Published in final edited form as:

Clin Chem Lab Med. 2011 November ; 49(11): 1907–1910. doi:10.1515/CCLM.2011.679.

Plasma symmetric dimethylarginine reference limits from the Framingham Offspring Cohort

Edzard Schwedhelm^{1,2,*}, Vanessa Xanthakis^{2,3}, Renke Maas⁴, Lisa M. Sullivan^{3,5}, Dorothee Atzler¹, Nicole Lüneburg¹, Nicole L. Glazer^{5,7}, Ulrich Riederer⁶, Ramachandran S. Vasan^{5,7,8}, and Rainer H. Böger^{1,2}

¹Institute of Clinical Pharmacology and Toxicology, University of Hamburg, Hamburg, Germany

²Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf, University of Hamburg, Hamburg, Germany

³Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA

⁴Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen, Erlangen, Germany

⁵Framingham Heart Study, 73 Mount Wayte Ave, Framingham

⁶Institute of Pharmacy, University of Hamburg, Hamburg, Germany

⁷Preventive Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

⁸Cardiology Sections, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

Abstract

BACKGROUND—Symmetric dimethylarginine (SDMA) is a by-product of protein methylation. Once released from proteins, SDMA is eliminated by the kidneys; consequently, plasma concentration has been suggested as a sensitive marker of renal function. Furthermore, recent work implicates SDMA in the pathogenesis of cardiovascular disease. To date, reference limits for SDMA plasma concentrations in healthy individuals are lacking.

METHODS—We defined reference limits for plasma SDMA concentrations in 840 relatively healthy individuals of the Offspring Cohort from Framingham Heart Study (mean age 56 years, 61 % women). Plasma SDMA concentrations were determined by LC-MS/MS using a stable isotope dilution assay.

RESULTS—The median SDMA concentration in the reference sample was 0.37 $\mu\text{mol/L}$ [Q1, Q3: 0.32, 0.43 $\mu\text{mol/L}$], and the reference limits were 0.225 and 0.533 (2.5th and 97.5th percentile). In a multivariable regression model, serum creatinine, age, and total homocysteine were positively associated with SDMA ($p < 0.001$ for all), whereas the body mass index and diastolic blood pressure were inversely related to SDMA (p -values < 0.01 and 0.03 respectively).

CONCLUSIONS—We report plasma SDMA reference limits from the community-based Framingham Heart Study. Plasma SDMA concentration was related positively to advancing age, but inversely to renal function. These reference limits may allow the identification of individuals with raised plasma SDMA concentrations.

* Author for correspondence: fax 49-40-741059757, schwedhelm@uke.uni-hamburg.de.

Keywords

Framingham Heart Study; LC-MS/MS; symmetric dimethylarginine

Introduction

We have recently reported reference limits for asymmetric dimethylarginine (ADMA) using a reference sample from the Framingham Offspring Cohort (1). ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), a cardiovascular risk factor, and higher levels are linked to greater risk of mortality and morbidity in humans (2). Unlike ADMA, symmetric dimethylarginine (SDMA) is only a weak endogenous inhibitor of NOS, and its pathophysiological role is unclear (3). Nevertheless, plasma SDMA is elevated in patients with impaired renal function, higher concentrations predict adverse outcomes in patients with prevalent cardiovascular disease, and levels are also inversely associated with insulin resistance (4–8). Analytical methods for the measurement of plasma SDMA concentrations are based on fluorescence and mass spectrometric (MS) detection. We have recently reported a liquid chromatography (LC)-tandem MS method for the simultaneous quantification of circulating ADMA and SDMA concentrations using stable isotope dilution (9). Applying this method, we now formulate reference intervals for plasma SDMA in healthy participants attending the 6th examination cycle of the Framingham Offspring Cohort.

Material and Methods

The design and selection criteria of the original Framingham Heart Study (FHS) have been described elsewhere (www.framinghamheartstudy.org/). The Offspring Cohort represents the children of the original participants and their spouses - the 6th examination cycle being performed from 1995 through 1998 as described previously (1). All participants provided written informed consent and the study protocol was approved by the institutional review board at the Boston University Medical Center. Of the 3,532 participants, we initially excluded participants from the present investigation for the following reasons: 1,079 with a serum creatinine level outside the creatinine reference limits (i.e. 64 – 105 $\mu\text{mol/L}$ for men and 49 – 90 $\mu\text{mol/L}$ for women), 15 with missing SDMA, and 93 with missing covariate data. From the 2,345 remaining participants, we established a healthy reference sample by excluding in a hierarchical fashion those with: a history of cardiovascular disease ($n=229$), hypertension ($n=753$), diabetes mellitus ($n=70$), smokers ($n=226$), obesity ($n=217$), or outlying values of SDMA (outliers were defined using Tukey's fences as values below $Q1-1.5*IQR$ or above $Q3+1.5*IQR$ [IQR = inter-quartile range], $n=10$), so that 840 participants comprised our healthy reference sample. Unthawed plasma sample aliquots were stored at -80°C prior to LC-tandem MS analysis. Method details and stability of dimethylarginines are described elsewhere [1,9,16]. All other investigated biochemical parameters were analysed at the FHS core laboratories (www.framinghamheartstudy.org/). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate an estimated glomerular filtration rate (eGFR, 10). The SAS statistical program (SAS Institute) was used for statistical analyses. Data are presented as means and standard deviations (SD) or means and standard errors (SE) unless otherwise indicated. Reference limits were calculated according to the recommendations of the International Federation of Clinical Chemistry (IFCC) as described elsewhere (1).

Results

The biochemical and clinical characteristics of our reference sample are given in Table 1A. The mean age of the reference sample was 56 years, 61% of participants were women. Compared to men, women had a lower body mass index, blood pressure, fasting glucose, total cholesterol/HDL ratio, total homocysteine, eGFR, serum creatinine (all $p<0.001$), as LDL cholesterol and triglycerides ($p\leq 0.01$). Plasma ADMA concentrations were similar in men (mean $\pm$ SD, 0.53 ± 0.11 $\mu\text{mol/L}$; median (Q1, Q3) = 0.51 ($0.45, 0.59$) $\mu\text{mol/L}$) and women (mean $\pm$ SD, 0.53 ± 0.12 $\mu\text{mol/L}$, median (Q1, Q3) = 0.51 ($0.45, 0.60$) $\mu\text{mol/L}$) in the SDMA reference sample. The mean SDMA concentration in the reference sample was 0.38 ± 0.08 $\mu\text{mol/L}$ [median (Q1, Q3) = 0.37 ($0.32, 0.43$)], and the reference limits were 0.225 and 0.533 $\mu\text{mol/L}$ (2.5th and 97.5th percentile, 95% CI 0.217 – 0.232 and 0.526 – 0.541 $\mu\text{mol/L}$, respectively). The histogram of the SDMA distribution in the reference sample is depicted in Figure 1. Plasma SDMA values were 39% lower than ADMA concentrations. The SDMA plasma concentrations were similar in men and women.

A positive correlation between age and SDMA was observed (unadjusted Pearson's $r=0.16$, $p<0.001$ in women; $r=0.11$, $p<0.05$ in men). In women, SDMA levels varied with menopausal status ($p<0.001$): in the subgroup of women before menopause (mean age 47 ± 5 years), mean SDMA concentration was 0.35 ± 0.07 $\mu\text{mol/L}$ as compared to postmenopausal women, in whom mean SDMA concentrations were 0.38 ± 0.08 $\mu\text{mol/L}$ (mean age 60 ± 8 years). Also, statistically significant age- and sex-adjusted correlations were observed between plasma SDMA and body mass index, serum creatinine, eGFR, and total homocysteine ($r=-0.13, 0.12, -0.12$, and 0.14 , respectively, $p<0.001$ for all) and diastolic blood pressure ($r=-0.10$, $p<0.01$).

The results of the multivariable regression models with SDMA as the dependent variable are shown in Table 1B. All candidate independent variables were included in a stepwise selection procedure (cut-off for model entry $p<0.1$) and a final model was created. Neither sex, alcohol intake, systolic blood pressure, blood lipids, fasting glucose, nor high sensitive CRP met the inclusion criteria. Data for men and women were combined since plasma SDMA concentrations were similar in the two sexes. In the final regression model, serum creatinine, age, and total homocysteine were positively associated with SDMA, whereas the body mass index and diastolic blood pressure were inversely related (Table 1B). An alternative regression model that included log eGFR (instead of serum creatinine) yielded similar results with total homocysteine being positively associated with SDMA, whereas the body mass index and log eGFR were inversely related. Of note, the explanatory power of both models was comparable ($R^2=0.08$ for both).

Discussion

The structural isomer of ADMA, i.e. SDMA, is regarded to be biochemically inert. It is eliminated by the kidney and enzymes metabolizing SDMA are unknown to date. However, recent work from our and other groups suggests that SDMA may interact with L-arginine transport and metabolism and moreover, that plasma SDMA is a cardiovascular risk factor independent of its association with other markers of renal (dys)function (5–7, 17). Of note, the association of SDMA for a composite cardiovascular end-point (stroke, TIA, myocardial infarction and vascular death) was stronger in the population-based Bruneck study as compared with the association of ADMA with the same composite outcome (5). In this community-based cohort, plasma ADMA concentration was found to be higher as compared with that of SDMA, a finding consistent with our observations. Our reference limits for plasma SDMA are within the range of values reported in healthy individuals by other investigators (12,13), but lower than that reported from the Bruneck study (5).

The relations of plasma SDMA concentrations to sex have been inconsistent in the published literature (5,13). In our sample, sex was not a statistically significant correlate of SDMA plasma concentrations in multivariable analyses. Interestingly, pre-menopausal women exhibited lower SDMA plasma concentrations than post-menopausal women in our cohort, possibly related to their lower age. Plasma SDMA was inversely associated with body mass index in multivariable models. In South African people with European ancestors, we have previously reported an inverse association between circulating SDMA and insulin resistance (8). However, in our reference cohort we did not observe an association between SDMA and fasting glucose.

It has been reported that SDMA activates store-operated calcium channels (SOCs, 14). This activation of SOCs by SDMA is accompanied by an increased oxidative burden. Oxidative stress also links SDMA with homocysteine and ADMA (15). We previously reported a close link between homocysteine and ADMA in the Framingham reference sample; in the present investigation we extend this association to SDMA (1). Also, SDMA increases with age, which is also evident for ADMA (1). A close link of SDMA with renal function has been well established in patients with chronic kidney and cardiovascular disease (11). Data are now provided from our and other cohorts that this notion at least partly turns true for healthy individuals.

In summary, we report reference limits for plasma SDMA concentration using a reference sample from the FHS and suggest that circulating levels are influenced by renal function, age, blood pressure and body mass index in apparently healthy subjects. These reference limits may form the basis for identification of elevated SDMA plasma concentrations. Our study population is comprised of predominantly white middle aged individuals and it is important to examine reference limits in other ethnicities.

Acknowledgments

This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195)

References

1. Schwedhelm E, Xanthakis V, Maas R, Sullivan LM, Schulze F, Riederer U, Benndorf RA, Böger RH, Vasan RS. Asymmetric dimethylarginine reference intervals determined with liquid chromatography-tandem mass spectrometry: results from the Framingham offspring cohort. *Clin Chem*. 2009; 55:1539–45. [PubMed: 19541865]
2. Böger RH, Maas R, Schulze F, Schwedhelm E. Elevated levels of asymmetric dimethylarginine (ADMA) as a marker of cardiovascular disease and mortality. *Clin Chem Lab Med*. 2005; 43:1124–9. [PubMed: 16197309]
3. Mangoni AA. The emerging role of symmetric dimethylarginine in vascular disease. *Adv Clin Chem*. 2009; 48:73–94. [PubMed: 19803415]
4. Schwedhelm E, Böger RH. The role of asymmetric and symmetric dimethylarginines in renal disease. *Nat Rev Nephrol*. 2011; 7:275–85. [PubMed: 21445101]
5. Kiechl S, Lee T, Santer P, Thompson G, Tsimikas S, Egger G, Holt DW, Willeit J, Xu Q, Mayr M. Asymmetric and symmetric dimethylarginines are of similar predictive value for cardiovascular risk in the general population. *Atherosclerosis*. 2009; 205:261–5. [PubMed: 19100547]
6. Wang Z, Tang WH, Cho L, Brennan DM, Hazen SL. Targeted metabolomic evaluation of arginine methylation and cardiovascular risks: potential mechanisms beyond nitric oxide synthase inhibition. *Arterioscler Thromb Vasc Biol*. 2009; 29:1383–91. [PubMed: 19542023]
7. Schulze F, Carter AM, Schwedhelm E, Ajjan R, Maas R, von Holten RA, Atzler D, Grant PJ, Böger RH. Symmetric dimethylarginine predicts all-cause mortality following ischemic stroke. *Atherosclerosis*. 2010; 208:518–23. [PubMed: 19700158]

8. Schutte A, Schutte R, Huisman H, Van Rooyen J, Fourie C, Malan L, Malan N, Schwedhelm E, Strimbeanu S, Anderssohn M, Böger R. Dimethylarginines: their vascular and metabolic roles in Africans and Caucasians. *Eur J Endocrinol.* 2010; 162:525–33. [PubMed: 19996198]
9. Schwedhelm E, Maas R, Tan-Andresen J, Schulze F, Riederer U, Böger RH. Highthroughput 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; 851:211–9.
10. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009; 150:604–12. [PubMed: 19414839]
11. Kielstein JT, Salpeter SR, Bode-Boeger SM, Cooke JP, Fliser D. Symmetric dimethylarginine (SDMA) as endogenous marker of renal function a meta-analysis. *Nephrol Dial Transplant.* 2006; 21:2446–51. [PubMed: 16766542]
12. Blackwell S, O'Reilly DS, Talwar D. Biological variation of asymmetric dimethylarginine and related arginine metabolites and analytical performance goals for their measurement in human plasma. *Eur J Clin Invest.* 2007 May; 37(5):364–71. [PubMed: 17461982]
13. Hov GG, Sagen E, Bigonah A, Asberg A. Health-associated reference values for arginine, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) measured with high-performance liquid chromatography. *Scand J Clin Lab Invest.* 2007; 67(8):868–76. [PubMed: 17852822]
14. Schepers E, Glorieux G, Dhondt A, Leybaert L, Vanholder R. Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes. *Nephrol Dial Transplant.* 2009; 24:1429–35. [PubMed: 19059932]
15. Böger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, Bode-Böger SM. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ Res.* 2000; 87:99–105. [PubMed: 10903992]
16. Shin S, Fung SM, Mohan S, Fung HL. Simultaneous bioanalysis of L-arginine, L-citrulline, and dimethylarginines by LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2011; 879:467–74.
17. Tsikas D, Sandmann J, Savva A, Luessen P, Böger RH, Gutzki FM, Mayer B, Frölich JC. Assessment of nitric oxide synthase activity in vitro and in vivo by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl.* 2000; 742:143–53. [PubMed: 10892593]

Figure 1. Distribution of SDMA plasma concentrations in the reference sample

A histogram of SDMA with reference intervals is depicted for ten classes. The 95 % CI for 2.5 and 97.5th percentile is (0.217,0.232) and (0.526,0.541) $\mu\text{mol/L}$, respectively. SDMA was determined from EDTA plasma samples with LC-MS/MS using stable isotope dilution.

Table 1A

Clinical characteristics of the reference sample

	Women (N=509)	Men (N=331)
Age, years	56±9	55±9
Body mass index, kg/m ²	24.4±2.8	26.2±2.4
Systolic blood pressure, mm Hg	117±12	120±10
Diastolic blood pressure, mm Hg	71±8	74±7
Fasting glucose, mmol/L	5.1±0.4	5.4±0.4
LDL cholesterol, mmol/L	3.18±0.90	3.33±0.86
Total cholesterol/HDL	3.5±1.1	4.6±1.3
Triglycerides, mmol/L	1.22±0.66	1.38±0.79
Log high sensitive CRP	0.31±1.1	0.19±1.03
Total homocysteine, µmol/L	8.2±2.6	9.6±3.3
Serum creatinine, µmol/L	79±7	92±8
Log eGFR	4.28±0.13	4.38±0.12
ADMA, µmol/L	0.53±0.12	0.53±0.11

Table 1B

Multivariable correlates of SDMA plasma concentrations in the reference

sample Variable, unit	Beta (SE)	p-value
Age, per 1 year increase	0.0010 (0.0003)	<0.001
Serum creatinine, per 1 $\mu\text{mol/L}$ increase	0.0010 (0.0003)	<0.001
Total homocysteine, per 1 $\mu\text{mol/L}$ increase	0.0037 (0.0009)	<0.001
Body mass index, per 1 Kg/m^2 increase	-0.0031 (0.001)	<0.01
Diastolic blood pressure, per mm Hg increase	-0.0008 (0.0004)	0.03

Values are means $\pm$ SD. Abbreviations used: LDL, low density lipoproteine; HDL, high density lipoproteine; eGFR, estimated glomerular filtration rate. The log eGFR was calculated using the CKD-EPI formula. Candidate variables that were considered for model entry were age, sex, BMI, SBP, DBP, total/HDL cholesterol ratio, triglycerides, serum creatinine, fasting glucose, total homocysteine, *alcohol consumption and log CRP*. Regression coefficients (beta estimates) represent the estimated mean change in SDMA ($\mu\text{mol/L}$) per 1-unit increase in the corresponding covariate.