Association of the Endogenous Nitric Oxide Synthase Inhibitor ADMA With Carotid Artery Intimal Media Thickness in the Framingham Heart Study Offspring Cohort

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Background and Purpose—Higher plasma concentrations of the endogenous nitric oxides synthase inhibitor asymmetrical dimethylarginine (ADMA) are associated with increased risk of cardiovascular and cerebrovascular events and death, presumably by promoting endothelial dysfunction and subclinical atherosclerosis. We hypothesized that plasma ADMA concentrations are positively related to common carotid artery intimal-media thickness (CCA-IMT) and to internal carotid (ICA)/bulb IMT.

Methods—We investigated the cross-sectional relations of plasma ADMA with CCA-IMT and ICA/bulb IMT in 2958 Framingham Heart Study participants (mean age, 58 years; 55% women).

Results—In unadjusted analyses, ADMA was positively related to both CCA-IMT (β per SD increment, 0.012; P=0.001) and ICA/bulb IMT (β per SD increment, 0.059; P=0.001). In multivariable analyses (adjusting for age, sex, systolic blood pressure, antihypertensive treatment, smoking status, diabetes, BMI, total-to-HDL cholesterol ratio, log C-reactive protein, and serum creatinine), plasma ADMA was not associated with CCA-IMT (P=0.991), but remained significantly and positively related to ICA/bulb IMT (β per SD increment, 0.0246; P=0.002).

Conclusions—In our large community-based sample, we observed that higher plasma ADMA concentrations were associated with greater ICA/bulb IMT, but not with CCA-IMT. These data are consistent with the notion that ADMA promotes subclinical atherosclerosis in a site-specific manner, with a greater proatherogenic influence at known vulnerable sites in the arterial tree. (Stroke. 2009;40:2715-2719.)

Key Words: carotid intimal medial thickness ■ epidemiology ■ risk factors

Carotid artery intimal-media thickness (IMT) is a widely accepted indicator of subclinical atherosclerosis burden, with higher values being associated with an adverse cardiovascular prognosis. Consequently, carotid IMT has also been proposed as a surrogate end point for therapeutic interventions directed at lowering atherosclerotic burden.1,2 Accumulating scientific evidence links asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of all major isoforms of nitric oxide (NO) synthase, to cardiovascular and cerebrovascular disease.3,4 Higher plasma ADMA concentrations are associated with increased risk of myocardial infarction, stroke, and total mortality in a broad spectrum of people in the general population,5-7 and in patients with prevalent coronary heart disease,8,9 septic shock,10 and renal failure.11 It is widely assumed that the association of ADMA and adverse clinical events in these diverse samples is largely related to the attenuation of the vasculoprotective effects of NO, leading to endothelial dysfunction and subsequent atherosclerosis.4,12-15 Few clinical studies have directly related endogenous ADMA concentrations to the extent of atherosclerosis in community-based samples; thus, ADMA has been related to CT coronary artery calcification in young adults.16 In 1999 Miyazaki et al17 reported a strong positive correlation of ADMA with common carotid artery (CCA) intimal media thickness (IMT) in 122 clinically healthy Japanese, a finding that has been replicated in another Japanese sample,18 and in smaller studies19-21 investigating patients with prevalent disease. Another recent report22 noted an inverse association of

Received March 14, 2009; accepted April 15, 2009.
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Stroke is available at http://stroke.ahajournals.org
DOI: 10.1161/STROKEAHA.109.552539
ADMA concentrations with IMT, thereby rendering the issue unclear. These previous studies were limited by modest sample sizes and referral biases. More recently, ADMA was related to baseline CCA-IMT and to progression in CCA-IMT over the course of 6 years in 2 community-based cohorts23,24 of Native American and Japanese persons. However, no study has assessed the association of ADMA with IMT of the carotid bulb and the proximal part of the internal carotid artery (ICA), a surprising omission given abundant clinical and experimental data suggesting that this vascular region may be especially vulnerable to attenuation of NO synthesis.25

We hypothesized that higher ADMA concentrations are associated with greater IMT in the CCA, as well as in the ICA/bulb, and we tested this hypothesis in the large, community-based Framingham Offspring Study cohort.

Subjects and Methods

Participants and Covariates

We have detailed the selection criteria and design of the Framingham Heart Study elsewhere.26 The Framingham Offspring Study began in 1971 with the enrollment of 5124 participants who were either the children of the original cohort participants or spouses of these children. The study protocol was approved by the Boston Medical Center Institutional Review Board, and all participants provided written informed consent.

Offspring cohort participants undergo routine examinations at the Heart Study clinic approximately once every 4 years. At each Heart Study visit, attendees undergo anthropometric measurements, medical history, physical examination, and laboratory assessment of cardiovascular risk factors. Of 3532 attendees at the sixth examination cycle (1995–1998), 3453 (98%) participants had plasma ADMA concentration measured. We excluded 413 individuals from the present investigation because of prevalent cardiovascular disease including stroke. 124 were excluded for missing carotid information, 29 were excluded for missing ADMA, and 8 were excluded for creatinine >2 mg/dL. After these exclusions, 2958 individuals (1622 women) remained eligible for the present investigation. Participants with prevalent cardiovascular disease were excluded to avoid confounding by prevalent disease status. Also, if ADMA is associated with prevalent cardiovascular disease and prevalent cardiovascular disease is associated with greater IMT, then we could find a positive association between ADMA and IMT that is simply an epiphenomenon.

Carotid Ultrasonography

At the sixth examination cycle, carotid ultrasonography was performed and images were analyzed according to a standardized protocol by a single trained sonographer using a single ultrasound machine (Toshiba Medical Systems).27,28 A high-resolution 7.5-MHz transducer was used for imaging the CCA, whereas a 5.0-MHz transducer was used for the carotid bulb and the ICA. The trained sonographer was blinded to the clinical information of the participants and made IMT measurements, which were over-read by 1 of the investigators (J.P.). Carotid IMT measurements were obtained from 2 diastolic images at 3 sites in each (right and left) carotid artery. These sites were at the level of the distal CCA, at the carotid artery bulb, and at the proximal ICA (defined as the first 2 cm). At each site the maximal IMT was assessed in the right near and far walls, and in the left near and far walls, thus giving 4 wall-segment measurements at each site. These 12 measurements were grouped into 2 sets. The mean of the 4 measurements recorded in the distal CCA was estimated as the mean maximal CCA-IMT. Thus, the mean maximum wall thickness of the CCA was estimated as the mean of the maximum wall thicknesses for near and far wall on both the left and right sides [mLNW + mLFW + mRNW + mRFW]/4]. The mean of the 8 measurements recorded at the carotid bulb and proximal ICA sites was estimated as the mean maximal carotid bulb/ICA-IMT. We have previously reported27 good reproducibility for our measurement protocols, with intraclass correlation coefficients for mean maximal CCA-IMT and mean maximal ICA/bulb IMT of 0.86 and 0.74, respectively.

Determination of ADMA

ADMA was measured (as detailed elsewhere) using a fully validated commercially available high-throughput liquid chromatography-tandem mass spectrometry assay (DLD Diagnostika).29,30 The intra-assay and the interassay coefficients of variation were 3.2% and <5%, respectively.

Statistical Analyses

Means and SD are presented to summarize continuous clinical, imaging, and biochemical characteristics, and percentages are presented for categorical characteristics. We evaluated the distributional properties of the variables graphically, and log-transformed variables that were skewed. Pearson correlation coefficient was calculated to assess correlations among the variables; t-tests were used for the baseline comparisons of continuous data. It has previously been shown that the CCA and the ICA segments may differ with regard to their associations with risk common vascular risk factors.1,25 We, therefore, defined 2 vascular targets to be assessed, ie, CCA-IMT and the ICA/bulb IMT. The corresponding null hypotheses to be tested was that in a multivariable-adjusted model neither CCA-IMT nor the ICA/bulb IMT is related to continuous ADMA concentrations. To account for the inflation of the α error attributable to analysis of 2 carotid IMT measurements, we applied a Bonferroni correction and defined P < 0.025 (0.05 divided by 2) as indicating statistical significance, and as the threshold required to reject the null hypothesis. All additional analyses were deemed exploratory, and P < 0.05 was considered statistically significant for these analyses.

We used multiple linear regression models to relate plasma ADMA concentrations (independent variable) to CCA-IMT and ICA/bulb IMT (dependent variables), both sets of measurements being modeled as continuous untransformed variables, given their normal distributions. We did not observe effect modification by sex on formal statistical testing for an interaction between ADMA and sex for CCA-IMT or ICA/bulb IMT measurements. Accordingly, all analyses were performed for pooled sexes.

We constructed 2 sets of multivariable models: model 1 adjusted for age, sex, and BMI; model 2 adjusted for age, sex, systolic blood pressure, antihypertensive treatment, smoking status, diabetes, BMI, total-to-HDL cholesterol ratio, C-reactive protein (log-transformed), and serum creatinine (fully adjusted model). We used a general linear model for analysis of covariance to estimate adjusted least-squares means of the IMT measures across quartiles of plasma ADMA. We further evaluated the effect modification of ADMA by age (dichotomized at the median for the sample), diabetes (yes/no), smoking, and BMI (≥30 kg/m²; yes/no) by incorporating corresponding interaction terms into the multivariable model 2; none of these interactions was statistically significant. We also evaluated effect modification by a composite measure of vascular risk, the Framingham risk score.23 All analyses were performed with SAS statistical software version 9.1 (SAS Institute 2002).

Results

The baseline clinical and biochemical characteristics, as well as distributions of carotid IMT measures of the 2958 participants (55% women), in the present investigation are displayed in Table 1.

Association of ADMA Concentrations With CCA and ICA/Bulb IMT

In unadjusted analyses, ADMA was positively related to both CCA-IMT (β per SD increment, 0.012; P < 0.001) and ICA/bulb IMT (β per SD increment, 0.059; P < 0.001). On adjustment for other risk factors, ADMA remained positively
related to ICA/bulb IMT ($P=0.002$), but not to CCA-IMT ($P=0.99$). Table 2 displays the results of the multivariable-adjusted regression analyses relating ADMA concentrations (modeled as a continuous variable and as quartiles) to CCA-IMT and ICA/bulb IMT. Correspondingly, we observed an increase of the ICA/bulb IMT from the first to the fourth quartile of ADMA ($P=0.029$ for trend), whereas there was no significant trend for CCA-IMT ($P=0.39$), as shown in Tables 2 and 3.

Using Generalized Linear Models procedures, we also assessed whether the ADMA concentration retains an independent association with ICA/bulb IMT when accounting for the individual cardiovascular risk based on the Framingham Risk Score. The interaction between ADMA quartiles and Framingham Risk Score groups was not found significant ($P=0.32$); therefore, the association of ADMA with ICA/bulb IMT does not depend on the Framingham Risk Score. Subsequently, in a multivariable model controlling for Framingham Risk Score group, ADMA was associated with ICA/bulb IMT ($P<0.001$).

### Discussion

In our cross-sectional study of a large community-based sample, we observed a positive association of plasma ADMA concentrations with ICA/bulb IMT but not with CCA-IMT.

### Mechanisms Underlying the Association of Plasma ADMA and ICA/Bulb IMT

The L-arginine–NO pathway is crucial for the regulation of vascular tone and protection of vascular integrity by preventing endothelial activation. Premature atherosclerosis and spontaneous myocardial infarction were observed in a new mouse model deficient in all 3 NO synthase isoforms, which elegantly verifies the concept that failure of NO synthesis can promote generalized atherosclerosis. In a concentration range matching that observed in vivo inside cells, ADMA has discernible inhibitory effects on NO synthesis. Mice with partial ablation of the ADMA-degrading enzyme dimethylarginine dimethylaminohydrolase 1 also have only moderately (30% to 50%) elevated plasma ADMA concentrations, but demonstrate endothelial dysfunction, increased vascular resistance, elevated blood pressure, and reduced cardiac output. In contrast, mice overexpressing dimethylarginine dimethylaminohydrolase 1 or dimethylarginine dimethylaminohydrolase 2 appear to be protected from vascular damage. This is further supplemented by several clinical studies. Thus, our cross-sectional observations are consistent with the known association of ADMA with subclinical atherosclerosis in some previous reports.

An alternative explanation for the association of ADMA and ICA IMT would be that in patients with subclinical atherosclerosis, higher ADMA concentrations are simply a marker of associated processes such as oxidative stress or concomitant renal dysfunction that impairs its generation, metabolism, or excretion. We controlled for renal function by excluding individuals with overt renal dysfunction at baseline and by adjusting for serum creatinine in all multivariable analyses.

### Site-Specific Associations of ADMA

Several investigations have documented the differential responses of different anatomic vascular sites to systemic risk

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**Table 1. Characteristics of Study Sample**

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>All, N=2958</th>
<th>Women, N=1622</th>
<th>Men, N=1336</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>58±10</td>
<td>59±10</td>
<td>58±10</td>
</tr>
<tr>
<td>BMI, kg/m</td>
<td>27.9±5.2</td>
<td>27.3±5.7</td>
<td>28.5±4.4</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128±19</td>
<td>126±20</td>
<td>130±17</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76±9</td>
<td>74±9</td>
<td>78±9</td>
</tr>
<tr>
<td>Hypertension treatment, %</td>
<td>717 (24%)</td>
<td>368 (23%)</td>
<td>349 (26%)</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>441 (15%)</td>
<td>243 (15%)</td>
<td>198 (15%)</td>
</tr>
<tr>
<td>Diabetes*, %</td>
<td>234 (8%)</td>
<td>112 (7%)</td>
<td>122 (9%)</td>
</tr>
</tbody>
</table>

**Carotid ultrasound**

- CCA-IMT (mm): 0.72±0.16
- ICA/bulb-IMT (mm): 0.94±0.47

**Biochemical features**

- Total to HDL cholesterol ratio: 4.4±1.8
- Creatinine in mg/dL: 1.0
- C-reactive protein mg/dL: 4.2
- ADMA, μmol/L: 0.54±0.13

Values are expressed as mean±SD or percentages of patients.

*Diabetes: blood glucose ≥126 mg/dL or use of insulin or hypoglycemic prescription.

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**Table 2. Multivariable-Adjusted Regression of CCA-IMT and ICA/Bulb IMT on Plasma ADMA Levels**

<table>
<thead>
<tr>
<th>Models</th>
<th>CCA-IMT</th>
<th>ICA/bulb-IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (SE)</td>
<td>P</td>
<td>β (SE)</td>
</tr>
<tr>
<td>Models with continuous ADMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADMA, per SD increment</td>
<td>0.0000297 (0.0026)</td>
<td>0.002</td>
</tr>
<tr>
<td>β (SE)</td>
<td>P</td>
<td>P for Trend</td>
</tr>
<tr>
<td>Models with ADMA quartiles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Q2</td>
<td>-0.015 (0.007)</td>
<td>0.046</td>
</tr>
<tr>
<td>Q3</td>
<td>-0.010 (0.008)</td>
<td>0.176</td>
</tr>
<tr>
<td>Q4</td>
<td>-0.008 (0.007)</td>
<td>0.266</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, systolic blood pressure, antihypertensive treatment, smoking status, diabetes, BMI, total-to-DL cholesterol ratio, log-C-reactive protein, and serum creatinine.

SE indicates standard error of β. β is the regression coefficient per 1 SD increase in ADMA (model A) or with quartile change in ADMA (model B).
Table 3. Unadjusted and Adjusted Mean IMT Values by Quartile Categories of ADMA

<table>
<thead>
<tr>
<th>IMT Variable</th>
<th>Covariates Adjusted</th>
<th>Unadjusted and Adjusted Mean IMT Values† (mm) by Quartile Categories of ADMA</th>
<th>P Q4 vs Q1</th>
<th>P for Trend Across Quartiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA-IMT, mm</td>
<td>Unadjusted</td>
<td>Q1 0.71 Q2 0.71 Q3 0.73 Q4 0.74</td>
<td>0.004</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>Age, sex, BMI</td>
<td>Q1 0.73 Q2 0.72 Q3 0.72 Q4 0.72</td>
<td>0.332</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td>Multivariable*</td>
<td>Q1 0.73 Q2 0.72 Q3 0.72 Q4 0.72</td>
<td>0.266</td>
<td>0.391</td>
</tr>
<tr>
<td>ICA/bulb-IMT, mm</td>
<td>Unadjusted</td>
<td>Q1 0.86 Q2 0.91 Q3 0.97 Q4 1.00</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age, sex, BMI</td>
<td>Q1 0.91 Q2 0.93 Q3 0.94 Q4 0.96</td>
<td>0.016</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Multivariable*</td>
<td>Q1 0.91 Q2 0.93 Q3 0.94 Q4 0.96</td>
<td>0.036</td>
<td>0.029</td>
</tr>
</tbody>
</table>

*Multivariable adjusted models include adjustment for age, sex, systolic blood pressure, antihypertensive treatment, smoking status, diabetes, BMI, total-to-HDL cholesterol ratio, log-C-reactive protein, and serum creatinine.
†According to model IMT values are unadjusted and adjusted least-square means.

Factors. The varying association of ADMA with atherosclerotic lesions in carotid artery locations that are only centimeters apart is intriguing. This finding should, however, be interpreted with caution, because some previous studies did relate ADMA to CCA-IMT; the absence of an association between ADMA and CCA-IMT in the present study therefore may be attributable to chance or may reflect differences in the stage of the disease at both sites, as well as ethnicity and other differences between our sample and the previous cohort studies. Yet, for the carotid artery, it has frequently been reported that the region of the bulb and the adjacent part of the ICA appear to be more sensitive to circulating risk factors for atherosclerosis than the common carotid artery. A major physiological distinction between the CCA and the ICA/bulb are differences in flow patterns. For anatomic reasons, laminar flow in the carotid bulb is frequently disturbed, leading to insufficient stimulation of NO synthase expression and activation. Thus, it is conceivable that this location may be especially sensitive to further impairment of NO synthesis by higher ADMA concentrations. The differential association of ADMA with CCA-IMT and ICA-IMT might also reflect a higher prevalence of atherosclerosis and plaque formation in the ICA-IMT and could suggest that ADMA is associated with more severe lesions.

Study Strength and Limitations
The strengths of our investigation include the large, community-based, well-characterized sample, and the determination of ADMA blinded to IMT measurements. Key limitations of our study include its cross-sectional design that precludes any causal inferences, and the predominantly white sample, which limits the generalizability of our observations to other ethnicities.

Conclusions
In our large, community-based sample, we observed that higher plasma concentrations of the endogenous NO synthase inhibitor ADMA were associated with greater ICA/bulb IMT but not with greater CCA-IMT. These findings are consistent with the promotion of subclinical atherosclerosis in a site-specific manner (ie, at sites of arterial vulnerability) by circulating ADMA. Further, ADMA may serve as a biomarker for carotid disease, and additional exploration of this pathway could further our understanding of the pathophysiology underlying the development of carotid atherosclerosis.

Sources of Funding
This work was supported by National Institutes of Health/National Heart, Lung, and Blood Institute Contract N01-HC-25195, N01HV28178, and 2K24HL04334, Dr Vasan), the National Institute on Aging (R01 AG16495; AG08122, Dr Wolf; and AG033193, Dr Seshadri), the National Institute of Neurological Disorders and Stroke (R01 NS17950, Dr Wolf), and the Deutsche Forschungsgemeinschaft grant (Bo1431-4/1, Dr Büger).

Disclosures
Drs. Böger, Schwedhelm, and Maas are named as inventors on patents relating to analytical assays for methylarginines and receive modest royalties from these.

References


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*Stroke*. 2009;40:2715-2719; originally published online June 4, 2009;
doi: 10.1161/STROKEAHA.109.552539

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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