Asymmetric Dimethylarginine and Cardiovascular Risk: Systematic Review and Meta-Analysis of 22 Prospective Studies

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Background—Asymmetric dimethylarginine (ADMA) inhibits the production of nitric oxide, a key regulator of the vascular tone, and may be important in the development of cardiovascular disease (CVD). Our aim was to reliably quantify the association of ADMA and its isomer symmetric dimethylarginine (SDMA) with the risk of CVD outcomes in long-term cohort studies.

Methods and Results—Data were collated from 22 prospective studies involving a total of 19,842 participants, which have recorded 2339 CVD, 997 coronary heart disease, and 467 stroke outcomes during a mean follow-up of 7.1 years. In a comparison of individuals in the top with those in the bottom third of baseline ADMA values, the combined risk ratios were 1.42 (95% confidence interval: 1.29 to 1.56) for CVD, 1.39 for coronary heart disease (1.19 to 1.62), and 1.60 for stroke (1.33 to 1.91). Broadly similar results were observed according to participants’ baseline disease status (risk ratios for CVD: 1.35 [1.18 to 1.54] in general populations; 1.47 [1.16 to 1.87] in individuals with pre-existing CVD; and 1.52 [1.26 to 1.84] in individuals with pre-existing kidney disease) and by different study characteristics, including geographical location, sample type, assay method, number of incident outcomes, and level of statistical adjustment (all $P$ values $>0.05$). In contrast, in 8 prospective studies involving 9070 participants and 848 outcomes, the corresponding estimate for SDMA concentration was 1.32 (0.92 to 1.90) for CVD.

Conclusions—Available prospective studies suggest associations between circulating ADMA concentration and CVD outcomes under a broad range of circumstances. Further research is needed to better clarify these associations, particularly in large general population studies. (J Am Heart Assoc. 2015;4:e001833 doi: 10.1161/JAHA.115.001833)

Key Words: asymmetric dimethylarginine • cardiovascular diseases • meta-analysis • prospective studies

A symmetric dimethylarginine (ADMA) is a naturally occurring modified amino acid in human blood. It inhibits the production of nitric oxide, a key regulator of the vascular tone, and may thereby contribute importantly to the process of atherosclerosis.1–3 ADMA has been shown to correlate with various measures of subclinical atherosclerosis, including carotid intima-media thickness4 and flow-mediated dilatation.5–8 Additionally, a growing number of studies suggest that high values of circulating ADMA concentration are associated with the incidence of cardiovascular disease (CVD) outcomes. However, interpretation of these studies has been complicated because they differ in relation to the population studied (eg, approximately general population versus patients with pre-existing CVD or kidney disease), the disease outcomes assessed (eg, “hard” CVD composed of coronary heart disease and stroke versus wider definitions), and/or the analytical approaches used (eg, different adjustment for potential confounders).9 Furthermore, as previously published reports typically comprised only a few hundred incident CVD outcomes, they were insufficiently powered to investigate associations by clinically relevant characteristics. Finally, the extent to which associations of ADMA are consistent with those of its related isomer symmetric dimethylarginine (SDMA) has never been quantitatively reviewed.

To help clarify the evidence, we have conducted a systematic review and meta-analysis of available data on ADMA and SDMA in relation to CVD outcomes. We had 3 principal aims. First, to quantify associations of circulating...
ADMA concentration with incident CVD, coronary heart disease (CHD), and stroke in a consistent manner. Second, to evaluate these associations under a wide range of circumstances. Third, to compare associations for ADMA with those for SDMA.

Methods

Literature Search and Study Selection

We sought prospective studies that had been published between January 1970 and January 2015 and reported on associations of dimethylarginines with incident CVD (defined as CHD or stroke). Systematic searches of PubMed, Web of Science, and EMBASE were supplemented by scanning reference lists of articles identified (including relevant reviews) and by correspondence with several study investigators. The search strategy is detailed in Table 1. Studies were eligible for inclusion if they had recorded events over at least 1 year of follow-up and involved any of the following types of study populations: approximately general population (ie, participants not selected on the basis of preexisting disease at baseline), populations with pre-existing cardiovascular diseases (eg, people with CHD or stroke or peripheral artery disease), or populations with pre-existing kidney disease (eg, people with chronic kidney disease or a kidney transplant). We only included studies that conformed to our pre-specified CVD outcome definition, and excluded studies that used broader outcome definitions (involving incident heart failure, cardiac arrhythmia, peripheral arterial disease, venous thrombosis, pulmonary embolism, or all-cause mortality),10–22 had a follow-up of less than 1 year,23 or both.24–26 The meta-analysis was conducted following the PRISMA guidelines.

Data Extraction

Descriptive and quantitative data were extracted by consensus among 2 independent reviewers using standardized data extraction protocols. If multiple publications on the same study were available, the most up-to-date or comprehensive information was used. Retrieved study characteristics included study design, geographical location, population source (ie, population registers, general practice registers, or hospital-based registers), baseline disease status, study size, average age at baseline, and proportion of male participants. Additionally, information on the measurement of dimethylarginines was obtained, including sample type (ie, plasma/serum), storage temperature, and assay details (ie, assay method and manufacturer/source). Finally, data in relation to follow-up were extracted, including duration of follow-up, the specific composition of reported endpoints, number of incident outcomes, effect sizes, and degree of statistical adjustment of reported associations. The degree of adjustment was classified as “o” when risk ratio (RRs) estimates were unadjusted; “+” when RRs were adjusted for age and sex; “++” when further adjusted for at least 2 conventional CVD risk factors (ie, smoking, diabetes, blood pressure, or circulating lipid levels); and “+++” when additionally adjusted for other factors.

Statistical Analysis

Analyses involved only within-study comparisons (ie, cases and controls were directly compared only within each cohort) to limit potential biases. To enable a consistent approach to analysis, RRs and 95% confidence intervals (CIs) in each study were standardized to a common scale, ie, to reflect a comparison of the top third with the bottom third of the population’s baseline distribution of circulating ADMA or SDMA concentrations, employing statistical methods described.
elsewhere. These comparisons correspond approximately to a difference of 0.67 μmol/L in ADMA and 0.53 μmol/L in SDMA concentrations, respectively. Summary RRs were calculated by pooling study-specific estimates by random-effects meta-analysis, with hazard ratios and odds ratios assumed to approximate the same measure of relative risk. One study provided supplementary unpublished tabular data on 10 years of follow-up (as opposed to 5 years in the original published report). When studies reported RRs of various levels of adjustment, the most adjusted estimate was used. Consistency of findings across studies was assessed with standard χ² tests and the I² statistic. Subgroup analyses were conducted using meta-regression across pre-specified study-level characteristics. Evidence of publication bias was assessed using funnel plots and Egger’s asymmetry test. Duval and Tweedie’s nonparametric “trim and fill” method was applied to take into account the effect of publication bias on pooled RRs. All analyses were performed using Stata release 12.1 (StataCorp, College Station, TX). All statistical tests were 2-sided and used a significance level of P<0.05.

Figure 1. Study flow diagram. ADMA indicates asymmetric dimethylarginine; CVD, cardiovascular disease; SDMA, symmetric dimethylarginine.
Table 2. Study Design and Assay Methods of the 22 Prospective Studies

| Study Acronym | Country | Study Baseline | Population Source | Baseline Disease | No. of Participants | Mean Age, y | Male, % | Sample Type | Storage Temperature, °C | Assay Method | Manufacturers or Assay Source | Mean ADMA, μmol/L | Mean SDMA, μmol/L |
|---------------|---------|----------------|-------------------|------------------|---------------------|------------|--------|-------------|--------------------------|-------------|---------------------------|----------------|----------------|
| General population |         |                |                   |                  |                     |            |        |             |                          |             |                           |                |                |
| BRUNECK28     | Italy   | 2000–2010      | Population register | —                | 685                 | 66         | 48     | Plasma      | −70°                     | LC-MS/MS    | ABSciex API 4000           | 0.97           | 0.65           |
| DHS33         | US      | 2000–2002      | Population register | —                | 3523                | 43         | 44     | Plasma      | −70°                     | LC-MS/MS    | Varian 1200L              | N/R            | N/R            |
| GetABI (no PAD)34 | Germany | 2001–2006     | GP register        | —                | 1187                | 72         | 42     | Plasma      | N/R                     | LC-MS/MS    | Varian 1200L              | 0.60           | 0.48           |
| INCHIANTI35   | Italy   | 1998–2000      | Population register | —                | 1025                | 75         | 44     | Serum       | −80°                     | LC-MS/MS    | Varian 1200L              | 0.5            | —              |
| KIHD36        | Finland | 1991–1993      | Population register | —                | 150                 | 58         | 100    | Serum       | −80°                     | HPLC        | In house                  | 0.51           | —              |
| KVINNO STUDIEN37 | Sweden | 1968–1969   | Population register | —                | 880                 | 46         | 0      | Serum       | −20°                    | HPLC        | In house                  | 0.62           | —              |
| MDC38         | Sweden  | 1991–1996      | Population register | —                | 506                 | 58         | 40     | Plasma      | N/R                     | LC-MS/MS    | N/R                       | N/R            | —              |
| MONICA/KORA29 | Germany | 1989–1995     | Population register | —                | 342                 | 61         | 100    | Plasma      | −70°                    | ELISA       | DLD Diagnostica            | 0.79           | —              |
| Populations with pre-existing CVD |         |                |                   |                  |                     |            |        |             |                          |             |                           |                |                |
| AtheroGene40  | Germany | 1999–2004      | Hospital           | Confirmed CAD    | 1874                | 61         | 79     | Serum       | −80°                     | ELISA       | DLD Diagnostica            | 0.68           | —              |
| BECAC41       | Norway  | 2000–2004      | Hospital           | Suspected CAD    | 1364                | 61         | 75     | Plasma      | −80°                     | LC-MS/MS    | Bevital AS                | 0.59*          | —              |
| Cavalca et al42 | Italy  | 2005–2007      | Hospital           | NSTEMI           | 104                 | 67         | 74     | Plasma      | −80°                     | HPLC        | ESA Biosciences            | 0.43           | 0.49           |
| Cavusoglu et al43 | US     | 1999–2002      | Hospital           | Suspected CAD    | 182                 | 65         | 100    | Plasma      | −70°                     | ELISA       | DLD Diagnostica            | N/R            | —              |
| GeneBank44    | US      | N/R            | Hospital           | Suspected CAD    | 1011                | 64         | 47     | Plasma      | −80°                     | HPLC        | In-house                  | 1.03*          | 0.65*          |
| GetABI (PAD)34 | Germany | 2001–2006     | GP register        | PAD              | 1260                | 74         | 46     | Plasma      | N/R                     | LC-MS/MS    | Varian 1200L              | 0.63           | 0.51           |
| KAROLA45      | Germany | 1999–2000      | Hospital           | CHD              | 1148                | 59         | 85     | Plasma      | −80°                     | LC-MS/MS    | Varian 1200L              | 0.57           | 0.53           |

*Indicates significant difference from baseline values.
| Study Acronym | Study Design | Population Source | Baseline Disease | No. of Participants | Mean Age, y | Male, % | Sample Type | Storage Temperature, °C | Assay Method | Manufacturers or Assay Source | Mean ADMA, µmol/L | Mean SDMA, µmol/L |
|--------------|-------------|--------------------|------------------|---------------------|-------------|--------|-------------|-------------------------|--------------|--------------------------|-----------------|----------------|
| Lu et al⁴⁶   | Taiwan 1999–2001 Hospital | Suspected CAD | 103 | 71 | 87 | Plasma | −70° | HPLC | Waters 470 | 0.56 | 0.58 |
| Lu et al⁴⁷   | Taiwan 2006–2009 Hospital | Suspected CAD | 997 | 67 | 79 | Plasma | −70° | HPLC | Waters 470 | 0.45 | — |
| ALERT⁴⁸      | Multi-national 1996–1997 Hospital | KTx | 1847 | 50 | 66 | Serum | −80° | HPLC | N/R | 0.77* | — |
| CREED⁴⁹,⁵⁰   | Italy 1997–1998 Hospital | CKD stage 5 | 283 | 61 | 56 | Plasma | −80° | HPLC | Varian | 3.03* | — |
| Ignjatovic et al⁵¹ | Serbia N/R Hospital | Hemodialysis | 153 | 58 | 70 | Plasma | N/R | HPLC | Agilent 1200 | 0.44 | 0.94 |
| MDRD⁵²       | US 1989–1993 Hospital | CKD stage 3/4 | 821 | 52 | 60 | Serum | −70° | ELISA | DLD Diagnostica | 0.73 | — |
| SDC⁵³        | Denmark 1993 Hospital | Diab. nephropathy | 397 | 42 | 61 | Plasma | N/R | HPLC | N/R | 0.46 | — |
| Total         | 1968–2010 | | 19 842 | 58 | 58 | | | | 0.71 | 0.56 |

Full study names: ALERT, Assessment of Lescol in Renal Transplantation Study; BECAC, Bergen coronary angiography cohort; BRUNECK, Bruneck Study; CREED, Cardiovascular Risk Extended Evaluation in Dialysis; DHS, Dallas Heart Study; GetABI, German Epidemiological Trial on Ankle Brachial Index; INCHIANTI, Invecchiare in Chianti Study; KAROLA, Langzeiterfolge der KArDiologischen Anschlussbehandlung; KIHd, Kuopio Ischaemic Heart Disease Study; KVINNOSTUDIEN, Kvinnostudien Population Study of Women in Gothenburg; MDC, Malmö Diet and Cancer Cardiovascular Cohort; MDRD, Modification of Diet in Renal Disease Study; MONICA/KORA, Monitoring of Trends and Determinants in Cardiovascular Disease Augsburg; SDC, Steno Diabetes Center. ADMA indicates asymmetric dimethylarginine; CAD, coronary artery disease; CHD, coronary heart disease; CKD, chronic kidney disease; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; GP, general practitioner; HPLC, high-performance liquid chromatography; KTx, kidney transplant; LC-MS/MS, liquid chromatography with tandem mass spectrometry; N/R, not reported; NSTEMI, non ST-elevation myocardial infarction; PAD, peripheral arterial disease; SDMA, symmetric dimethylarginine. * Median.
Results

General Characteristics of the Included Studies

We screened 3180 records (Figure 1) and identified 22 eligible prospective studies reporting on a total of 19,842 participants (Tables 2 and 3). During a mean follow-up duration of 7.1 years, a total of 2339 CVD, 997 CHD, and 467 stroke outcomes were recorded. Sixteen studies were based in Europe, 4 in North America, and 2 in Asia. Participants were typically sourced from population registers (7111 participants), general practitioner registers (2447 participants), or from hospitals (10,284 participants). Eight studies were based on participants from general populations (8298 participants); 9 studies involved people with pre-existing CVD (8043 participants); and 5 studies involved people with pre-existing kidney disease (3501 participants). All but 3 studies reported effect estimates adjusted for age, sex, and at least 2 other conventional CVD risk factors (ie, smoking, diabetes, blood pressure, or circulating lipid levels). Fourteen studies reported effect estimates further adjusted for additional characteristics, such as:

Table 3. Follow-Up and Incident Outcomes in the 22 Prospective Studies

| Study Acronym | Median Duration of Follow-Up, Years | Definition of Incident Cardiovascular Outcomes | No. of Incident Cardiovascular Outcomes |
|---------------|------------------------------------|-----------------------------------------------|----------------------------------------|
|               |                                    | Fatal CVD Non-Fatal MI Fatal MI Coronary Revascularization Ischemic Stroke Hemorrhagic Stroke CVD CHD Strokes |
| General population |                                   |                                             |                                        |
| BRUNECK28      | 10.0*                              | No                                           | Yes                                     | Yes                                     | Yes                                     | Yes                                     | No                                      | 90                                      | 39                                      | 46                                      |
| DHS33          | 7.4                                | Yes                                          | No                                       | No                                       | No                                       | No                                       | No                                      | 62                                      | —                                       | —                                       |
| GetABI (No PAD)34 | 5.0*                              | No                                           | Yes                                     | Yes                                     | Yes                                     | Yes                                     | Yes                                     | 131                                     | 99                                      | 33                                      |
| INCHIANTI35    | 9.2                                | Yes                                          | No                                       | No                                       | No                                       | No                                       | No                                      | 141                                     | —                                       | —                                       |
| KIHD36         | 6.0*                              | Yes                                          | No                                       | No                                       | No                                       | No                                       | No                                      | —                                       | 45                                      | —                                       |
| KVINNOSTUDIEN37| 24.0*                             | No                                           | Yes                                     | Yes                                     | No                                       | No                                       | No                                      | 101                                     | 58                                      | 43                                      |
| MDC38          | 12.0*                             | Yes                                          | Yes                                     | Yes                                     | No                                       | No                                       | Yes                                     | 253                                     | —                                       | —                                       |
| MONICA/KORA29  | 6.2                                | Yes                                          | Yes                                     | No                                       | No                                       | No                                       | No                                      | —                                       | 88                                      | —                                       |

Populations with pre-existing CVD

| AtheroGene40   | 2.6†     | Yes                                          | Yes                                     | No                                       | No                                       | Yes                                     | Yes                                     | 159                                     | —                                       | 45                                      |
| BECAC41        | 5.3†     | Yes                                          | Yes                                     | No                                       | No                                       | No                                       | No                                      | —                                       | 129                                     | —                                       |
| Cavala et al42 | 1.8      | Yes                                          | Yes                                     | No                                       | No                                       | No                                       | No                                      | —                                       | 24                                      | —                                       |
| Cavusoglu et al43 | 2.0* | Yes                                          | Yes                                     | No                                       | No                                       | No                                       | No                                      | —                                       | 37                                      | —                                       |
| GeneBank44     | 3.0*     | No                                           | Yes                                     | No                                       | No                                       | No                                       | Yes                                     | 64                                      | —                                       | —                                       |
| GetABI (PAD)34 | 5.0*     | No                                           | Yes                                     | Yes                                     | Yes                                     | Yes                                     | Yes                                     | 263                                     | 197                                     | 65                                      |
| KAROLA45       | 8.1      | Yes                                          | Yes                                     | No                                       | No                                       | Yes                                     | Yes                                     | 150                                     | —                                       | —                                       |
| Lu et al46     | 1.3      | Yes                                          | Yes                                     | Yes                                     | Yes                                     | No                                       | No                                      | 51                                      | —                                       | —                                       |
| Lu et al47     | 2.4      | Yes                                          | Yes                                     | No                                       | Yes                                     | Yes                                     | Yes                                     | 144                                     | —                                       | —                                       |

Populations with pre-existing kidney disease

| ALERT48        | 6.7†     | No                                           | Yes                                     | Yes                                     | Yes                                     | Yes                                     | Yes                                     | 455                                     | 281                                     | 174                                     |
| CREED50,50     | 10.9*    | No                                           | No                                       | No                                       | Yes                                     | Yes                                     | Yes                                     | —                                       | —                                       | 61                                      |
| Ignjatovic et al51 | 3.0* | Yes                                          | No                                       | No                                       | No                                       | No                                       | No                                      | 37                                      | —                                       | —                                       |
| MDRD52         | 9.5      | Yes                                          | No                                       | No                                       | No                                       | No                                       | No                                      | 122                                     | —                                       | —                                       |
| SDC53          | 11.3     | No                                           | Yes                                     | Yes                                     | Yes                                     | Yes                                     | Yes                                     | 116                                     | —                                       | —                                       |
| Total          | 7.1      |                                               |                                         |                                         |                                         |                                         |                                         | 2339                                    | 997                                     | 467                                     |

ALERT indicates Assessment of Lescol in Renal Transplantation Study; AtheroGene; BECAC, Bergen coronary angiography cohort; BRUNECK, Bruneck Study; CHD, coronary heart disease; CKD, chronic kidney disease; CREED, Cardiovascular Risk Extended Evaluation in Dialysis; CVD, cardiovascular disease; DHS, Dallas Heart Study; GetABI, German Epidemiological Trial on Ankle Brachial Index; INCHIANTI, Invecchiare in Chianti Study; KAROLA, Langzeitfolge der KardioLogischen Anschlussbehandlung; KIHD, Kuopio Ischaemic Heart Disease Study; KVINNOSTUDIEN, Kvinnestudien Population Study of Women in Gothenburg; MDC, Malmö Diet and Cancer Cardiovascular Cohort; MDRD, Modification of Diet in Renal Disease Study; MI, myocardial infarction; MONICA/KORA, Monitoring of Trends and Determinants in Cardiovascular Disease Augsburg; PAD, peripheral arterial disease; SDC, Steno Diabetes Center.

*Maximum.
†Mean.
as body mass index, C-reactive protein, social status, physical activity, or estimated glomerular filtration rate. On average, participants were 58 years old at baseline; 58% were male. The pooled mean and standard deviation was 0.71 ± 0.31 μmol/L for circulating ADMA and 0.56 ± 0.24 μmol/L for SDMA concentrations.

Circulating ADMA Concentration And Risk of Cardiovascular Outcomes

In a comparison of individuals in the top with those in the bottom third of baseline values of ADMA, the combined RRs were 1.42 (95% confidence interval: 1.29 to 1.56) for CVD, 1.39 (1.19 to 1.62) for CHD, and 1.60 (1.33 to 1.91) for stroke (Figures 2 and 3). The level of between-study heterogeneity was low with I² values ranging from 0% to 16%. The magnitude of association was comparable in studies conducted in the general population (RR for CVD, 1.35 [1.12 to 1.57]) and studies in individuals with pre-existing kidney disease (1.31 [1.07 to 1.60]) (Figure 4). Furthermore, there was no evidence for publication bias across studies reporting on stroke outcomes.

Circulating SDMA Concentration and Risk of Cardiovascular Outcomes

In a subset of 9 studies with available information on baseline SDMA concentration, the combined RRs comparing the top with the bottom third of SDMA concentration were 1.32 (0.92 to 1.90) for CVD, 1.44 (0.77 to 2.67) for CHD, and 1.31 (0.83 to 2.07) for stroke (Figures 2 and 6). The level of between-study heterogeneity was moderate with I² values ranging from 27% to 77%.

Discussion

The present review of about 20 000 non-overlapping participants from 22 prospective cohort studies across 9 countries has assessed strength and consistency of associations between circulating levels of ADMA and SDMA concentration and subsequent risk of cardiovascular outcomes. Overall, compared with individuals in the bottom third of baseline ADMA concentration, those in the top third of baseline ADMA concentration were at increased risk for CVD and CHD outcomes (P_{Egger}=0.009 and 0.033) (Figure 5). Application of the “trim and fill” method suggested that ≈5 studies for CVD and ≈2 studies for CHD were missing due to publication bias. Addition of these theoretical studies to the meta-analyses would attenuate RRs slightly, with RRs of 1.35 (1.20 to 1.51) and 1.35 (1.11 to 1.64) for CVD and CHD, respectively. There was no evidence for publication bias across studies reporting on stroke outcomes.
concentration were at a ≈40% higher risk of CVD. This association was similar in participants with and without pre-existing CVD or kidney disease at baseline and across studies that used diverse methods to measure dimethylarginine levels. On the basis of somewhat limited existing data on SDMA, there was no significant association between SDMA concentration and the risk of cardiovascular outcomes.

Our epidemiological findings lend support to the suggested pathophysiologica role of ADMA in atherogenesis. ADMA inhibits the production of nitric oxide (NO), a potent

**Figure 3.** Reported RRs (95% CI) for cardiovascular outcomes in individuals in the top compared with those in the bottom third of ADMA concentration. I² (95% CI) was 16% (0%, 53% for CVD, 14% (0%, 55%) for CHD and 0% (0%, 71%) for stroke. ADMA indicates asymmetric dimethylarginine; ALERT, Assessment of Lescol in Renal Transplantation Study; BECAC, Bergen coronary angiography cohort; BRUNECK, Bruneck Study; CHD, coronary heart disease; CREED, Cardiovascular Risk Extended Evaluation in Dialysis; CVD, cardiovascular disease; DHS, Dallas Heart Study; GetABi, German Epidemiological Trial on Ankle Brachial Index; INCHIANTI, Invecchiare in Chianti Study; KAROLA, Langzeiterfolge der KARDiologische Anschlussheilbehandlung; KIHd, Kuopio Ischaemic Heart Disease Study; KVINNOSTUDIEN, Kvinnostudien Population Study of Women in Gothenburg; MDC, Malmö Diet and Cancer Cardiovascular Cohort; MDRD, Modification of Diet in Renal Disease Study; MONICA/KORA, Monitoring of Trends and Determinants in Cardiovascular Disease Augsburg; PAD, peripheral arterial disease; RRs, risk ratios; SDC, Steno Diabetes Center.
vasodilator, from L-arginine. Accordingly, mice with genetically and chemically elevated levels of ADMA exhibit prompt increases in systemic vascular resistance and blood pressure, whereas mice with low ADMA levels show decreases in these parameters. In addition to the effect on vascular tone, it has been proposed that the combination of high ADMA and low NO may promote vascular inflammation, low density lipoprotein oxidation, smooth muscle cell proliferation, endothelial cell apoptosis, generation of free radicals, and adhesion and aggregation of platelets. In the Framingham Study, elevated ADMA levels were associated with a higher risk of MRI-detected silent brain infarcts. Furthermore, compelling evidence from randomized controlled trials indicates that L-arginine supplementation...

| Subgroup                          | No. of CVD events | RR comparing top vs. bottom third (95% CI) | P value |
|-----------------------------------|-------------------|-------------------------------------------|---------|
| **Study population**              |                   |                                           |         |
| General populations               | 778               | 1.35 (1.18, 1.54)                         | 0.649   |
| Pre-existing CVD                  | 831               | 1.47 (1.16, 1.87)                         |         |
| Pre-existing kidney disease       | 730               | 1.52 (1.26, 1.84)                         |         |
| **Source of study population**    |                   |                                           |         |
| Population register               | 647               | 1.36 (1.18, 1.57)                         |         |
| GP register                       | 394               | 1.29 (1.07, 1.55)                         |         |
| Hospital                          | 1298              | 1.54 (1.29, 1.83)                         |         |
| **Location**                      |                   |                                           |         |
| Europe                            | 1896              | 1.37 (1.25, 1.49)                         |         |
| North America                     | 248               | 1.57 (1.16, 2.13)                         | 0.205   |
| Asia                              | 195               | 2.69 (0.83, 8.75)                         |         |
| **Sample type**                   |                   |                                           |         |
| Plasma                            | 1361              | 1.42 (1.22, 1.65)                         | 0.397   |
| Serum                             | 978               | 1.48 (1.30, 1.68)                         |         |
| **Assay method**                  |                   |                                           |         |
| HPLC                              | 968               | 1.71 (1.37, 2.14)                         |         |
| LC-MS/MS                          | 1090              | 1.28 (1.14, 1.44)                         | 0.102   |
| ELISA                             | 281               | 1.45 (1.15, 1.82)                         |         |
| **Median length of follow-up**    |                   |                                           |         |
| ≤5 years                          | 849               | 1.45 (1.21, 1.72)                         | 0.948   |
| >5 years                          | 1490              | 1.41 (1.26, 1.58)                         |         |
| **No. of events**                 |                   |                                           |         |
| ≤150 events                       | 1209              | 1.52 (1.30, 1.77)                         | 0.288   |
| >150 events                       | 1130              | 1.34 (1.21, 1.50)                         |         |
| **Level of adjustment**           |                   |                                           |         |
| α                                  | 64                | 1.61 (0.86, 3.00)                         | 0.848   |
| ++                                 | 431               | 1.55 (1.09, 2.22)                         |         |
| +++                                | 1844              | 1.42 (1.29, 1.57)                         |         |
| **Age at baseline**               |                   |                                           |         |
| ≤60 years                         | 1296              | 1.42 (1.26, 1.62)                         | 0.904   |
| >60 years                         | 1043              | 1.54 (1.28, 1.87)                         |         |
| **Male participants, proportion** |                   |                                           |         |
| ≤50%                              | 1105              | 1.34 (1.20, 1.50)                         | 0.285   |
| >50%                              | 1234              | 1.34 (1.21, 1.50)                         |         |
| **OVERALL**                       | 2339              | 1.42 (1.29, 1.56)                         |         |

Figure 4. Association of ADMA concentration with CVD risk according to different clinically relevant characteristics. + indicates adjusted for age and sex; ++, further adjusted for at least 2 conventional CVD risk factors; ++++, additionally adjusted for other factors; ADMA, asymmetric dimethylarginine; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; GP, general practitioner; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; o, unadjusted; RR, risk ratio.
reduces blood pressure and may recuperate vascular endothelial function. Altogether, ADMA and NO are regarded to play a pivotal role in endothelial dysfunction, the essential first step in atherogenesis.

On the other hand, despite its structural similarity to ADMA, the somewhat discrepant findings for SDMA may be explained by the notion that function and metabolism of SDMA are different. SDMA has very limited or no inhibitory

**Figure 5.** Funnel plots of reported associations between ADMA and cardiovascular outcomes. The dotted lines show pseudo 95% confidence intervals around the overall pooled estimate. P values are from Egger's asymmetry test of associations. ADMA indicates asymmetric dimethylarginine; ALERT, Assessment of Lescol in Renal Transplantation Study; BECAC, Bergen coronary angiography cohort; BRUNECK, Bruneck Study; CHD, coronary heart disease; CREED, Cardiovascular Risk Extended Evaluation in Dialysis; CVD, cardiovascular disease; DHS, Dallas Heart Study; GetABI, German Epidemiological Trial on Ankle Brachial Index; KAROLA, Langzeitfolge der KARDiologischen Anschlussheilbehandlung; KVINNOSTUDIEN, Kvinnostudien Population Study of Women in Gothenburg; MDC, Malm Cardiovascular Cohort; MDRD, Modification of Diet in Renal Disease Study; MONICA/KORA, Monitoring of Trends and Determinants in Cardiovascular Disease Augsburg; PAD, peripheral arterial disease; RR, risk ratio; SDC, Steno Diabetes Center.

**Figure 6.** Reported RRs (95% CI) for cardiovascular outcomes in individuals in the top compared with those in the bottom third of SDMA concentration. I² (95% CI) was 69% (36%, 85%) for CVD, 77% (37%, 92%) for CHD and 27% (0%, 92%) for stroke. BRUNECK indicates Bruneck Study; CHD, coronary heart disease; CVD, cardiovascular disease; DHS, Dallas Heart Study; GetABI, German Epidemiological Trial on Ankle Brachial Index; KAROLA, Langzeitfolge der KARDiologischen Anschlussheilbehandlung; PAD, peripheral arterial disease; RRs, risk ratios; SDMA, symmetric dimethylarginine.
effect on NO synthase.72 While >80% of circulating ADMA is eliminated through enzymatic degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH),73 SDMA is primarily eliminated through renal filtration.74 A meta-analysis of 18 studies suggested that SDMA strongly correlates with both measured and estimated glomerular filtration rate and therefore can be regarded as an endogenous marker of renal function.75 Nonetheless, it is also possible that the apparent lack of significant associations for SDMA observed in the current review is due to low statistical power because concurrent data on this marker were available in only a handful of studies.

The strengths and limitations of our study merit consideration. We present the first meta-analysis on circulating ADMA and SDMA concentrations in relation to subsequent risk of cardiovascular outcomes. We applied pre-defined inclusion criteria to identify solely prospective, long-term studies (>1 year follow-up), thereby limiting potential misleading results owing to “reverse causation”. In the absence of individual-participant-level data, we used standardized estimates of ADMA and SDMA to allow consistent comparisons, and focused on clearly defined cardiovascular outcomes to meaningfully characterize the etiological associations. We have also presented data on a wide range of clinically relevant subgroups, which allowed us to explore in detail any potential sources of heterogeneity.

Nevertheless, our meta-analysis was still limited by the moderate amount of available data on cardiovascular outcomes. For example, there were only around 400 stroke events recorded among the ADMA studies. All studies measured dimethylarginines only once at baseline and were therefore unable to assess within-person variability of these biomarkers over time.76 Given these limitations in the existing literature, further investigation in large general population studies is needed, which would enable: (1) a further increase in the precision of estimates; (2) characterization of the shape of any dose-response relationships; (3) direct comparison of the magnitude of effect sizes for ADMA to those for traditional cardiovascular risk factors; (4) a more consistent approach to statistical adjustment; and (5) exploration of usefulness of these markers in CVD risk prediction by calculation of risk prediction metrics such as risk discrimination and risk reclassification. Finally, our meta-analysis was based on published data from prospective observational studies and therefore does not allow inferences to be made on the causal involvement of ADMA in cardiovascular disease development. Intervention studies that specifically target ADMA-related pathways (eg, via L-arginine and DDAH) will help judge causality.77,78 Furthermore, genetic loci discovered in recent genome-wide association studies of circulating levels of ADMA79,80 should provide further opportunities to evaluate specifically the causal association of ADMA with CVD through the “Mendelian randomization” approach81 (in analogy to the existing evidence for a causal role of NO signaling in the development of myocardial infarction82).

In conclusion, available evidence suggests significant positive associations of ADMA with cardiovascular disease outcomes under a broad range of circumstances. Further research is needed to better clarify these associations, particularly in large general population studies.

Acknowledgments

We thank Professors Stefan Kiechl and Johann Willeit for providing additional unpublished information for the Bruneck Study.

Sources of Funding

Dr Willeit is supported by an Erwin Schrödinger Fellowship in Epidemiology sponsored by the Austrian Science Fund (J 3679-B13). Professor Mayr is a Senior Fellow of the British Heart Foundation.

Disclosures

None.

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