Arterial elasticity is associated with endothelial vasodilatory function and asymmetric dimethylarginine level in healthy subjects

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ORIGINAL ARTICLE

Arterial elasticity is associated with endothelial vasodilatory function and asymmetric dimethylarginine level in healthy subjects

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Abstract
Arterial stiffening may be linked to the reduced bioactivity of nitric oxide (NO) and increased plasma concentrations of the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA). The aim of this study was to investigate whether large (C1) and small artery (C2) elasticity is associated with endothelial function index (EFI) and plasma concentration of ADMA. We included 63 healthy subjects, aged 19 to 70 years, in the study. EFI, C1 and C2 were assessed by pulse wave analysis (PWA) and ADMA level was measured using an enzyme-linked immunoassay. Linear regression analysis revealed significant positive correlation between EFI and both C1 and C2 ($R=0.29$, $p=0.02$; $R=0.38$, $p=0.002$, respectively). A significant inverse association occurred between ADMA and C1 as well as C2 ($R=0.32$, $p=0.03$; $R=0.37$, $p=0.009$, respectively). In multiple regression analysis, C2 was determined by EFI, ADMA, age and BMI, and C1 was correlated with EFI, age and BMI. These findings suggest that endothelial vasodilatory dysfunction and accumulation of ADMA may be important mechanisms underlying reduced arterial elasticity in healthy subjects.

Key Words: ADMA, arterial stiffness, endothelium, pulse wave analysis

Introduction
Nitric oxide (NO) is an important anti-atherogenic molecule and is involved in regulation of the biomechanical properties of the human arteries [1]. Accumulation of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of NO synthase (NOS), may be causally involved in vascular dysfunction. By blocking NO generation, ADMA may contribute to endothelial vasodilatory dysfunction [2] and arterial stiffening as well as...
have an important role in the progression of coronary artery disease and renal damage [3,4].

The atherosclerotic process [5] and the risk factors for atherosclerosis [6] can alter arterial wall elasticity. Assessment of the elastic behaviour of the arteries may provide an insight into early functional and structural abnormalities of atherosclerosis as well as serve as a surrogate end-point for prediction and treatment of cardiovascular disease [7,8]. Although vascular parameters can be measured using several devices, pulse wave analysis (PWA) is a non-invasive, simple and reproducible method for assessing arterial elasticity and endothelial function [5,7–11]. Conditions associated with endothelial dysfunction [12] are correlated with increased arterial stiffness [13]; therapeutic interventions which improve endothelial function also reduce arterial stiffness [14]; similarly, stimulation of NO production leads to a reduction in arterial stiffness [5,11]. These data suggest a direct link between endothelium-mediated arterial tone and pulsatile vascular function. However, only a few data have been published about the interrelationships between arterial elasticity and endothelial vasomotor properties [15–17], but relevant studies have not used PWA for endothelial assessment. Furthermore, although ADMA could be involved pathophysiologically in the process of arterial stiffening, only a few data [18] are available about associations between ADMA and arterial elasticity in humans. We hypothesized that arterial elasticity could be related to ADMA level and endothelial vasodilatory function in healthy volunteers. Thus, the aim of present study was to test this hypothesis in a group of healthy individuals.

Material and methods

Subjects

The study group (17 F, 46 M) was recruited from the general population. The exclusion criteria for the control group were the following (based on clinical examination, ECG and blood tests): any acute or chronic inflammatory disease, coronary artery disease, cardiac arrhythmias or valve pathologies, hypertension (blood pressure >140/90 mmHg), cerebral or peripheral atherosclerotic disease, diabetes mellitus (fasting serum glucose level >6 mmol/L), malignancies, renal failure and regular use of any medications. This study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of the University of Tartu. Informed written consent was obtained from each participant.

Study protocol

The subjects were studied and the plasma samples were collected between 0800 and 1000 h after an overnight fast and abstinence from any medications, tobacco, alcohol and tea or coffee [19,20]. After 15 min rest in a quiet, temperature-controlled room, blood pressure was measured and PWA was performed. Thereafter, venous blood samples were drawn from the antecubital fossa, height and weight were recorded, and body mass index (BMI) was calculated.

Biochemical analyses

Blood samples were centrifuged and plasma for ADMA stored at −70°C until analysis. ADMA was determined from plasma by a competitive enzyme-linked immunoassay
(ELISA) using a commercially available kit (catalogue no. EA201/96 (DLD Gesellschaft für Diagnostika und Medizi-nische Geräte mbH; Adlerhorst 15 D-22459 Hamburg, Germany) in 49 individuals. The competitive ADMA-ELISA uses the microtitre plate format. ADMA is bound to the solid phase of the microtitre plate. In samples, ADMA is acylated and competes with solid-phase-bound ADMA for a fixed number of rabbit anti-ADMA anti-serum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase ADMA is detected by anti-rabbit/peroxidase. The substrate TMB/peroxidase reaction is monitored at 450 nm.

Plasma glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride levels and creatinine concentrations were determined by standard laboratory methods using certified assays in a local clinical laboratory. Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease formula, equation MDRD 1 [21].

Assessment of arterial elasticity and endothelial function

The arterial waveform was measured in the dominant arm by the Cardiovascular Profiling Instrument (HDI/Pulse Wave CR-2000, Hypertension Diagnostics Inc®, Eagan, USA) as described previously [9,10]. The cuff for blood pressure measurement was placed on the contralateral arm and inflated concurrently with pulse waveform recording for calibration. The elasticity indices of the arteries were quantified during the diastolic portion of the cardiac cycle (mean of 30 s recording). According to the modified Windkessel model of circulation, C1 is a marker for large artery elasticity and C2 is a marker for small artery elasticity. Mean arterial pressure (MAP) was also calculated from the radial pressure waveform using the HDI/Pulse Wave CR-2000 software.

Endothelial function was assessed by PWA using a Sphygmocor apparatus, as described in detail previously [5,11]. Briefly, the peripheral pressure waveforms were recorded from the radial artery of the dominant arm at the wrist employing a high fidelity micromanometer (SPT-301B; Millar Instruments®, Texas, USA). Using the Sphygmocor apparatus (SCOR Px, 7.0; AtCor Medical®, Sydney, Australia), the corresponding ascending aortic waveforms were then generated and augmentation index (AIx) was calculated. The AIx was corrected for a heart rate of 75 beats per minute. Next, a 500-μg tablet of nitroglycerine (NTG) (Nycomed®, Roskilde, Denmark) and, after restoration of haemodynamics, 400 μg of β2 agonist salbutamol (Salb) (GlaxoWellcome Production®, Évreux, France) were administered. A maximum improvement in AIx following Salb administration (due to stimulation of NO synthesis) was defined as endothelium-dependent vasodilation (EDV), while an improvement in AIx after NTG was interpreted as a marker for endothelium-independent vasodilation (EIDV). We used endothelial function index (EFI), defined as the EDV/EIDV ratio, to represent endothelial function. This index can evaluate vasodilation depending on the activity of endothelial NOS (eNOS) in relation to non-specific vasodilation. All PWA recordings were performed consecutively by a single operator.

Statistical analysis

All data were tested for normality. The continuous data were expressed as means ± standard deviation. Correlations between the variables were examined using univariate
linear regression and multiple regression analysis (free software R, version 2.2.1 for Windows). A $p < 0.05$ was considered significant.

Results

The main characteristics of the study population are listed in Table I. The subject’s age was in the range 19 to 70 years. Linear regression analysis was used to establish whether endothelial function correlated with large artery and small artery elasticity. There was a significant positive association of EFI with C1 and C2 (Figure 1). Significant inverse

![Figure 1. Scatterplot of large artery (C1) and small artery elasticity (C2) and endothelial function index (EFI) in 63 healthy subjects. C1 is positively correlated to EFI ($R=0.29$, $p=0.02$) (A), and C2 is positively associated with EFI ($R=0.38$, $p=0.002$) (B).](image)
Correlations were observed between ADMA and C1 as well as C2 (Figure 2). The results of univariate correlation analysis encouraged elucidation of relationships between arterial elasticity and EFI or ADMA in multivariate models adjusted for age and BMI. After adjustment for potential confounders in multivariate models, the final model demonstrated that C1 was significantly inversely related to age and positively to BMI and EFI (Table IIa) but not to ADMA level (Table IIb). The data were also used in multivariate models to find any independent determinants of C2. The final model (Table III) showed that C2 was correlated inversely to age and ADMA and positively to EFI and BMI.

Discussion

The current study established whether NO-mediated vascular tone and ADMA were associated with arterial elasticity in healthy subjects. The significant associations in multiple

Table IIa. Multiple regression model with C1 as the dependent variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.009</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.05</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EFI</td>
<td>0.37</td>
<td>0.14</td>
<td>0.01</td>
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</tbody>
</table>

$R^2=0.37, p<0.001$.

Table IIb. Multiple regression model with C1 as the dependent variable.

<table>
<thead>
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<th>Variable</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Age (years)</td>
<td>-0.007</td>
<td>0.003</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.04</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>ADMA (µmol/L)</td>
<td>-0.44</td>
<td>0.42</td>
<td>0.3</td>
</tr>
</tbody>
</table>

$R^2=0.27, p<0.003$. 

Figure 2. Scatterplot of large artery (C1) and small artery elasticity (C2) and asymmetric dimethylarginine (ADMA) level in 49 healthy subjects. C1 is inversely correlated to ADMA ($R=-0.32, p=0.03$) (A), and C2 is inversely associated with ADMA ($R=-0.37, p=0.009$) (B).
regression models between C1 and EFI as well as between C2 and EFI indicate that alterations in arterial elasticity are related to the degree of endothelial vasodilatory function. The strong correlation between C2 and ADMA after adjustment for potential confounders suggests that elevated ADMA has a more pronounced effect on the vascular stiffening of the small arteries rather than the large conduit arteries.

Interest in the biomechanical properties of the arteries has markedly increased in recent years. Early identification of pathological changes in the vasculature is of great importance in stratification of cardiovascular risk, as the risk factors for atherosclerosis, endothelial dysfunction and reduced arterial elasticity are modifiable [14]. It has been demonstrated that altered elastic properties of the arteries, measured by PWA (i.e. increased AIx or decreased C2), are significantly associated with cardiovascular events [7,8], which renders PWA methodology applicable to identification and monitoring of subjects with cardiovascular pathology [5,7–11].

In the current study, we demonstrated correlations between endothelial vasomotor function and arterial elasticity. Although there appears to be a link between pulsatile arterial function and NO-mediated arterial tone, the extent to which EFI, C1 and C2 are derived from systolic or diastolic PWA has not been studied previously. In multiple regression model C1 was negatively correlated with age and positively with EFI and BMI. Small artery elasticity was inversely associated with ADMA and age and positively with EFI and BMI. It was expected that age had a significant impact on the stiffening of the large and small arteries [9,10]. Our results also support previously reported data about a close association between arterial elasticity and endothelial function, most likely relating to the endothelial release of NO [15–17]. The stronger correlations between EFI or ADMA and C2 compared with C1 can be explained by the fact that the smaller arteries, close to the arterial branching points, have a thinner media layer and NO is therefore more important in producing dilation in them. Furthermore, it has been demonstrated that inhibition of NO synthesis with NG-nitro-L-arginine methyl ester caused a significant reduction in C2, while following L-arginine administration restored the baseline [22], indicating a closer possible link between small artery elasticity and endothelium-mediated arterial tone. In addition, it has been shown previously in humans that infusion of ADMA decreases renal plasma flow [23] and increases pulmonary vascular resistance [24] as those vascular beds have a high density of thin-walled small arteries. However, the significant relationship between C1 and

<table>
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<th>Standard error</th>
<th>p-value</th>
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<tr>
<td>Age (years)</td>
<td>-0.11</td>
<td>0.03</td>
<td>&lt;0.001</td>
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<td>BMI (kg/m²)</td>
<td>0.44</td>
<td>0.12</td>
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<tr>
<td>EFI</td>
<td>3.85</td>
<td>1.46</td>
<td>0.01</td>
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$R^2=0.41$, $p<0.001$.

<table>
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<th>Variable</th>
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<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.08</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.28</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>ADMA (μmol/L)</td>
<td>-6.9</td>
<td>4.08</td>
<td>0.04</td>
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</table>

$R^2=0.26$, $p<0.004$.
EFI provides evidence that endothelial function is also associated directly with elasticity of the large arteries, considering that NO itself can modify the elastic properties of the large arteries in humans [1,5,11].

ADMA has emerged in recent years as a novel cardiovascular risk factor [25]. Increased ADMA concentrations represent a strong and independent risk marker for progression of coronary artery disease [4] as well as of renal damage [3], in which arterial stiffness is also pathophysiologically involved and serves as an excellent indicator of future cardiovascular events [8,26]. Furthermore, the relationship between ADMA and arterial stiffness found by us could also explain how elevated ADMA can be linked to left ventricular hypertrophy [27]. The most obvious consequences of arterial stiffening, caused at least in part by accumulation of ADMA, are higher central systolic and pulse pressure. These haemodynamic changes increase left ventricle afterload, reduce coronary perfusion and are associated with left ventricular hypertrophy, which is \textit{per se} a cardiovascular risk factor.

Despite the fact that ADMA may be causally involved in vascular dysfunction, no data are available about associations between arterial elastic properties and ADMA concentration in humans. We have demonstrated in the current study that deterioration of large and small artery elasticity is associated with increased ADMA level, suggesting that elevated ADMA plays a significant role in vascular stiffening. Increased ADMA may affect vascular function and structure through various mechanisms. Evidence has accumulated to the effect that elevation in ADMA may at least in part cause eNOS uncoupling, increase vascular superoxide level and contribute to oxidative stress [25], which \textit{per se} may be a major mechanism of vascular impairment [13,28]. Increased levels of ADMA also reduce bioavailability of NO and cause endothelial dysfunction [25] \textit{via} blocking all three isoforms of NOS and enhance NO degradation due to eNOS-mediated superoxide production. It has been demonstrated that ADMA causes vascular arteriosclerotic lesions also in an eNOS-independent manner. Direct upregulation of the angiotensin-converting enzyme and increased oxidative stress \textit{via} the angiotensin II type 1 receptor might also be involved in the long-term vascular effects of ADMA [29]. However, elevated ADMA levels promote endothelial-monocyte interaction [30], related to carotid intima-media thickness [31], and correlate with severity of PAD [32], suggesting that an increase in ADMA level is associated with critical processes in atherogenesis.

It has recently been demonstrated that NO may contribute a functional component to arterial stiffness [1] and endothelial dysfunction is closely related to decreased arterial elasticity [15–17]. Thus, reduced bioavailability of NO and endothelial dysfunction, most likely due to accumulation of ADMA, might be one potential mechanism underlying alterations in the elasticity of the arteries. However, whether there occurs a causal relationship between increased ADMA levels, eNOS-mediated superoxide production, endothelial dysfunction and reduced arterial elasticity, remains to be established.

Surprisingly, in both of our multiple regression models, BMI correlated positively with arterial elasticity. This unexpected finding might have been caused by the relatively small number of subjects. We have no plausible explanation for this observation. However, it has been demonstrated previously that within the non-obese range BMI is positively associated with vascular function in healthy subjects [33].

In conclusion, we have provided novel evidence regarding the link between endothelium-related functional and biochemical properties and arterial elasticity. The above observations support the notion that the increase in ADMA level and endothelial dysfunction may be an important mechanism involved in arterial stiffening in healthy subjects. A better understanding of the associations between biochemical and functional vascular correlates
may also lead to effective assessment of subclinical cardiovascular pathology. We believe that measurement of ADMA combined with assessment of vascular physiologic properties by PWA might provide useful information for evaluation of vascular health.

Acknowledgments

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