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Asymmetric dimethylarginine and hemodynamic regulation in middle-aged men

Hannu Päivä[a,b,*], Juha Laakso[c], Mika Kähönen[d,e], Väinö Turjanmaa[d,e], Tiit Kööbi[d,e], Silja Majahalme[f], Terho Lehtimäki[b,g], Inkeri Ruokonen[h], Reijo Laaksonen[b,h]

[a]*Department of Internal Medicine, Tampere University Hospital, 33521 Tampere, Finland*
[b]*Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Centre for Laboratory Medicine, Tampere University Hospital, 33521 Tampere, Finland*
[c]*Institute of Biomedicine, Pharmacology, University of Helsinki, 00014 Helsinki, Finland*
[d]*Department of Clinical Physiology, Tampere University Hospital, 33521 Tampere, Finland*
[e]*Department of Clinical Physiology, Medical School, University of Tampere, 33521 Tampere, Finland*
[f]*Appleton Heart Institute, Appleton, WI 54911, USA*
[g]*Medical School, University of Tampere, 33521 Tampere, Finland*
[h]*Department of Clinical Pharmacology, University of Helsinki, 00014 Helsinki, Finland*

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Abstract

The goal of this study was to evaluate the role of asymmetric dimethylarginine (ADMA) in the regulation of hemodynamic functions in hypertensive men. It has been suggested that ADMA, as an endogenous nitric oxide synthase inhibitor, is linked to hypertension and vascular reactivity. Sixty-seven men aged 51.1 years (range, 45-55 years) were studied. Plasma ADMA and symmetric dimethylarginine were determined by high-performance liquid chromatography–tandem mass spectrometry. Blood pressure (BP) was measured by 24-hour ambulatory recordings and casual measurements. Hemodynamic regulation was assessed by noninvasive methods. The nitric oxide production was estimated based on plasma nitrate (NO_3^-) determination. Results showed that plasma arginine derivatives or L-arginine/ADMA ratio was not associated with BP values observed during 24-hour monitoring or in casual measurements. Systemic vascular resistance, pulse wave velocity, or cardiac output was not associated with plasma ADMA or plasma NO_3^- levels. No association was found between plasma ADMA and NO_3^- either. Interestingly, subjects on antihypertensive treatment had lower plasma ADMA concentrations than nontreated subjects (0.30 ± 0.08 and 0.36 ± 0.11 μmol/L, respectively, $P = .04$) despite higher BP values. In conclusion, these results suggest that plasma ADMA does not have a determinative role in the regulation of hemodynamic functions in Finnish middle-aged men.

1. Introduction

Diminished bioavailability of nitric oxide (NO) impairs endothelium-dependent vasodilation and activates other mechanisms that may play an important role in the pathogenesis of atherosclerosis [1]. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase that can modulate NO production and thereby endothelial function [2]. An association between ADMA and blood pressure (BP) has been reported in some studies [3,4] but not in others [5,6]. Moreover, myocardial vasodilator capacity and impaired endothelium-dependent brachial artery vasodilation have been found to have an association with ADMA in plasma [7,8]. In addition, intravenously dosed ADMA has been observed to significantly increase systemic vascular resistance (SVR) and arterial BP [9]. It has been suggested that high plasma ADMA will lead to diminished NO bioavailability and thereby increased vascular resistance and elevated BP [4].

This study was performed to assess the role of plasma ADMA on physiological regulation of BP and hemodynamic functions. To increase the reliability of our BP recordings, we used not only casual measurements but also ambulatory BP

* Corresponding author. Department of Internal Medicine, University Hospital of Tampere, PO Box 2000, 33521 Tampere, Finland. Tel.: +358 3 311 611; fax: +358 3 311 64362.
E-mail address: hannu.paiva@uta.fi (H. Päivä).

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monitoring. Other hemodynamic measurements were assessed by using noninvasive methods. Also, NO production was estimated by determining plasma nitrate (NO_3^-) levels.

2. Subjects and methods

2.1. Selection of subjects

Sixty-seven hypertensive otherwise healthy males were studied (Table 1). We classified the participants on the basis of repeated casual BP measurements according to the Joint National Committee classification [10]. Twenty-three of the participants were prehypertensive (PREHT) (systolic BP [SBP] 120-139 mm Hg or diastolic BP [DBP] 80-89 mm Hg, or both), 29 belonged to the stage 1 hypertensive group (ST1HT) (SBP 140-159 mm Hg or DBP 90-99 mm Hg, or both), and 15 belonged to the stage 2 hypertensive group (ST2HT) (SBP $\geq$ 160 mm Hg or DBP $\geq$ 100 mm Hg, or both). Twenty-five percent of the subjects were on antihypertensive treatment. The study was approved by the Ethics Committee of Tampere University Hospital. All participants gave a written informed consent.

2.2. Blood pressure monitoring

Casual BP was measured with the participants in the sitting position after 10 minutes of rest, using a calibrated aneroid barometer (Speidel and Keller, Jungingen, Germany). Systolic BP was read at the first Korotkoff sound and DBP at the disappearance of the Korotkoff sounds (phase V). The deflation rate was 2 mm Hg/s. Blood pressure was recorded on 2 consecutive days, before the ambulatory recording (3 measurements at least 1 minute apart) and after it (2 measurements at least 1 minute apart) The average of 5 readings was used for analysis. Ambulatory BP monitoring was performed with the previously validated [11,12] DIASYS 200 device (Novacor, Rueil-Malmaison, France). Blood pressure was measured at 15-minute intervals between 6:00 AM and 10:00 PM, and at 30-minute intervals between 10:00 PM and 6:00 AM. Twenty-four–hour BP was calculated using hourly means. Only recordings with less than 10% missing or inappropriate values were accepted. The raw data were checked manually and inappropriate readings [13] removed.

2.3. Dynamic exercise test

Dynamic exercise testing was performed in an upright position using a bicycle ergometer (Siemens, Elema, Germany). The starting workload was 50 W, and the workload was increased in a stepwise manner with increments of 50 W every 4 minutes until 85% of the age-specific maximum heart rate was reached. The pedaling frequency was 60 rpm. The mean values of BP during the final minute of the preexercise period (the subject sitting on the bicycle ergometer before test initiation), the second workload, the final workload, and 10 minutes after the exercise testing were used for comparisons.

2.4. Noninvasive hemodynamic study

Blood pressure was monitored by a finger BP measurement device (Finapres 2300, series FAX, Ohmeda, Louisville, Colo) which provides continuous noninvasive monitoring of beat-to-beat BP and is therefore a useful noninvasive alternative to intra-arterial BP measurements [14,15].

Cardiac output (CO) (CO = heart rate $\times$ stroke volume [SV]) was measured using whole-body impedance cardiography (CircMon, Model B202, JR Medical, Tallinn, Estonia). This method is described in detail elsewhere [16,17]. Briefly, the whole-body impedance cardiography channel of CircMon B202 is based on the Tishchenko [18] SV equation with a correction factor for tetrapolar registration and includes also a correction of SV by hematocrit and body mass index. Disposable electrocardiogram electrodes (Blue sensor type R-00-S, Medicotest, Ølstykke, Denmark) were used. A pair of electrically connected current electrodes was placed on extremities, just proximal to the wrists and ankles. Voltage electrodes

Table 1
Characteristics of the participants

Variable	BP group			
	PREHT (n = 23)	ST1HT (n = 29)	ST2HT (n = 15)	All (N = 67)
Casual SBP (mm Hg)	130 ± 7	147 ± 6*	160 ± 12*,**	144 ± 14
Casual DBP (mm Hg)	82 ± 6	91 ± 5*	100 ± 9*,**	90 ± 9
Age (y)	49.8 ± 4.4	51.8 ± 3.8	51.4 ± 4.4	51.1 ± 4.2
BMI	28.4 ± 3.2	26.7 ± 3.6	28.1 ± 4.1	27.6 ± 3.6
TC (mmol/L)	5.63 ± 1.10	5.44 ± 1.14	5.39 ± 0.94	5.49 ± 1.07
LDL cholesterol (mmol/L)	3.62 ± 1.10	3.28 ± 1.02	3.37 ± 0.79	3.42 ± 1.00
TGs (mmol/L)	1.72 ± 0.99	1.67 ± 0.93	1.35 ± 0.57	1.61 ± 0.88
CL (mL/[s · 1.73 m^2])	2.02 ± 0.47	2.01 ± 0.39	1.84 ± 0.37	1.97 ± 0.41
Tobacco smoking (Y/N)	7/16	8/21	11/4	26/41
Antihypertensive medication (Y/N)	1/22	7/22	9/6	17/67
On ACE inhibitors (n)	1	2	4	7

Values are mean ± SD. BMI indicates body mass index; CL, creatinine clearance.

* $P < .0001$ vs PREHT subjects.

** $P < .0001$ vs ST1HT subjects.

were placed proximal to the current electrodes with a 5-cm distance between the centers of the electrodes. Whole-body impedance cardiography reliably measures CO and has an excellent agreement with invasive thermodilution and direct oxygen Fick methods for measuring CO in subjects without cardiac shunts and valvular lesions [16,17]. Thus, this method is a feasible and handy technique for a noninvasive and continuous analysis of CO and its changes in different conditions. Systemic vascular resistance was calculated from CO and mean arterial pressure (MAP) as SVR = MAP/CO × 80. Left cardiac work (LCW) was calculated from the equation LCW = 0.0143 × MAP × CO. In addition, total arterial compliance was estimated by the SV to pulse pressure (SV/PP) ratio, which has been proposed to be a rough measure for total arterial compliance [19,20]. Arterial pulse wave velocity (PWV) was obtained from the time delay between simultaneously recorded flow pulses and the distance between recording sites, that is, between the root of aorta and popliteal artery, a method which we have found to show a good agreement with measurements of pulse transition in arterial tree by ultrasound-determined Doppler-flow method [21]. Arterial pulse waves were recorded with the voltage-sensing channels of CircMon B202 and analyzed automatically with the same device. Pulse transition in aortic root was estimated from the whole-body impedance cardiogram at the point where a sharp systolic upstroke commenced. The pulse wave arrival to the popliteal artery was similarly estimated from the sharp systolic upstroke of the second channel with the active electrode placed at the knee joint level. The distance between the aortic root and the knee joint was estimated from the subject's height by using a ratio of height/1.61.

Systemic vascular resistance was related to the surface area of the subjects and was transformed to the respective index: SVR index (SVRI = SVR × m^2), LCW index (LCWI = LCW/m^2), and total arterial compliance index (SI/PP = SV/PP/m^2).

2.5. Blood sampling

Blood samples were drawn into EDTA tubes on ice. After participants had fasted 12 hours, plasma was separated by low-speed ultracentrifugation and stored at −80°C. Plasma total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride (TG), ADMA, and symmetric dimethylarginine (SDMA) concentrations were analyzed.

2.6. Determinations of plasma lipids and arginine derivatives

The concentrations of plasma TG, TC, and HDL cholesterol were determined using the Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). The LDL concentration was calculated by using Friedewald's formula [22], because the plasma TG levels did not exceed 4.0 mmol/L. The interassay coefficients of variation were 1.4% for the TC, 1.0% for the TG, and 3.7% for the HDL cholesterol assessments.

Asymmetric dimethylarginine and SDMA measurement was carried out by using a high-performance liquid chromatography (HPLC)–tandem mass spectrometry. A 200-μL aliquot of plasma was diluted and applied on a solid-phase straight-phase silica column (100 mg Bond Elut, Varian, Palo Alto, CA). After washing with methanol, arginine and its methylated derivatives were eluted into 4 mol/L NH_4OH in 50% acetonitrile and the eluent was evaporated with a stream of N_2. Before analysis, dry residue was dissolved in high-performance liquid chromatography mobile phase. Tandem mass spectrometry (Applied Biosystems, Foster City, CA) was used to detect arginine and its metabolites. The mass spectra observed were similar to those found by Vishwanathan et al [23]. Transitions yielding best signal-to-noise ratios on Perkin-Elmer instrument were m/z 203 → 70 for ADMA and SDMA, m/z 189 → 70 for (monomethylarginine) LMMA, and m/z 175 → 60 for arginine. Vishwanathan et al used Micro-Meritics tandem mass spectrometry equipment (Micrometrics Analytical Services, Northcross, GA) and found m/z 203 → 158 for ADMA and m/z 203 → 172 for SDMA to give the best response. Normalized difference in SDMA peaks of samples run with and without added SDMA was calculated. That is, the peak height of arginine and other peaks except SDMA was adjusted to exactly the same level in the 2 samples. The procedure allowed the normalized difference in SDMA between samples containing endogenous SDMA and endogenous plus added SDMA to be calculated. The difference thus gave a response for a known SDMA supplement, allowing calculation of SDMA concentration in the sample containing only endogenous SDMA; that is, for determination of SDMA levels calibration was performed by the method of standard additions for each sample. For other analytes, the difference in normalized SDMA peak height was used as an internal standard. For ADMA, total coefficient of variation was 10.3% and respective intra- and interday component coefficient of variation were 7.0% and 7.6% obtained by analysis of a pooled serum sample. Arginine and its metabolites used for calibration were obtained from Sigma Chemicals (St Louis, MO).

2.7. Determination of plasma nitrate

The NO production in plasma was monitored by The Nitric Oxide Quantitation Kit (Active Motif, CA) according to the manufacturer's instructions. The kit is based on nitrate and nitrite determination. The absorbances were detected with Multiskan Ascent spectrophotometer (Thermo Labsystems, Vantaa, Finland). The detection limit of the assay is less than 1 μmol of nitrite/nitrate.

2.8. Statistical methods

The data were analyzed using Statistica for Windows (StatSoft, Tulsa, Okla). The normality of each variable was

Table 2
Results of BP measurements and other hemodynamic parameters in different BP groups

Variable	PREHT (n = 23)	ST1HT (n = 29)	ST2HT (n = 15)
24-h AMB SBP (mm Hg)	117 ± 10	129 ± 12*	129 ± 13*
24-h AMB DBP (mm Hg)	81 ± 7	88 ± 9*	88 ± 8*
Daytime AMB SBP (mm Hg)	123 ± 12	138 ± 15**	137 ± 13**
Daytime AMB DBP (mm Hg)	86 ± 8	94 ± 9***	94 ± 8***
Nighttime AMB SBP (mm Hg)	100 ± 10	110 ± 13**	113 ± 15**
Nighttime AMB DBP (mm Hg)	71 ± 8	76 ± 8***	78 ± 10***
Max SBP in dynamic exercise test (mm Hg)	192 ± 15	211 ± 18*	224 ± 25*,****
Max DBP in dynamic exercise test (mm Hg)	101 ± 10	110 ± 11***	115 ± 13**
SVRI (dyn · s/[cm^5 · m^2])	2893 ± 440	2937 ± 597	3009 ± 479
PWV (m/s)	12.6 ± 1.9	12.8 ± 2.4	12.8 ± 1.9
CO (L/min)	5.64 ± 1.01	5.60 ± 0.99	5.60 ± 0.95

Values are mean ± SD. AMB indicates ambulatory.
* $P < .0001$ vs PREHT subjects.
** $P < .001$ vs PREHT subjects.
*** $P < .01$ vs PREHT subjects.
**** $P < .05$ vs ST1HT subjects.

studied by Kolmogorov-Smirnov test. Univariate correlation analysis was carried out with Pearson's correlation test for normally distributed variables. One-way analysis of variance was used to assess the statistical differences between the BP groups. In the dynamic exercise test, overall differences in responses of BP over time between ADMA subgroups were compared with analysis of variance for repeated measurements. Data are presented as mean ± SD unless otherwise stated. A P value of less than .05 was considered statistically significant.

3. Results

3.1. Arginine derivatives and lipids

The average plasma L-arginine, ADMA, and SDMA concentrations in all subjects were 144 ± 29, 0.34 ± 0.10, and 0.74 ± 0.23 μmol/L, respectively. Plasma lipids and lipoproteins were not associated with plasma L-arginine, ADMA, and SDMA concentrations.

3.2. Arginine derivatives, nitrate, and BP

Plasma ADMA concentrations or L-arginine/ADMA ratios were not associated with SBP and DBP values recorded by casual measurements (Table 2). No correlation was observed between plasma arginine derivatives or L-arginine/ADMA ratios and BP values observed during 24-hour monitoring, including daytime and nighttime recordings. The difference between daytime and nighttime BP values was not related to plasma ADMA concentrations and L-arginine/ADMA ratios. Similarly, during the dynamic exercise test, plasma ADMA was not related to the BP response during the test.

Plasma L-arginine, ADMA, and SDMA concentrations were comparable in all BP groups (PREHT, ST1HT, ST2HT) (Table 3). Lack of a direct association between ADMA and BP was also observed in a subgroup analysis of subjects not receiving antihypertensive treatment. To determine whether higher ADMA levels, that is, above a certain threshold value, would affect BP readings, we compared subjects in the highest quartile of ADMA distribution (n = 16, ADMA > 0.40 μmol/L) to the others (n = 51). Both casual and 24-hour BP measurements were comparable in both ADMA groups. Plasma nitrate was not directly associated with BP, and the levels were comparable in all BP groups. No association either was observed between plasma ADMA and nitrate or L-arginine/ADMA ratio and nitrate. This was analyzed in both smokers and nonsmokers.

Subjects receiving antihypertensive treatment had significantly higher SBP (152 ± 11 vs 140 ± 13 mm Hg, $P = .0006$) and DBP (95 ± 9 vs 87 ± 10 mm Hg, $P = .004$) values than other subjects. However, they had lower plasma

Table 3
Concentrations of plasma arginine and its derivatives and nitrate in different BP groups

Variable	PREHT (n = 23)	ST1HT (n = 29)	ST2HT (n = 15)
ADMA (μmol/L)	0.37 ± 0.12	0.32 ± 0.08*	0.32 ± 0.10*
L-Arginine (μmol/L)	143 ± 37	141 ± 19*	149 ± 32*
L-Arginine/ADMA ratio	424 ± 174	464 ± 195*	543 ± 219*
SDMA (μmol/L)	0.82 ± 0.32	0.70 ± 0.15*	0.70 ± 0.16*
NO_3^- (μmol/L)	7.15 ± 3.13	8.11 ± 5.05*	7.03 ± 2.87*

Values are mean ± SD.
* P = not significant vs PREHT subjects.

ADMA concentrations than nontreated subjects (0.30 ± 0.08 and 0.36 ± 0.11 μmol/L, respectively, $P = .04$), but had comparable plasma L-arginine/ADMA ratios and SDMA and nitrate levels.

3.3. Arginine derivatives, nitrate, and other hemodynamic parameters

Systemic vascular resistance index and PWV were correlated with SBP ($R = 0.42$, $P < .001$ and $R = 0.25$, $P < .05$) and DBP ($R = 0.34$, $P < .01$ and $R = 0.26$, $P < .05$) but did not differ significantly in defined BP groups. Subjects on antihypertensive treatment had significantly higher SVRI than others (3196 ± 544 and 2851 ± 481 dyn · s/[cm^5 · m^2], respectively; $P < .02$). Arginine derivatives or plasma nitrate was not associated with SVR, PWV, or CO in the whole study population or different subgroups as described above. The results were similar in smokers and nonsmokers.

4. Discussion

The results of this study show no association between plasma ADMA and noninvasively recorded hemodynamic parameters, suggesting that plasma ADMA is not playing a major independent role in the regulation of hemodynamic functions in middle-aged men with borderline or clear hypertension.

Based on 2 earlier studies, elevated levels of plasma ADMA have been thought to play a role in the development of essential hypertension. Miyazaki et al [3] observed in subjects without any known vascular disease that plasma ADMA levels were associated with MAP. Furthermore, Surdacki et al [4] demonstrated that men with newly diagnosed and untreated hypertension had increased plasma ADMA levels and depressed systemic NO formation compared to normotensive controls. In addition, intravenously dosed ADMA significantly increased SVR and arterial BP [9], and lowered CO [9,24]. However, Delles et al [5] did not find any correlation between plasma ADMA concentration and BP in young men with mild essential hypertension. In the present study, we aimed to evaluate further the association between plasma ADMA and hemodynamic regulation. Thus, we measured BP values carefully with 2 different methods to avoid misinterpretations. To strengthen our data, we also did hemodynamic profiling of the study subjects including SVRI, PWV, and CO measurements. In addition, BP values were measured also during a maximal dynamic exercise test. Also, nitrate production was evaluated by determining NO_3^- levels in plasma.

This study demonstrated a well-known [25] association between BP and vascular resistance. Because ADMA is an endogenous competitive inhibitor of NO synthase [2] and can modulate endogenous vasodilator NO [26] production, there were theoretical reasons to assume that plasma ADMA and nitrate (metabolite of NO) are also associated with BP and other hemodynamic regulation. However, our extensive investigation package indicated no apparent association between plasma ADMA or nitrate and hemodynamic regulation in our study group. Previously, the reduced L-arginine/ADMA ratio has been shown to be both significantly and more closely correlated than ADMA to endothelial dysfunction in healthy volunteers [27]. However, in this study L-arginine/ADMA ratio was not associated with BP or other hemodynamic parameters (which are only partly endothelium-dependent parameters). Most hypertensive subjects had even slightly but not significantly higher L-arginine/ADMA ratios than the others. Most of the evidence from clinical studies [28] but not all [29,30] indicates that there is a deficiency in the release of NO by the endothelium in hypertension. In fact, some previous studies have demonstrated reduced NO production in hypertensive subjects compared to normotensive controls by measuring decreased levels of urinary nitrate [31,32]. Because NO is short-lived in vivo, direct measurement of NO production is difficult [33]. Plasma and urinary nitrate measurements have been used to estimate NO production [31], but these assays may be confounded especially by dietary nitrates. In the present study, standardized diets were not used, which may in part explain the lack of association between measured plasma nitrate concentrations and hemodynamic parameters. Nitric oxide inhaled via tobacco smoke is another source of nitrate [32]. However, in the present study, plasma nitrate levels was not associated with BP or vascular resistance even after smokers were excluded from analyses.

In vitro, ADMA inhibits NO formation by the endothelial cells in a concentration-dependent manner [34]. Asymmetric dimethylarginine concentrations in this study were relatively low compared for example to the threshold value related to elevated risk of acute myocardial infarction (0.40 vs 0.62 μmol/L) or to the threshold value related to elevated BP (0.40 vs 0.58 μmol/L) in our earlier studies [8,35]. Thus, it is possible that in a clinical setting only significantly elevated plasma ADMA levels may modulate NO production and are independent predictors of vascular functions. Such high plasma ADMA values may be related, for example, to dysfunctional ADMA-degrading enzymes, DDAH-1 and DDAH-2, because of specific genetic mutations [36] or renal dysfunction [37,38]. Furthermore, plasma ADMA levels may not be associated with ADMA levels in tissues where ADMA may have a direct and more potent regulatory role.

We observed that subjects on antihypertensive treatment had slightly but significantly lower plasma ADMA levels than other subjects. It is possible that antihypertensive treatment has reduced ADMA levels in these subjects as it has been demonstrated at least for angiotensin-converting enzyme (ACE) inhibitors and angiotensin II AT 1 receptor blockers in earlier studies [5,39]. In the present study, 7 patients were on ACE inhibitors. On the other hand, these patients, despite antihypertensive treatment, were still clearly hypertensive. Thus, ADMA levels in these patients

may have also been lowered to compensate for the inappropriately high BP. We are therefore suggesting that ADMA may have a dual role in the regulation of BP. As suggested earlier, high ADMA levels, for example, because of genetic reasons, may cause elevated BP levels in some persons, whereas in others ADMA levels could be lowered, for example, because of hypertension caused by down-regulation of the DDAH enzymes.

5. Conclusions

In a physiological setting, plasma ADMA is not associated with BP or other hemodynamic parameters in apparently healthy middle-aged men.

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