was no association in those with diabetes. Third, neither ADMA nor Arg (or their ratio) was associated with CVD incidence in our sample.

Table 4. Biomarkers and Risk of Death

	No. of Events/No. at Risk (%)	Unadjusted HR (95% CI)	*P*	Multivariable-Adjusted HR (95% CI)*	*P*
ADMA (per 1-SD increase)		1.10 (0.99–1.22)	0.09	1.21 (1.07–1.37)	0.003
Quartile 1	50/832 (6.0)	Referent	...	Referent	...
Quartile 2	61/827 (7.4)	1.23 (0.85–1.79)	0.27	1.08 (0.71–1.64)	0.71
Quartile 3	79/834 (9.5)	1.38 (0.97–1.97)	0.07	1.15 (0.77–1.71)	0.51
Quartile 4	95/827 (11.5)	1.33 (0.95–1.88)	0.10	1.53 (1.05–2.23)	0.03
Trend		1.09 (0.98–1.21)	0.10	1.15 (1.02–1.30)	0.02
Arg (per 1-SD increase)		0.87 (0.77–0.98)	0.02	0.89 (0.77–1.02)	0.08
Quartile 1	71/821 (8.7)	Referent	...	Referent	...
Quartile 2	64/833 (7.7)	1.03 (0.73–1.44)	0.88	0.96 (0.66–1.41)	0.85
Quartile 3	88/850 (10.4)	0.98 (0.71–1.34)	0.88	0.86 (0.60–1.24)	0.43
Quartile 4	62/816 (7.6)	0.70 (0.50–0.98)	0.04	0.73 (0.50–1.07)	0.10
Trend		0.90 (0.81–0.997)	0.04	0.90 (0.80–1.02)	0.09
Arg/ADMA (per 1-SD increase)		0.82 (0.72–0.94)	0.003	0.80 (0.69–0.93)	0.004
Quartile 1	92/824 (11.2)	Referent	...	Referent	...
Quartile 2	77/827 (9.3)	0.85 (0.63–1.15)	0.30	0.76 (0.53–1.07)	0.12
Quartile 3	70/835 (8.4)	0.85 (0.62–1.16)	0.30	0.69 (0.48–1.01)	0.05
Quartile 4	46/834 (5.5)	0.52 (0.36–0.74)	0.0003	0.49 (0.33–0.74)	0.0007
Trend		0.83 (0.75–0.93)	0.0006	0.80 (0.71–0.91)	0.0007

*Adjusted for age, sex, systolic and diastolic BPs, hypertension treatment, smoking, diabetes, total/HDL cholesterol, creatinine, prevalent CVD, and log BNP, log renin, log homocysteine, log urinary UACR, and log CRP. n=2750 for the multivariable-adjusted model. Mean was 0.55 μmol/L (SD, 0.13 μmol/L) for ADMA, 78.9 μmol/L (SD, 20.7 μmol/L) for Arg, and 149.4 (SD, 44.4) for the Arg/ADMA ratio.

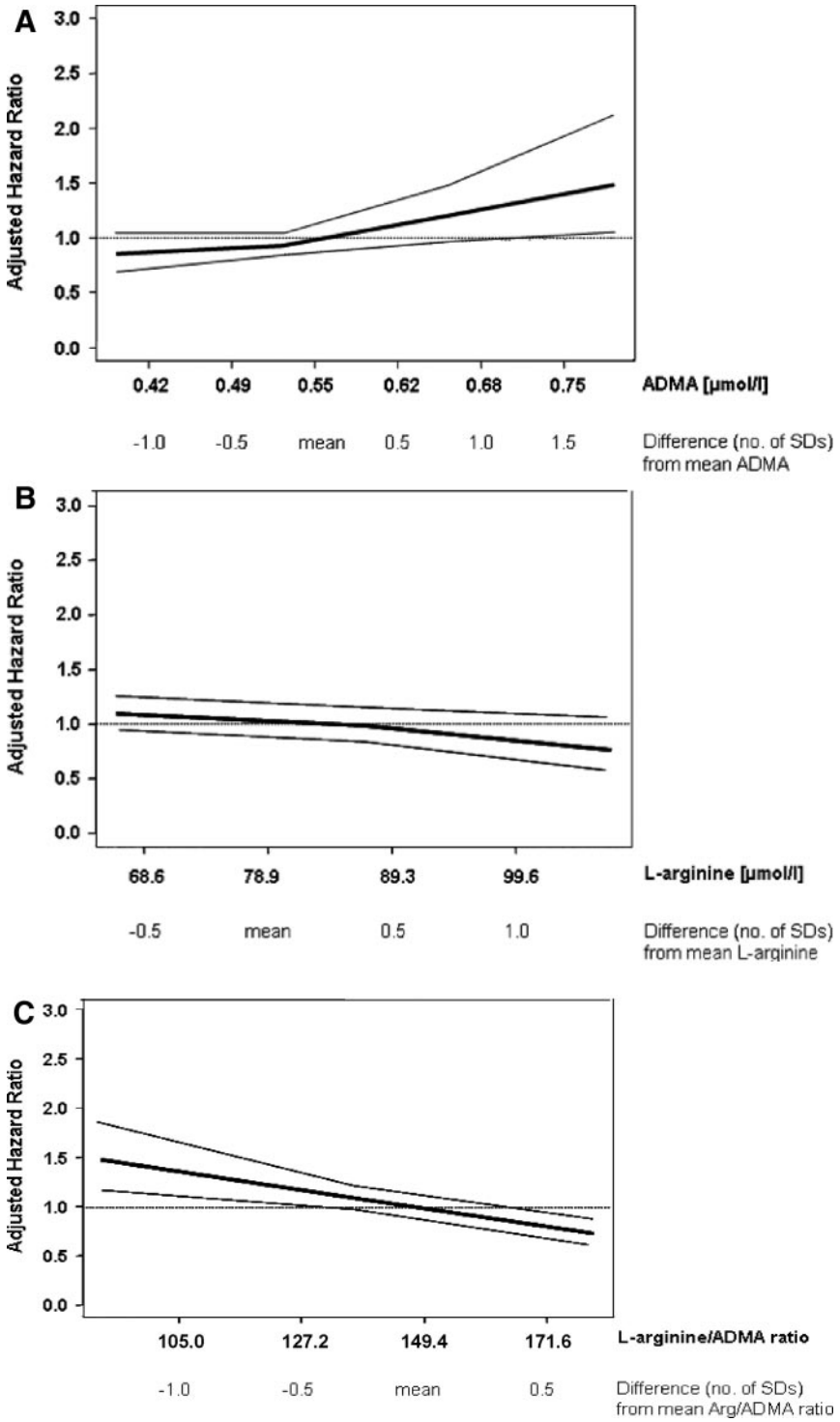

Figure 1. Spline graphs displaying the relationship between death and ADMA (A), Arg (B), and the Arg/ADMA ratio (C) at baseline. Means were as follows: ADMA, 0.55 μmol/L (SD, 0.13 μmol/L); Arg, 78.9 μmol/L (SD, 20.7 μmol/L); Arg/ADMA ratio, 149.4 (SD, 44.4). Data are expressed as the SD difference from the mean for each biomarker. The bold line shows the association of each biomarker with mortality risk; 95% CIs are plotted as fine gray lines. The horizontal gray line is the line of no association between the biomarker and risk.

Potential Mechanisms Underlying the Association With Death

ADMA is an endogenous inhibitor of all 3 NOS isoforms. ADMA impairs NO production, possibly by competing with Arg for the substrate-binding site of NOS.[19]

Clinical studies indicate that reduced NO release in the presence of elevated ADMA results in endothelial dysfunction that is reversible by Arg.[20,21] A recent study demonstrated that ADMA is a strong determinant of flow-mediated, endothelium-dependent vasodilation in healthy people,[22] similar to findings reported in hypercholesterolemic individuals.[20] Patients with endothelial dysfunction are at increased risk of CVD/death.[23] A systemic infusion of ADMA results in increased peripheral resistance, elevated BP, and impaired cerebral blood flow in healthy people.[24,25] Recent studies also indicate that higher ADMA increases death risk in patients who are critically ill as a result of cardiac (eg, cardiogenic shock)[26] or noncardiac (eg, septic shock) illnesses,[27] suggesting that depletion of Arg accompanied by increased ADMA may be a marker of death risk resulting from cardiovascular

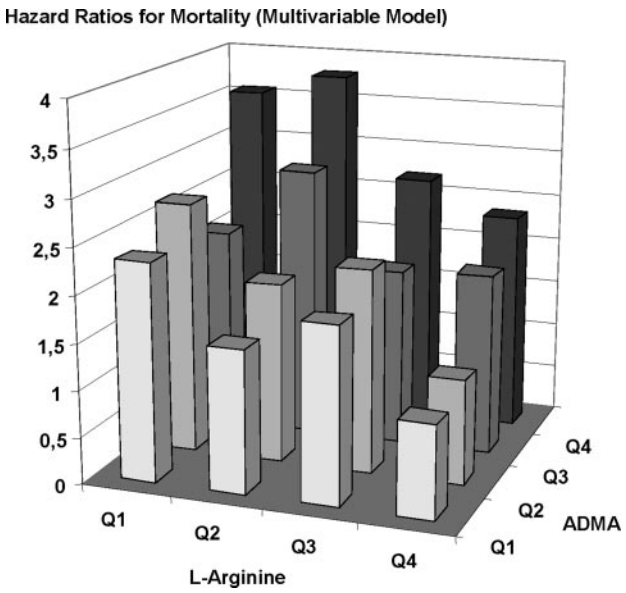

Figure 2. HRs for death according to cross-classification of quartiles of plasma ADMA and Arg at baseline. The first quartile of ADMA and fourth quartile of Arg were defined as the referent.

or noncardiovascular causes. In secondary analyses, we observed an association of higher ADMA with non-CVD death but not with CVD death. The latter observations are hypothesis generating and warrant evaluation in further studies with a greater number of outcome events.

Interestingly enough, mice that lack all 3 NOS isoforms develop spontaneous myocardial infarction, renal disease, ileus, and glucose intolerance more frequently than wild-type mice or mice lacking a single NOS isoform.[28] Because ADMA is a nonselective inhibitor of all 3 NOS isoforms, slight changes in its concentration may affect multiorgan function much more than anticipated from reduced endothelial NOS activity alone, a phenomenon that might also explain the stronger association of ADMA with death than with incident CVD in our cohort. Indeed, recent data show that even small changes in ADMA in the extracellular space, which correspond to the difference between the lowest and highest quartiles of ADMA, or in those between wild-type and dimethylarginine dimethylaminohydrolase (DDAH) transgenic mice are sufficient to induce significant changes in the Arg/ADMA ratio and in NOS activity in cultured endothelial cells.[21]

In a recent study, ADMA was predictive of death in patients with coronary disease but not in those without coronary artery disease.[29] In the Population Study of Women in Gothenburg, ADMA was associated with an HR of 1.12 for death in healthy women,[30] which is consistent with our study.

Experimental studies have substantiated the adverse consequences of higher ADMA. Genetically modified mice that overexpress DDAH-1 (the enzyme that clears ADMA) show enhanced NO production,[31] whereas heterozygous DDAH-1 knockout mice show impaired NO signaling and pulmonary hypertension, and homozygotes are not viable.[32]

Taken together, these data may explain the relation between high ADMA and death in our cohort. Our data suggest that ADMA may be a marker of death risk, but its role as a causal factor or as a promising therapeutic target merits further study.

Lack of Association With CVD Incidence: Comparison With the Literature

We did not observe an association of ADMA with CVD incidence. This may be related to a true lack of association or to limited statistical power to discern modest hazards in our sample. In 2 recent studies,[10,11] ADMA was positively related to coronary events in nonsmoking men. A recent population-based study of women revealed that those in the highest ADMA quintile (>0.71 μmol/L) had a 29% increased risk of myocardial infarction and stroke over 24 years of follow-up[30]; however, the incidence curves for women with ADMA above versus below this threshold did not separate until 13 years of follow-up. It is possible that the follow-up period in our study was too short to demonstrate a significant increase

Table 5. ADMA and Risk of Death in Participants With Diabetes Versus Those Without Diabetes

ADMA (per 1-SD Increase)	No. of Events/ No. at Risk (%)	Unadjusted HR (95% CI)	*P*	Multivariable-Adjusted HR (95% CI)*	*P*
Subjects with no diabetes (n=2948)		1.16 (1.03–1.31)	0.02	1.30 (1.13–1.50)	0.0002
Quartile 1	36/746 (4.8)	Referent		Referent	...
Quartile 2	41/737 (5.6)	1.16 (0.74–1.82)	0.51	1.04 (0.63–1.73)	0.88
Quartile 3	62/736 (8.4)	1.62 (1.07–2.44)	0.02	1.44 (0.90–2.31)	0.12
Quartile 4	77/729 (10.6)	1.45 (0.97–2.15)	0.07	1.75 (1.13–2.72)	0.01
Trend		1.14 (1.01–1.28)	0.04	1.23 (1.07–1.41)	0.004
Subjects with diabetes (n=372)		0.92 (0.74–1.15)	0.47	0.85 (0.62–1.16)	0.30
Quartile 1	14/86 (16.3)	Referent		Referent	...
Quartile 2	20/90 (22.2)	1.25 (0.63–2.49)	0.52	0.84 (0.37–1.91)	0.68
Quartile 3	17/98 (17.4)	0.83 (0.41–1.69)	0.61	0.44 (0.18–1.07)	0.07
Quartile 4	18/98 (18.4)	0.98 (0.48–1.97)	0.94	0.71 (0.30–1.71)	0.45
Trend		0.95 (0.76–1.18)	0.62	0.84 (0.63–1.12)	0.24

SD in subjects with no diabetes, ADMA=0.13. SD in subjects with diabetes, ADMA=0.13.

*Adjusted for age, sex, systolic and diastolic BPs, hypertension treatment, smoking, total/HDL cholesterol, creatinine, prevalent CVD, log BNP, log renin, log homocysteine, log UACR, and log CRP.

Table 6. Arg/ADMA Ratio and Risk of Death in Participants With Diabetes Versus Those Without Diabetes

ARG/ADMA (per 1-SD Increase)	No. of Events/No. at Risk (%)	Unadjusted HR (95% CI)	*P*	Multivariable-Adjusted HR (95% CI)*	*P*
Subjects with no diabetes (n=2948)		0.77 (0.66–0.90)	0.0009	0.72 (0.60–0.87)	0.0005
Quartile 1	66/697 (9.5)	Referent		Referent	...
Quartile 2	62/735 (8.4)	0.86 (0.61–1.22)	0.40	0.68 (0.45–1.02)	0.06
Quartile 3	57/765 (7.5)	0.86 (0.60–1.22)	0.39	0.66 (0.43–1.00)	0.05
Quartile 4	31/751 (4.1)	0.43 (0.28–0.67)	0.0001	0.37 (0.23–0.62)	0.0001
Trend		0.80 (0.71–0.90)	0.0003	0.75 (0.65–0.87)	0.0002
Subjects with diabetes (n=372)		0.99 (0.80–1.22)	0.91	1.07 (0.82–1.40)	0.62
Quartile 1	26/127 (20.5)	Referent		Referent	...
Quartile 2	15/92 (16.3)	0.77 (0.41–1.46)	0.42	0.79 (0.35–1.75)	0.56
Quartile 3	13/70 (18.6)	0.87 (0.44–1.70)	0.67	0.59 (0.24–1.45)	0.25
Quartile 4	15/83 (18.1)	0.90 (0.48–1.71)	0.75	0.97 (0.43–2.16)	0.93
Trend		0.97 (0.79–1.19)	0.76	0.95 (0.73–1.24)	0.71

SD in subjects with no diabetes, Arg/ADMA=43.77. SD in subjects with diabetes, Arg/ADMA=48.20.

*Adjusted for age, sex, systolic and diastolic BPs, hypertension treatment, smoking, total/HDL cholesterol, creatinine, prevalent CVD, log BNP, log renin, log homocysteine, log UACR, and log CRP.

in CVD incidence in our sample, which overall had an intermediate risk of vascular events. Previous studies in high-risk samples have shown that ADMA is associated with CVD events in coronary artery disease patients,[5,7] patients with peripheral arterial occlusive disease,[33] chronic renal disease patients,[8] and unselected patients undergoing major elective surgery.[34]

ADMA and Death: Effect Modification by Diabetes

In prespecified analyses, we observed significant association of ADMA and the Arg/ADMA ratio with death in individuals without diabetes but no association in those with diabetes. There are several possible explanations for these observations. First, studies suggest that in patients with diabetes, ADMA may change with progression of the disease; lower ADMA concentrations are observed during the initial period of mild renal damage and increased GFR,[35] whereas levels are higher in diabetes-associated chronic renal failure.[36] Second, in some studies, the elevation of ADMA in diabetes may actually have been overestimated because of the technical difficulty of separating ADMA and its biologically inactive isomer, symmetric dimethylarginine, by chromatography.[37] Our method was designed to specifically separate dimethylarginines by differences in their mass spectra rather than by differences in retention times and thus allows highly reliable and accurate quantitation of ADMA.[16] Third, physiological effects of ADMA in diabetes may be complex. In diabetes, the burden of reactive oxygen species is generated by hyperglycemia in the vessel wall.[38] Reactive oxygen species such as superoxide anion or hydrogen peroxide almost instantly react with NO, forming hazardous species like peroxinitrite and nitroxyl anion. In addition, there are conditions in which NOS turns into a generator of oxygen-derived free radicals rather than NO, eg, during substrate depletion or cofactor deficiency.[38,39] There is evidence for enhanced NOS-derived free radical production in diabetes.[40,41] ADMA competes with Arg for binding to NOS and to the amino acid transporter and thereby reduces intracellular availability of substrate for NOS. Thus, inhibition of NO production by ADMA may result in variable effects in terms of oxidative damage to the vessel wall in diabetes. Additional studies evaluating a larger sample of individuals with diabetes are warranted.

Strengths and Limitations

The strengths of our investigation are its prospective design, longitudinal surveillance for outcomes, and adjustment for novel biomarkers. The biological plausibility that higher ADMA, lower Arg, or a low Arg/ADMA ratio mediates death risk is reflected by the strength of the associations, temporal relations, and the overall consistency of the associations across analyses. Several limitations merit comment. Establishing that ADMA is a "risk factor" for death would require additional mechanistic studies that assess systemic oxidative/nitrosative stress and cause-specific mortality. The modest sample size of participants with diabetes and the limited number of deaths in this group may have limited our statistical power to detect associations in this subgroup. In addition, future studies that compare the relative predictive utilities of endothelial function measures with ADMA may help clarify whether factors other than NO availability may influence CVD risk. Finally, our sample was white of European descent and comprised middle-aged individuals with a vascular risk factor profile consistent with this age group, limiting the generalizability to other ethnicities and to younger and healthier samples.

Conclusions

In our large community-based sample, higher ADMA was significantly associated with all-cause mortality but not with CVD incidence. This relation was observed in individuals without baseline diabetes, whereas no association was noted in persons with diabetes. Additional investigations are warranted to confirm these observations and to elucidate the mechanisms underlying the associations in select strata.

Acknowledgments

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

Sources of Funding

This work was supported through National Institutes of Health/National Heart, Lung, and Blood Institute contracts N01-HC-25195, K23-HL074077-01 (Dr Wang), N01HV28178, and 2K24HL04334 (Dr Vasan) and by the Deutsche Forschungsgemeinschaft grant Bo 1431/4-1 (Dr Böger).

Disclosures

Drs Böger, Schwedhelm, and Maas are named as inventors on patents relating to analytical assays for methylarginines and receive royalties from them. The other authors report no conflicts.

References

1. Napoli C, Ignarro LJ. Nitric oxide and atherosclerosis. *Nitric Oxide*. 2001;5:88–97.
2. Palmer RM, Rees DD, Ashton DS, Moncada S. L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun*. 1988;153:1251–1256.
3. Boger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. *Cardiovasc Res*. 2003;59:824–833.
4. 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;339:572–575.
5. Krempl TK, Maas R, Sydow K, Meinertz T, Boger RH, Kahler J. Elevation of asymmetric dimethylarginine in patients with unstable angina and recurrent cardiovascular events. *Eur Heart J*. 2005;26:1846–1851.
6. 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;24:1912–1919.
7. Schnabel R, Blankenberg S, Lubos E, Lackner KJ, Rupprecht HJ, Espinola-Klein C, Jachmann N, Post F, Peetz D, Bickel C, Cambien F, Tiret L, Munzel T. 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–e59.
8. Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frolich J, Boger R. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet*. 2001;358:2113–2117.
9. Boger RH. Asymmetric dimethylarginine (ADMA): a novel risk marker in cardiovascular medicine and beyond. *Ann Med*. 2006;38:126–136.
10. Maas R, Schulze F, Baumert J, Lowel H, Hamraz K, Schwedhelm E, Koenig W, Boger RH. Asymmetric dimethylarginine, smoking, and risk of coronary heart disease in apparently healthy men: prospective analysis from the population-based Monitoring of Trends and Determinants in Cardiovascular Disease/Kooperative Gesundheitsforschung in der Region Augsburg study and experimental data. *Clin Chem*. 2007;53:693–701.
11. Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, Laaksonen R. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet*. 2001;358:2127–21278.
12. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med*. 2006;355:2631–2639.
13. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281–290.
14. Kannel WB, Wolf PA, Garrison RJ, eds. *Section 34: Some Risk Factors Related to the Annual Incidence of Cardiovascular Disease and Death in Pooled Repeated Biennial Measurements: Framingham Heart Study, 30 Year Follow-Up*. Bethesda, Md: US Department of Health and Human Services; 1987.
15. Schwedhelm E, Tan-Andresen J, Maas R, Riederer U, Schulze F, Boger RH. Liquid chromatography-tandem mass spectrometry method for the analysis of asymmetric dimethylarginine in human plasma. *Clin Chem*. 2005;51:1268–1271.
16. Schwedhelm E, Maas R, Tan-Andresen J, Schulze F, Riederer U, Boger RH. High-throughput liquid chromatographic-tandem mass spectrometric determination of arginine and dimethylated arginine derivatives in human and mouse plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;851:211–219.
17. 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;130:461–470.
18. Cox DR. Regression models and life tables (with discussion). *J Royal Stat Soc*. 1972;34(series B):187–220.
19. Boger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, Bode-Boger SM. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ Res*. 2000;87:99–105.
20. Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation*. 1998;98:1842–1847.
21. Cardounel AJ, Cui H, Samouilov A, Johnson W, Kearns P, Tsai AL, Berka V, Zweier JL. Evidence for the pathophysiological role of endogenous methylarginines in regulation of endothelial NO production and vascular function. *J Biol Chem*. 2007;282:879–887.
22. Ardigo D, Stuehlinger M, Franzini L, Valtuena S, Piatti PM, Pachinger O, Reaven GM, Zavaroni I. ADMA is independently related to flow-mediated vasodilation in subjects at low cardiovascular risk. *Eur J Clin Invest*. 2007;37:263–269.
23. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101:1899–1906.
24. Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, Vallance P. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arterioscler Thromb Vasc Biol*. 2003;23:1455–1459.
25. Kielstein JT, Impraim B, Simmel S, Bode-Boger SM, Tsikas D, Frolich JC, Hoeper MM, Haller H, Fliser D. Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation*. 2004;109:172–177.
26. Nicholls SJ, Wang Z, Koeth R, Levison B, DelFraino B, Dzavik V, Griffith OW, Hathaway D, Panza JA, Nissen SE, Hochman JS, Hazen SL. Metabolic profiling of arginine and nitric oxide pathways predicts hemodynamic abnormalities and mortality in patients with cardiogenic shock after acute myocardial infarction. *Circulation*. 2007;116:2315–2324.
27. O'Dwyer MJ, Dempsey F, Crowley V, Kelleher DP, McManus R, Ryan T. Septic shock is correlated with asymmetrical dimethyl arginine levels, which may be influenced by a polymorphism in the dimethylarginine dimethylaminohydrolase II gene: a prospective observational study. *Crit Care*. 2006;10:R139.
28. Nakata S, Tsutsui M, Shimokawa H, Suda O, Morishita T, Shibata K, Yatera Y, Sabanai K, Tanimoto A, Nagasaki M, Tasaki H, Sasaguri Y, Nakashima Y, Otsuji Y, Yanagihara N. Spontaneous myocardial infarction in mice lacking all nitric oxide synthase isoforms. *Circulation*. 2008;117:2211–2223.
29. Meinitzer A, Seelhorst U, Wellnitz B, Halwachs-Baumann G, Boehm BO, Winkelmann BR, Marz W. Asymmetrical dimethylarginine independently predicts total and cardiovascular mortality in individuals with angiographic coronary artery disease (the Ludwigshafen Risk and Cardiovascular Health study). *Clin Chem*. 2007;53:273–283.
30. Leong T, Zylberstein D, Graham I, Lissner L, Ward D, Fogarty J, Bengtsson C, Bjorkelund C, Thelle D. Asymmetric dimethylarginine independently predicts fatal and nonfatal myocardial infarction and stroke in women: 24-year follow-up of the Population Study of Women in Gothenburg. *Arterioscler Thromb Vasc Biol*. 2008;28:961–967.
31. Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuehlinger MC, Wang BY, Tsao PS, Kimoto M, Vallance P, Patterson AJ, Cooke JP. Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: genetic and physiological evidence. *Circulation*. 2003;108:3042–3047.
32. Leiper J, Nandi M, Torondel B, Murray-Rust J, Malaki M, O'Hara B, Rossiter S, Anthony S, Madhani M, Selwood D, Smith C, Wojciak-Stothard B, Rudiger A, Stidwill R, McDonald NQ, Vallance P. Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat Med*. 2007;13:198–203.

33. Mittermayer F, Krzyzanowska K, Exner M, Mlekusch W, Amighi J, Sabeti S, Minar E, Muller M, Wolzt M, Schillinger M. Asymmetric dimethylarginine predicts major adverse cardiovascular events in patients with advanced peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2006;26:2536–2540.
34. Maas R, Dentz L, Schwedhelm E, Thoms W, Kuss O, Hiltmeyer N, Haddad M, Kloss T, Standl T, Boger RH. Elevated plasma concentrations of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine predict adverse events in patients undergoing noncardiac surgery. *Crit Care Med*. 2007;35:1876–1881.
35. Paiva H, Lehtimaki T, Laakso J, Ruokonen I, Rantalaiho V, Wirta O, Pasternack A, Laaksonen R. Plasma concentrations of asymmetric-dimethyl-arginine in type 2 diabetes associate with glycemic control and glomerular filtration rate but not with risk factors of vasculopathy. *Metabolism*. 2003;52:303–307.
36. Krzyzanowska K, Mittermayer F, Shnawa N, Hofer M, Schnabler J, Etmuller Y, Kapiotis S, Wolzt M, Schernthaner G. Asymmetrical dimethylarginine is related to renal function, chronic inflammation and macroangiopathy in patients with type 2 diabetes and albuminuria. *Diabet Med*. 2007;24:81–86.
37. Schwedhelm E. Quantification of ADMA: analytical approaches. *Vasc Med*. 2005;10(suppl 1):S89–S95.
38. Pritchard KA Jr, Groszek L, Smalley DM, Sessa WC, Wu M, Villalon P, Wolin MS, Stemerman MB. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res*. 1995;77:510–518.
39. Forstermann U. Janus-faced role of endothelial NO synthase in vascular disease: uncoupling of oxygen reduction from NO synthesis and its pharmacological reversal. *Biol Chem*. 2006;387:1521–1533.
40. Dixon LJ, Hughes SM, Rooney K, Madden A, Devine A, Leahey W, Henry W, Johnston GD, McVeigh GE. Increased superoxide production in hypertensive patients with diabetes mellitus: role of nitric oxide synthase. *Am J Hypertens*. 2005;18:839–843.
41. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. *Free Radic Biol Med*. 2006;40:183–192.

CLINICAL PERSPECTIVE

The endothelium plays a major role in regulating vascular tone by secreting the potent vasodilator nitric oxide, which is synthesized from L-arginine (Arg) by endothelial nitric oxide synthase. Nitric oxide synthase is competitively inhibited by asymmetric dimethylarginine (ADMA), an endogenous compound that is elevated in renal failure, cardiovascular disease, and diabetes mellitus. Higher ADMA and low ratio of Arg to ADMA are markers of endothelial dysfunction. Prospective investigations have highlighted the role of ADMA as a predictor of cardiovascular disease events and death in patients with coronary artery disease, renal failure, and other high-risk conditions. Data are limited regarding the relations of ADMA and Arg/ADMA ratio to cardiovascular disease incidence and death in the general population. We related plasma ADMA, Arg, and the Arg/ADMA ratio to the incidence of cardiovascular disease and death in 3320 participants from the community-based Framingham study cohort who were followed up for 10.9 years. In multivariable models adjusting for established risk factors, ADMA was associated positively with death, whereas the Arg/ADMA ratio was inversely related. We noted effect modification by diabetes status; ADMA was associated with death in individuals without diabetes but not in individuals with diabetes. ADMA and the Arg/ADMA ratio were not associated with cardiovascular disease incidence. Additional studies evaluating a larger sample of individuals (including those with diabetes) and with longer follow-up are warranted to confirm these observations.