Asymmetric Dimethylarginine, Blood Pressure, and Renal Perfusion in Elderly Subjects

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Background—Reduced availability of nitric oxide (NO) is thought to contribute to the age-associated increase of renovascular tone and blood pressure. We assessed blood concentrations of the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) as well as renal hemodynamics, comparing young (n=24, 13 men, 25±1 years) and elderly (n=24, 13 men, 69±2 years) healthy subjects and elderly subjects with essential hypertension (n=24, 13 men, 70±2 years).

Methods and Results—Plasma ADMA concentration and renovascular resistance (RVR) were significantly higher (P<0.05) and effective renal plasma flow (ERPF) significantly lower (P<0.05) in elderly (2.77±0.20 μmol/L, 125±10 mm Hg/mL per minute, 487±26 mL/min per 1.73 m²) than in young healthy subjects (1.30±0.11, 77±3, 654±18). Both ADMA levels and RVR were higher and ERPF lower in the hypertensive elderly subjects (3.53±0.23, 163±11, 427±19; P<0.05 versus both groups). In contrast, plasma concentrations of the biologically inactive stereoisomer symmetric dimethylarginine, L-arginine, and homocysteine were similar in the 3 groups studied. In the logistic regression analysis only ADMA was an independent determinant of both ERPF (P<0.001; r²=0.80) and RVR (P<0.002; r²=0.86). In addition, ADMA (P<0.002) and serum glucose (P<0.036) were independently related (r²=0.67) to the level of blood pressure.

Conclusions—These results are compatible with the notion that accumulation of the endogenous NO synthase inhibitor ADMA in senescent individuals is involved in the decrease of renal perfusion and increase of blood pressure. (Circulation. 2003;107:1891-1895.)

Key Words: aging • nitric oxide synthase • kidney • hypertension

Reduced nitric oxide (NO)-dependent vasodilation as an early indicator of atherosclerotic disease has been documented in elderly subjects, particularly if cardiovascular risk factors such as smoking or hypertension were present.1–3 NO synthase (NOS) synthesizes NO from the amino acid L-arginine. Guanidino-substituted analogues of L-arginine such as asymmetric dimethylarginine (ADMA) can selectively inhibit NOS by competitive blockade of its active site.4 Several clinical studies examined different populations and found that increased plasma ADMA levels are not only correlated with the severity of atherosclerotic disease but also predict increased cardiovascular mortality rates.5–11 Thus, ADMA is thought to be not only a biochemical marker of atherosclerosis but potentially a pathogenic mediator.4,9,12

Past and more recent studies have documented that aging is accompanied by changes in renal hemodynamics, particularly by an increase in renovascular tone with reduced ability of postglomerular vessels to dilate in response to stimuli such as acetylcholine or amino acids.13–16 Furthermore, in senescent individuals, reduced availability of NO is thought to be linked to the increase in blood pressure and in renovascular resistance, possibly a reflection of arteriosclerosis.17,18 So far, no specific information on plasma ADMA concentration and its potential relation to abnormal renal hemodynamics in the elderly is available. To address this issue, we measured renal hemodynamics and blood concentrations of dimethylarginines in young and elderly healthy normotensive subjects and in elderly patients with mild to moderate essential hypertension. True glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were assessed by using the inulin- and PAH-clearance techniques, respectively.

Methods

Participants and Protocol

The local ethics committee approved the study protocol; all participants gave written informed consent. Twenty-four young and 24 elderly healthy normotensive subjects and, in addition, 24 elderly patients with mild to moderate essential hypertension were exam-
Hypertension was defined according to World Health Organization criteria as blood pressure $\geq 140/90$ mm Hg or antihypertensive medication. To exclude individuals with primary renal disease, sonography, urine analysis, and serum chemistry were performed in all participants and only subjects with normal plasma creatinine concentration were enrolled. In elderly subjects, manifest atherosclerotic vascular disease and/or heart failure were excluded by clinical examination and echocardiography. Thus, with the exception of essential hypertension, none of the elderly participants had relevant medical problems. All participants were nonsmoking whites. The three groups were matched with regard to gender and body weight.

Inulin and PAH were studied under open conditions. Preprandial counseling was given to all participants who were advised to ingest a standardized diet with regard to sodium chloride (100 mmol/d) and calorie content (30 kcal/kg body weight) 1 week before and during the examination. Cardiovascular drugs with the potential to confound the measurement of renal hemodynamics were discontinued in elderly hypertensive patients at least 1 week before the examination, in accordance with their respective pharmacodynamic half-life. GFR and ERPF were measured after 12 hours of fasting in a quiet room, in supine position, using the inulin ($C_i$) and para-aminohippurate ($C_{o,PAH}$) infusion clearance techniques as described before. In brief, a priming dose of 1500 mg inulin/m$^2$ (Inustest, Lævosen Co) and of 500 mg para-aminohippurate/m$^2$ (Nephrotest, BGA) was followed by continuous infusion of inulin (10 mg/m$^2$/min) and para-aminohippurate (8 mg/m$^2$/min) with ultraprecise pumps (Perfusor FT, Braun Melsungen). After an equilibration period of 100 minutes, blood samples for determination of $C_i$ and $C_{o,PAH}$ were taken at regular intervals. To calculate renovascular resistance, mean arterial blood pressure (MAP) was measured at the same time points during the clearance studies by using a noninvasive oscillometric technique (Dinamap, Criticon Inc). Blood samples for measurements of dimethylarginines, 1-arginine, homocysteine, glucose, total, HDL, and LDL cholesterol, and triglyceride concentrations were taken without venous compression at the start of the clearance measurement after at least 100 minutes of supine position. In addition, ambulatory 24-hour blood pressure was assessed on a separate day with the use of an automatic device (model 90207, SpaceLabs Inc).

### Measurements and Calculations

Inulin was measured enzymatically with inulinase$^{19}$ and PAH was measured photometrically. The clearances of inulin and PAH were calculated from the delivered dose: $C = (I_i - I_f)/S$; where $C$ is the clearance, $I$ is the infusion rate, $I_f$ is the concentration of the analyte in the infusion fluid, and $S$ is the plasma concentration of the analyte. Filtration fraction (FF) was calculated as the ratio $C_{i}/C_{o,PAH}$ and renovascular resistance (RVR) by using the equation $RVR = ([MAP - 12] \times 723)/ERPF$. Plasma levels of 1-arginine, ADMA, and the biologically inactive stereoisomer symmetric dimethylarginine (SDMA) were measured with high-performance liquid chromatography (HPLC), using precolumn derivatization with o-phthalaldehyde (OPA). Plasma samples and internal standards were extracted on C8 solid-phase extraction cartridges (MDU/L Varian) and thereafter were incubated for 30 seconds with the OPA reagent (5.4 mg/mL OPA in borate buffer, pH 8.5 containing 0.4% mercaptoethanol) before automatic injection into the HPLC. The OPA derivatives of 1-arginine, ADMA, and SDMA were separated on a C6H5 column (Macherey and Nagel) with the fluorescence monitor set at an excitation wavelength of 340 nm and an emission wavelength of 455 nm. The coefficients of variation of this method are 5.2% within assay and 5.5% between assays; the detection limit of the assay is 0.1 µmol/L. Plasma total homocysteine (Hcy) concentrations were measured with a fluorescence-polarization immunonassay. All other measurements were done with routine laboratory tests by certified assay methods.

### Statistics

The SPSS package was used for statistical analysis. Comparison between groups was done by using ANOVA after normality of data distribution was confirmed with the Shapiro-Wilk test. A 2-tailed $t$ test for comparison of random data between groups was used when ANOVA gave significant differences, and the Bonferroni correction was applied to account for multiple comparisons. The zero hypothesis was rejected at a probability level of 0.05. All data are presented as mean±SEM. In addition, a multinomial logistic regression analysis was performed with the three groups defined as part of the analysis to detect significant characteristics of individuals studied apart from the predetermined group variables of age and blood pressure. The regression model included body weight, ADMA, SDMA, 1-arginine, Hcy, triglycerides, glucose, and LDL and HDL cholesterol. Furthermore, independent predictors of ERPF, RVR, and the level of blood pressure were evaluated with the use of logistic regression analysis. Data of the dependent variables ERPF, RVR, and MAP were categorized, and forward stepwise inclusion (likelihood quotient) was applied to reveal significant independent determinants for each dependent variable. The Nagelkerke coefficient ($r^2$) indicates the percentage of variability of the dependent variable explained by the significant independent determinant(s).

### Results

The clinical characteristics of the three groups of subjects studied are presented in the Table. GFR and ERPF were significantly lower in both groups of elderly individuals than in young healthy subjects, and ERPF was significantly lower in both groups of elderly individuals, being significantly higher in healthy elderly subjects than in elderly subjects with hypertension. Conversely, RVR and FF were higher in both groups of elderly subjects as compared with young individuals, particularly in the elderly patients with high blood pressure. In addition, the elderly hypertensive patients not taking drugs had a significantly higher MAP and total and LDL cholesterol serum concentrations than young and elderly healthy subjects. Furthermore, both in healthy elderly and in hypertensive elderly, HDL cholesterol was significantly lower than in young healthy subjects. In contrast, serum glucose concentrations were not significantly different in all three groups studied. Plasma ADMA concentrations were significantly higher in healthy elderly than in healthy young subjects and were even higher in hypertensive elderly. Plasma SDMA, 1-arginine, and Hcy levels were not significantly different in young and healthy elderly and in hypertensive elderly subjects (Table). Individual data on plasma ADMA concentrations and RVR are shown in Figure 1 and Figure 2.

The multinomial logistic regression analysis revealed that ADMA ($P<0.001$) and LDL cholesterol ($P<0.004$) blood levels were important independent characteristics of individuals studied. The logistic regression analysis further revealed that ADMA blood levels were the only significant independent determinant of ERPF ($P<0.001$, $r^2=0.80$) after adjustment for potential confounding by age ($P=0.196$), body weight ($P=0.736$), MAP ($P=0.289$) and blood SDMA ($P=0.899$), 1-arginine ($P=0.596$), Hcy ($P=0.116$), LDL cholesterol ($P=0.887$), HDL cholesterol ($P=0.514$), triglyceride ($P=0.500$), and glucose ($P=0.152$) levels. In addition, after elimination of age in the second step of the analysis, only ADMA blood concentrations remained a significant determinant of RVR ($P=0.002$) in the final regression equation, explaining most of its variability ($r^2=0.86$). All other potential cofounders were not significantly related to RVR: age ($P=0.356$), body weight ($P=0.514$), MAP ($P=0.491$) and blood SDMA ($P=0.838$), 1-arginine ($P=0.251$), Hcy ($P=0.053$), LDL cholesterol ($P=0.669$), HDL cholesterol ($P=0.280$), triglyceride ($P=0.061$), and glucose ($P=0.226$).
In contrast, this was not the case with L-arginine, the substrate plasma flow and increased calculated renovascular resistance. Levels were a significant predictor of reduced effective renal filtration fraction, C_{EF}/C_{PAH} 0.18 \pm 0.00 0.22 \pm 0.01^* 0.25 \pm 0.01^{†\dagger} and serum ADMA (P=0.002) and serum glucose (P<0.036) concentrations were independent predictors of the blood pressure level after adjustment for age (P=0.672), body weight (P=0.841) and blood SDMA (P=0.608), L-arginine (P=0.889), Hcy (P=0.101), LDL cholesterol (P=0.189), HDL cholesterol (P=0.683), and triglyceride (P=0.665) levels. ADMA and glucose plasma concentrations together explained a large part of blood pressure variability (r^2=0.67) in the individuals that we studied.

### Discussion

Our findings are of interest with regard to the pathophysiology of the aging of the kidney. It is well known that even normal aging is associated with some loss of renal tissue accompanied by changes in renal hemodynamics. The observed decrease in ERPF and increase in FF and RVR is particularly pronounced in elderly persons with cardiovascular comorbidity such as hypertension and/or heart failure, that is, conditions in which the availability of NO is reduced. In this context, it has not been resolved whether age-related changes in renal hemodynamics are caused by structural abnormalities or whether there exists a functional abnormality as well, for example, reduced capacity of renal vessels to dilate as a consequence of reduced availability of (or responsiveness to) vasodilator substances. Experimental studies and studies in humans support the latter concept. In this context, it must be pointed out that the postglomerular renal (micro) vasculature is particularly sensitive to NO inhibition. This has been recently demonstrated by using ADMA in animal experiments and in studies with isolated organs. Increased blood levels of ADMA in the elderly may therefore reduce the availability of NO and thus contribute to endothelial dysfunction and arteriosclerosis and finally may lead to increased renovascular resistance and hypertension. This hypothesis is supported by the fact that the highest plasma ADMA levels have been found in elderly subjects with the highest FF and/or with the highest RVR, respectively. Furthermore, in line with this assumption are indirect observations related to biological effects of ADMA: In patients with kidney disease, high plasma ADMA concentrations correlate significantly with the ex vivo capability of the patient’s blood to inhibit NO production in cultured endothelial cells. Furthermore, intrabrachial ADMA

<table>
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<th>Characteristics of the Study Populations</th>
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<td><strong>Young</strong></td>
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<td><strong>Normotensive</strong></td>
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<td><strong>(n=24)</strong></td>
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<tr>
<td>Gender, male/female</td>
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<tr>
<td>Age, y</td>
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<td>Body weight, kg</td>
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<td>24-h MAP, mm Hg</td>
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<td>Serum creatinine, mg/dL</td>
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<td>GFR, mL/min per 1.73 m²</td>
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<td>ERPF, mL/min per 1.73 m²</td>
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<td>Filtration fraction, C_{EF}/C_{PAH}</td>
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<td>RVR, mm Hg/mL per minute</td>
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<td>Plasma L-arginine, μmol/L</td>
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<td>Plasma homocysteine, μmol/L</td>
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*P<0.05, young normotensive vs elderly normotensive; †P<0.05, young normotensive vs elderly hypertensive; ‖P<0.05, elderly normotensive vs elderly hypertensive.
infusion abolished endothelium-dependent vasodilation in healthy subjects. Further indirect support for the assumption that increased plasma ADMA levels reduce renal perfusion and increase blood pressure with senescence comes from our recent experiments in which systemic ADMA administration significantly decreased ERPF and increased RVR and MAP in healthy subjects [Kielstein JT et al, unpublished data, 2002]. Long-term intervention studies with substances that increase NO production and overcome NOS inhibition by ADMA within the renal vascular bed such as L-arginine are needed to clarify this issue. It is currently uncertain whether the moderate increase of plasma ADMA levels in the hypertensive elderly as compared with normotensive elderly subjects, as found in this study, has pathophysiological relevance. We emphasize, however, that according to several studies in different populations, in the long run even small differences in mean plasma ADMA levels (ie, 1 μmol/L) are associated with deterioration of endothelial function and a significant increase in the rate of cardiovascular events.10,32–34

What could be the explanation for the increase in ADMA blood levels with age? ADMA is released from proteins that have been posttranslationally methylated and hydrolyzed.4,9,12 These proteins are found in the nucleolus and appear to be involved in RNA processing and transcriptional control. Two types of enzymes methylate arginine residues—protein arginine methyltransferase type I (PRMT I) forms ADMA, whereas PRMT II forms SDMA. Increased activity of PRMT I could theoretically lead to increased appearance of ADMA with aging, although this is an unlikely explanation. On the other hand, decreased ADMA breakdown may be of importance. ADMA is excreted by the kidneys to some extent, but the predominant metabolic pathway is degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyzes ADMA (but not SDMA) to dimethylamine and L-citrulline.4,9,12 Colocalization of DDAH and NOS in various cell types including renal tubular cells supports the hypothesis that the intracellular concentration of ADMA is actively and cell-specifically regulated in NO-generating cells.35 To date, DDAH activity is difficult to assess, however, and no data are available on DDAH activity in persons of advanced age. Furthermore, the clearance of ADMA may be reduced, secondary to a decrease in GFR and/or reduced numbers of tubular cells containing DDAH as a consequence of renal tissue involution with age. The former explanation is less likely, however, because we and others have shown that plasma ADMA concentrations depend less on GFR than do plasma SDMA levels.36,37 Thus, SDMA accumulates proportionally more when GFR is reduced. This was not the case in our population of elderly individuals.

Another explanation for increased plasma ADMA levels with age might be increased generation of ADMA from the metabolism of Hcy, because the metabolic pathways generating Hcy and ADMA are closely linked.38 In addition, high Hcy levels have been shown to inhibit DDAH activity in vitro.39 Plasma Hcy levels were in the normal range, however, in our elderly and young subjects. In contrast, mean total and LDL cholesterol concentrations were significantly higher in the
elderly patients with essential hypertension. Hypercholesterolemia was shown to be associated with increased plasma ADMA concentrations in vivo,32 and an inhibitory effect of LDL cholesterol on DDAH activity was documented in vitro.40 Thus, increased cholesterol concentration could contribute to the increase of plasma ADMA levels, at least in hypertensive elderly subjects. In this regard, a recent observation of a significant relation between plasma ADMA levels and insulin resistance suggests that increased plasma ADMA levels may characterize patients with the metabolic syndrome.41

In conclusion, our finding of a significant relation between high blood ADMA levels with reduced renal perfusion and high blood pressure values is consistent with a causal role of ADMA in the pathophysiology of the age-related endothelial dysfunction, resulting in increased renovascular tone and blood pressure.

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References

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