Association Between Exaggerated Blood Pressure Response to Exercise and Serum Asymmetric Dimethylarginine Levels

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Background: The exaggerated blood pressure response to exercise (EBPR) is an independent predictor of hypertension. Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide inhibitor and higher plasma levels of ADMA are related to increased cardiovascular risk. The aim of this study is to identify the relationship between ADMA and EBPR.

Methods and Results: A total of 66 patients (36 with EBPR and 30 as controls) were enrolled in the study. EBPR is defined as blood pressure (BP) measurements ≥200/100 mmHg during the treadmill test. All the subjects underwent 24-h ambulatory BP monitoring. L-arginine and ADMA levels were measured using a high performance lipid chromatography technique. The serum ADMA levels were increased in the EBPR group compared to the healthy controls (4.0±1.4 vs 2.6±1.1 μmol/L respectively, P=0.001), but L-arginine levels were similar in the 2 groups (P=0.19). The serum ADMA levels were detected as an independent predictor of EBPR (odds ratio 2.28; 95% confidence interval 1.22–4.24; P=0.002).

Conclusions: Serum ADMA levels might play a role in EBPR to exercise. (Circ J 2010; 74: 1135–1141)

Key Words: ADMA; Cardiovascular risk; Exaggerated blood pressure response to exercise; Hypertension

In view of the importance of hypertension to morbidity and mortality, many investigators have tried to detect a predictor for the development of this disease. Some authors suggest that the exaggerated blood pressure response to exercise (EBPR) is an independent predictor of future hypertension, stroke, congestive heart failure, ischemic heart disease and cardiovascular mortality in apparently healthy individuals.1–4 Also, EBPR is associated with target-organ damage, such as left ventricular hypertrophy and carotid atherosclerosis.5,6 The prevalence of EBPR was reported to be 9% in a healthy population.7 EBPR is related to impaired endothelial function.8,9 Endothelial cells are responsible for the continuous basal production of nitric oxide (NO), which serves to counteract the neural vasoconstrictor tone and to regulate blood flow and blood pressure (BP).10 NO is synthesized by NO synthase. Asymmetric dimethylarginine (ADMA) is a major endogenous NO synthase inhibitor.11 ADMA mediates endothelial dysfunction.12 Increased ADMA levels have been related to diabetes, hypertension, preeclampsia, dyslipidemia, stroke, congestive heart failure, vasospastic angina and acute coronary events.13 In a PubMed search using the keyword “N, N-dimethylarginine” (the term defining ADMA), no articles about its concentrations in patients with EBPR were identified.

The aim of the present study was to test the hypothesis that elevated serum ADMA levels, as a potential mediator of endothelial dysfunction, might play a key role in EBPR. For this aim, the study compared the levels of ADMA in patients with EBPR vs age- and gender-matched non-EBPR patients.

Methods

Study Population

The subjects enrolled in our study were recruited from 452 patients, who were suspected of having coronary artery disease, and who underwent treadmill exercise testing but showing no ischemic changes during exercise (mean age 51.4±6.8 years).

Exaggerated BP response is defined as systolic BP (SBP) ≥200 mmHg or diastolic BP (DBP) ≥100 mmHg at the peak of exercise. Normotension was defined as both SBP <140 mmHg and DBP <90 mmHg measured in the physicians’ office. Ambulatory BP monitoring (ABPM) was recorded in both groups. The study was approved by the local Ethics Committee. Informed consent was obtained from each subject.

Criteria for exclusion were as follows: (1) the use of medications for hypertension; (2) cardiovascular diseases, such as coronary heart disease, severe valvular heart disease, congestive heart failure; (3) electrocardiographic abnormalities...
indicative of myocardial infarction; (4) diabetes mellitus, renal and hepatic diseases, active infectious diseases such as urinary tract, pulmonary, etc., infections; (5) women using hormone replacement therapy; (6) office BP measurement of >140/90 mmHg; and (7) a 24-h mean ABPM of >135/85 mmHg. Also excluded were any individuals with ischemic electrocardiographic changes (horizontal or downsloping ST-segment depression by 1 mm or more), complex dysrhythmia and symptoms of ischemia during the exercise stress test (EST). None of the patients and control subjects were treated with any drugs such as lipid-lowering drugs, antidiabetics, etc.

**Office BP Measurement**

Resting SBP and DBP were measured from the brachial artery using a mercury sphygmomanometer (ERKA D-83646 Bad Tölz, Kallmeyer Medizintechnik GmbH & Co KG, Germany) in the physicians’ office. After at least 5 min of sitting rest, BP was measured 2 times with a 1 min interval. If the readings differed by 5 mmHg, extra readings were obtained. If the DBP was over 100 mmHg at peak exercise time, the test was considered as either normal or EBPR whenever the SBP was over 200 mmHg or the DBP was over 100 mmHg at peak exercise time.

**Exercise Treadmill Testing**

EST was performed on a treadmill integrated with a computer and ECG system (Model 770 M, RAM Medical and Industrial Instruments & Suppl, Padova, Italy). The standard Bruce protocol was used for the EST. The subjects were encouraged to continue until 90% of their adjusted-age maximal heart rate was achieved. During exercise, a 12-lead electrocardiogram was recorded at the end of each 3-min stage, at peak exercise and in the 3rd min of the recovery period. SBP and DBP were non-invasively recorded with an automated BP monitor (Tango Stress BP, Sun Tech Medical Inc, Morrisville, NC, USA) when the subjects were sitting immediately before the testing and during the last 30 s of each 3-min exercise stage. At the end of study, BP was measured at the 3rd min of recovery phase in the sitting position. Based on BP response, the test was considered as either normal or EBPR whenever the SBP was over 200 mmHg or the DBP was over 100 mmHg at peak exercise time.

**Ambulatory BP Monitoring**

ABPM was performed 2.5±1.1 days after the EST. Twenty-four-hour ABPM was carried out with a non-invasive automated device (Tracker NIBP2, Del Mar Reynolds Ltd, Hertford, England, UK) and the cuff of the device was fitted on the non-dominant arm. Subjects were instructed to maintain the same daily routine and sleep patterns and to stop muscular activity (especially athletic activity) and keep their arms entirely still during BP measurements. The BP monitor was programmed to measure BP at intervals of 20 min during the day and 30 min at night. Each BP reading was edited by a computer and rejected if the SBP was less than 80 mmHg or more than 250 mmHg or if the DBP was less than 40 mmHg or more than 140 mmHg. Recordings for each subject were accepted if more than 80% of the raw data were valid. Average values were calculated for 2 periods of a whole day: a 6-h period between 1 AM and 6 AM (nighttime) and a 12-h period between 9 AM and 9 PM (daytime). An average 24-h ABPM recording of less than 135 mmHg systolic and 85 mmHg diastolic is considered normal. A 24-h ambulatory BP equal or above these limits was considered hypertensive.  

### Table 1. Baseline Characteristics of the Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD (Interquartile range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=30)</td>
<td>EBPR (n=36)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>46.2±11.5</td>
<td>46.9±9.8</td>
</tr>
<tr>
<td><strong>Gender (M/F)</strong></td>
<td>24/6</td>
<td>29/7</td>
</tr>
<tr>
<td><strong>Smoker (%)</strong></td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>27.1±4.1</td>
<td>28.0±4.9</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>91.1±13.0</td>
<td>99.8±11.9</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>94.7±8.8</td>
<td>103.1±10.3</td>
</tr>
<tr>
<td><strong>Waist circumference/Hip circumference</strong></td>
<td>0.96±0.07</td>
<td>0.97±0.06</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>118.4±8.5</td>
<td>23.1±8.1</td>
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<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>72.4±6.7</td>
<td>75.1±7.1</td>
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<tr>
<td><strong>HR (beats/min)</strong></td>
<td>88.9±8.1</td>
<td>84.0±12.1</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>98.0±4.9</td>
<td>96.8±10.0</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>0.9±0.1</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td><strong>GFR</strong></td>
<td>105.7±25.3</td>
<td>121.7±33.0</td>
</tr>
<tr>
<td><strong>TC (mg/dl)</strong></td>
<td>206.6±59.4</td>
<td>200.3±38.4</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
<td>133.4±51.0</td>
<td>122.2±36.0</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td>47.6±12.4</td>
<td>42.4±10.4</td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dl)</strong></td>
<td>128.3±83.2</td>
<td>165.8±82.3</td>
</tr>
<tr>
<td><strong>TC/HDL-C</strong></td>
<td>4.3±1.2</td>
<td>4.9±1.2</td>
</tr>
<tr>
<td><strong>ADMA (µmol/L)</strong></td>
<td>2.6±1.1</td>
<td>4.0±1.4</td>
</tr>
<tr>
<td><strong>L-Arginine (mmol/L)</strong></td>
<td>0.15±0.05</td>
<td>0.17±0.06</td>
</tr>
<tr>
<td><strong>hsCRP (mg/L)</strong></td>
<td>2.8±2.6</td>
<td>3.1±2.8</td>
</tr>
<tr>
<td><strong>WBC (K/µl)</strong></td>
<td>6.9±1.9</td>
<td>8.1±2.1</td>
</tr>
<tr>
<td><strong>Homocystein (µmol/L)</strong></td>
<td>13.4±4.9</td>
<td>15.4±7.0</td>
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</tbody>
</table>

EBPR, exaggerated blood pressure response to exercise; BMI, body mass index; SBP, systolic blood pressure (BP); DBP, diastolic BP; HR, heart rate; GFR, glomerular filtration rate; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ADMA, asymmetric dimethylarginine; hsCRP, high sensitive C-reactive protein; WBC, white blood cell count; NS, not statistically significant.
Anthropometric and Blood Analysis

Weight, height, waist and hip circumferences were measured and body mass index (BMI) was evaluated in all subjects. Blood samples were collected following a 12-h overnight fast and were analyzed by the hospital clinical laboratory. Fasting blood samples were obtained for glucose, lipids, high-sensitivity C-reactive protein (hsCRP), homocystein and ADMA analysis. All lipid determinations were done on serum using standard methods. Glomerular filtration rate (GFR) was calculated with the Cockcroft–Gault formula for every subject.15

Measurement of ADMA was accomplished by high performance liquid chromatography (HPLC), using the method described by Chen et al.16 In brief, to 1 ml serum, 20 mg 5-sulfosalisilic acid was added, and the mixture was left in an ice bath for 10 min. The precipitated protein was removed by centrifugation at 2,000×g for 10 min. Ten microliters of the supernatant, which was filtered through a 0.22-μm pore size filter, was mixed with 100 μl derivatization reagent [prepared by dissolving 10 mg o-phthalaldehyde in 0.5 ml methanol, 2 ml 0.4 M borate buffer (pH 10.0), and 30 μl 2-mercaptoethanol were added] and then injected into the chromatographic system. Separation of ADMA was achieved with a 250×4.6 mm interior diameter Supercosil C18 column with a particle size of 5 μm (Supelco Bellefonte, PA, USA) using 50 mm sodium acetate (pH 6.8), methanol, and tetrahydrofuran as the mobile phase (A, 82:17:1; B, 22:77:1) at a flow rate of 1.0 ml/min. Serum levels of ADMA were determined with HPLC (HP Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) with fluorescence detection. The areas of peaks detected by fluorescent detector (excitation, 338 nm; emission, 425 nm) were used for quantification.

Statistical Analysis
Data were analyzed by using SPSS software version 13.0 (SPSS, Chicago, IL, USA) and presented as mean±standard deviation. The distribution of the variables was analyzed with the Kolmogorov–Smirnow test. Correlation analysis was carried out with Pearson’s correlation test for normally distributed variables. Independent Student’s t-tests were used for comparing differences of normally distributed variables between the 2 groups. Adjusted (for hip circumference and waist circumference) means of ADMA were compared using a general linear model procedure. The relationship between the categorical variables was determined by the χ²-test for independence with Yates correction for continuity. Logistic regression analysis was performed for the detection of the predictors of EBPR. An univariated regression model was used separately for each of the following covariates: age, smoking, waist circumference/hip circumference ratio, BMI, GFR, CRP, WBC, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, fasting blood glucose, 24 h SBP and DBP measured with ABPM, daytime SBP and DBP, ADMA, and L-arginine. Only 6 covariates that were significantly associated with EBPR in an univariate model were included in the multivariate logistic
regression analysis (ADMA, hip circumference, triglyceride, day time SBP and DBP with ABPM values). Because of the small study sample size, and to prevent potentially overloading the model, all covariates were not entered into the multiple logistic regression models together. A P value of <0.05 was considered statistically significant for all the tests.

Results

The study enrolled 36 patients who had a normal office BP measurement at rest (<140/90 mmHg) but exercise-induced SBP ≥200 mmHg or DBP ≥100 mmHg (EBPR group), along with age- and gender-matched control subjects (n=30) who
showed normal BP response. The prevalence of EBPR was 8.0%. The demographic features of study participants such as age, smoking status, and BMI did not differ between the groups. Subjects with EBPR had higher waist and hip circumference values but waist circumference to hip circumference ratios were comparable between the groups. The main characteristics and laboratory findings of subjects are summarized in Table 1.

The serum ADMA levels were increased in the EBPR group compared to the control group (4.0±1.4 vs 2.6±1.1 μmol/L respectively, P=0.001) (Figure 1). The serum L-arginine levels were similar in the 2 groups (0.17±0.06 vs 0.15±0.05 mmol/L respectively, P=0.19). Also, corrected P values for ADMA according to the waist circumference and hip circumference were significantly different in the EBPR group than in the control group (P=0.001 and P=0.012 respectively). The serum ADMA levels were not correlated with any of the laboratory results.

The average BP values measured with ABPM during the EST are summarized in Table 2. The daytime and 24-h ABPM results were significantly higher in patients with EBPR than in control subjects, but the nighttime BP values were similar. ADMA levels were positively correlated with the SBP and DBP measured at peak exercise and recovery phase (Table 3; Figures 2A, B).

The factors affecting EBPR were analyzed by univariate and multivariate logistic regression models (Table 4). The independent predictor of EBPR was detected as the plasma ADMA levels (odds ratio 2.28; 95% confidence interval 1.22–4.24; P=0.002).

Table 4. ORs for Univariate and Multivariate Logistic Regression Analysis to the Association Between Hemodynamic Parameters and the EBPR Condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Univariate</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip circumference</td>
<td>1.11 (1.03–1.20)*</td>
<td>1.09 (0.98–1.19)</td>
</tr>
<tr>
<td>ADMA</td>
<td>2.00 (1.30–3.07)*</td>
<td>2.28 (1.22–4.24)*</td>
</tr>
<tr>
<td>ABPM total average SBP</td>
<td>1.08 (1.01–1.15)*</td>
<td>–</td>
</tr>
<tr>
<td>ABPM total average DBP</td>
<td>1.08 (1.01–1.17)*</td>
<td>–</td>
</tr>
<tr>
<td>ABPM day interval average SBP</td>
<td>1.07 (1.01–1.14)*</td>
<td>1.02 (0.93–1.12)</td>
</tr>
<tr>
<td>ABPM day interval average DBP</td>
<td>1.09 (1.01–1.18)*</td>
<td>1.03 (0.92–1.16)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; ABPM, ambulatory BP monitoring. Other abbreviations see in Tables 1, 3.

*ADMA, hip circumference, serum triglyceride levels, and ABPM day interval average SBP and DBP were included into the multivariate logistic regression model. Nagelkerke R²=0.45 for the multivariate regression model.

Discussion

The main finding of this study is that the serum ADMA levels were significantly increased in patients with EBPR compared to healthy controls and, they also predicted the EBPR. To the best of our knowledge, this is the first study dealing with the relationship between ADMA and EBPR.

Although, DBP might not increase or often decreases during exercise, SBP has been increasing at a steady rate as a function of the increase in cardiac output against peripheral adaptation during exercise. Total peripheral resistance in individuals with such BP reactivity did not fall adequately to compensate for the rise in cardiac output. This hemodynamic behavior can be explained by a hyperreactivity of the sympathetic nervous system, and an increased vascular response to the adrenergic stimulation or by a slight thickening of the arteriolar wall that alters its ability to respond to vasoconstrictor or vasodilator stimuli. Miyai et al reported elevated levels of plasma norepinephrine in subjects with EBPR than in those with a normal response. In youths, systolic EBPR resulted primarily from a failure to reduce total peripheral resistance during exercise and might be related to early structural vascular changes that precede to hypertension.

Chang et al showed that patients with EBPR have poor endothelium-dependent vasodilation due to an impaired NO/cyclic GMP pathway. Flow-mediated dilatation (FMD) of the brachial artery has been demonstrated to reflect systemic endothelium-dependent vasodilatory capacity mediated by NO. The short-term reduction of circulating ADMA by hemodialysis has shown to improve FMD. In addition, ADMA was a potential mediator of impaired FMD in experimental hyperhomocysteinemia in humans. It has been shown that by increasing plasma homocysteine concentrations using methionine infusion, FMD was impaired and the serum ADMA levels had increased. In a recent study, Stewart et al have reported that the SBP and pulse pressure responses to maximal exercise were associated with impaired endothelial vasodilator function assessed from the brachial artery by measuring FMD, independent of resting BP and arterial stiffness.

ADMA, an endogenous competitive inhibitor of NO synthesis, modulates NO production. Surdacki et al suggested that high plasma ADMA would lead to diminished NO bioavailability and, thereby, increased vascular resistance and elevated BP. In a recent experimental study, intravenously administered suppressor doses of ADMA increased arterial stiffness and decreased cerebral blood flow in young healthy men. In a randomized, double-blind, placebo-controlled study, Achan et al demonstrated that ADMA infusion increased the systemic vascular resistance and mean arterial BP in healthy subjects. The handgrip exercise increased cardiac output in control subjects but not in subjects who had been given ADMA. These results have suggested that ADMA had an important role in the regulation of vascular tone and cardiac hemodynamics. The serum ADMA levels have been found to be negatively associated with the systemic vascular resistance index at rest and during the hemodynamic reactivity test, and a positive association between serum ADMA levels and cardiac index has also been detected. These mentioned studies supported the hypothesis of the present study. The increased ADMA levels might play an important role in both the physiologic mechanism of and poor prognosis in EBPR via impaired NO bioavailability.
cise were more closely related to ABPM than office BP measurement. The present study has shown that daytime ABPM values were higher in the EBPR group than in the control subjects. However, nighttime ABPM values were similar in both groups. This could be because of increased sympathetic activity during daily physical stress. In addition, the autonomic dysregulation and vasoreactive abnormalities have been described in the early stages of hypertension. Therefore, it could be speculated that an EBPR is one of the manifestations of pathophysiological changes during the pre-hypertensive state.

In a small-randomised study, it has been shown that the serum ADMA level was decreased with perindopril and losartan monotherapy but not with bisoprolol in hypertensive patients. That is to say, β-blockers were not effective in lowering ADMA levels. Interestingly, in a recent report, nebivolol was seen to reduce ADMA in hypertensive patients when compared to atenolol. These studies suggested that the future development of drugs with specific ADMA-lowering effects could be more effective than the conventional drugs in the treatment of specific conditions which increased serum ADMA plays a crucial pathophysiological role. In view of the current literature, the data about the management of patients with EBPR is insufficient. Although increased sympathetic activity has been shown to be present during exercise in these patients, when the results were assessed together, the use of angiotensin-converting enzyme inhibitors/receptor blockers or nebivolol as a 3rd-generation β-blocker might be a reasonable medical therapy option owing to the suppressor effect on the sympathetic activity in these patients. Even though this is a more speculative hypothesis, it should be investigated with a randomized follow-up trial.

The conventional risk markers of coronary heart disease such as serum lipid levels, total cholesterol/HDL cholesterol ratio, hsCRP and homocyttein levels were similar in the EBPR and control groups. Because conventional risk markers failed to identify the patients with EBPR, ADMA might be an alternative marker of both EBPR and cardiovascular risk in this population. Interestingly, serum triglyceride concentration was increased in the EBPR group and it was negatively correlated with the L-arginine/ADMA ratio. Recent data showed that hypertriglyceridemia plays an important role in the development of cardiovascular disease but the importance of the association between triglycerides, EBPR and ADMA was unclear. Further investigations of ADMA in lipid disorders are mandatory.

The association between inflammatory markers and elevated BP in healthy patients have been investigated previously. Jae et al discussed that an EBPR might be the result of impaired endothelial function that can be detected by increased levels of low-grade inflammation markers. To test this, they assessed CRP levels and WBC count in 43 patients with EBPR and 42 control subjects. They reported that the WBC count was significantly higher in patients with EBPR (approximately 15%) than in those with a normal BP response. CRP levels were similar between groups, but they observed only a slight trend toward a positive association between CRP levels and WBC count. Similarly, the present study showed that a higher WBC count was observed in patients with EBPR compared to the control group. Also, hsCRP levels were similar in the control and in the EBPR groups. And the hsCRP level was not associated with the serum ADMA levels in the present study. In regression analysis, both hsCRP and WBC count were not associated with EBPR in the present study. In addition, ADMA level was not correlated with WBC count and hsCRP. Thus, ADMA levels were not directly related to inflammation. In our opinion, CRP is a more sensitive and specific indicator of systemic inflammation than the WBC count, and it needs further investigation for the probable relationship between EBPR and systemic inflammation.

Study Limitations

The small number of patients was the major limitation of this study. Another limitation of the study was that causality was not determined due to the cross-sectional design of the study. Therefore, predictive values for future diagnosed hypertension and the prognostic value of ADMA levels in EBPR patients should be investigated via large prospective follow-up trials.

In the present study, blood samples for the measurement of serum ADMA levels were taken after a 12-h overnight fast, in the morning, and none of the patients has been on a special diet. As a result, we think that the difference of ADMA levels between the 2 groups cannot be fully accounted for because of the different dietary habits of the subjects in the present study. However, we accepted that diet might partially affect the L-arginine and ADMA levels. Also, these results might not be generalized to female gender, elder people, and other ethnicities.

Conclusion

In spite of these limitations, the present study showed that the serum ADMA levels were significantly higher in subjects with EBPR during exercise. Impaired vasodilatory capacity could explain the unexpected hypertensive response during EST in otherwise healthy subjects. Inasmuch as the levels of conventional cardiovascular risk factors like homocysteine and hsCRP did not differ in subjects with EBPR compared to non-EBPR, ADMA might be used as a new risk marker.

Acknowledgments

The authors would like to express their special thanks to the staff who conducted the exercise tests at the Department of Cardiology, Faculty of Medicine Hospital, Selcuk University, Konya, Turkey, for their kind cooperation when conducting this study.

References