ADMA Increases Arterial Stiffness and Decreases Cerebral Blood Flow in Humans

Jan T. Kielstein, MD; Frank Donnerstag, MD; Sandra Gasper, MD; Jan Menne, MD; Anousheh Kielstein, MD; Jens Martens-Lobenhoffer, PhD; Fortunato Scalera, PhD; John P. Cooke, MD, PhD; Danilo Fliser, MD; Stefanie M. Bode-Böger, MD, MPH

Background and Purpose—Preclinical studies have revealed that the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA), increases vascular tone in cerebral blood vessels. Marked elevations of ADMA blood levels were found in patients with diseases characterized by decreased cerebral perfusion, such as ischemic stroke. Arterial stiffness is an independent predictor of stroke and other adverse cardiovascular events. The aim of this study was to investigate the influence of a systemic subpressor dose of ADMA on arterial stiffness and cerebral perfusion in humans.

Methods—Using a double-blind, vehicle-controlled study design, we allocated 20 healthy men in random order to infusion of either ADMA (0.10 mg ADMA/kg per min) or vehicle over a period of 40 minutes. Arterial stiffness was assessed noninvasively by pulse wave analysis. All volunteers underwent measurement of cerebral perfusion by dynamic contrast-enhanced perfusion magnetic resonance imaging of the brain.

Results—Infusion of ADMA significantly decreased total cerebral perfusion by 15.1 ± 4.5% (P = 0.007), whereas blood flow in the vehicle group increased by 7.7 ± 2.8% (P = 0.02). ADMA also increased arterial stiffness as assessed by measurement of the augmentation index (−12.6 ± 1.9 to −9.6 ± 1.5, P = 0.007).

Conclusions—Our results document for the first time that subpressor doses of ADMA increase vascular stiffness and decrease cerebral perfusion in healthy subjects. Thus, ADMA is an important endogenous modulator of cerebral vascular tone and may be involved in the pathogenesis of cerebrovascular disease. (Stroke. 2006;37:2024-2029.)

Key Words: cerebral blood flow □ hemodynamics □ nitric oxide □ stroke, ischemic □ vascular resistance

Stroke is a devastating disease that affects ~750,000 people per year in the United States, ranks as the third leading cause of death, causes long-term disability, and imposes an economic burden on the healthcare system.1 Since the discovery of neuronal nitric oxide synthase (NOS) in the brain and cerebral arteries,2 there has been increasing evidence that NO is a critical regulator of brain perfusion. Asymmetric dimethylarginine (ADMA) has been identified as an endogenous inhibitor of NOS. Clinical conditions that are associated with stroke are also associated with elevated blood levels of ADMA, ie, increasing age, diabetes, hypertension, carotid artery intima-media thickness, hyperlipidemia, hyperhomocyst(e)inemia, obesity, inflammation (for a review, see Kielstein and Zoccali3), and sickle cell disease.2 Finally, elevated ADMA levels have also been shown to be directly associated with an increased risk of stroke,5 and a recent study indicated that ADMA is an independent marker for acute stroke and for transient ischemic attacks.6 This may be explained through increased arterial stiffness, which is an independent predictor of stroke and other adverse cardiovascular events7,8 and which is related to NO availability.9 Alternatively, by decreasing endothelium-mediated NO-dependent vasodilation, ADMA might impair cerebral autoregulation and contribute to ischemic injury.

Several in vitro and animal studies have revealed that ADMA increases vascular tone in cerebral blood vessels.10–12 Recently, it was speculated that ADMA might participate in the pathogenesis of arterial stiffness.13 We therefore studied the effect of acute systemic ADMA infusion on arterial compliance and on cerebral perfusion in humans.

Subjects and Methods

Participants and Protocols

The study protocol was approved by the local ethics committee, and all participants gave written, informed consent. We recruited 20 healthy white males (mean ± SD age, 27.3 ± 3.2 years). All participants underwent a physical examination, routine blood chemistry analysis, and urinalysis. Smoking habit, allergies, hypertension (blood
increased arterial stiffness) and vice versa. The validity of the reflected wave as a result of increased pulse wave velocity (due to increased wave reflections from the periphery and/or earlier return of systolic shoulders of the central pressure waveform, and pulse pressure, ie, the difference in pressure between the early and late systolic phases of the central pressure waveform, and pulse pressure, was measured as an index of wave reflections. AIx was originally defined by Kelly et al.15 as the ratio of augmentation of pressure, ie, the difference in pressure between the early and late systolic phases of the central pressure waveform, and pulse pressure, expressed as a percentage. Larger values of AIx indicate increased wave reflections from the periphery and/or earlier return of the reflected wave as a result of increased pulse wave velocity (due to increased arterial stiffness) and vice versa. The validity of the noninvasively determined AIx has been previously confirmed.16 Using the Sphygmocor (AtCor Medical, version 6.2), a single operator obtained radial artery waveforms (derived from 20 sequentially recorded radial artery waveforms) that were fed for analysis. The AIx was adjusted for heart rate. Blood pressure was measured at regular intervals before, during, and after the infusion period but not during the MRI with a noninvasive oscillometric technique (Dinamap, Criticon Inc). In a subset of subjects, a venous blood gas analysis was performed immediately before and after the infusion.

**Measurement of Arterial Stiffness: AIx**

The augmentation index (AIx) of the central (aortic) pressure waveform was measured as an index of wave reflections. AIx was originally defined by Kelly et al.15 as the ratio of augmentation of pressure, ie, the difference in pressure between the early and late systolic phases of the central pressure waveform, and pulse pressure, expressed as a percentage. Larger values of AIx indicate increased wave reflections from the periphery and/or earlier return of the reflected wave as a result of increased pulse wave velocity (due to increased arterial stiffness) and vice versa. The validity of the noninvasively determined AIx has been previously confirmed.16 Using the Sphygmocor (AtCor Medical, version 6.2), a single operator obtained radial artery waveforms (derived from 20 sequentially recorded radial artery waveforms) that were fed for analysis. The AIx was adjusted for heart rate. Blood pressure was measured at regular intervals before, during, and after the infusion period but not during the MRI with a noninvasive oscillometric technique (Dinamap, Criticon Inc). In a subset of subjects, a venous blood gas analysis was performed immediately before and after the infusion.

**Magnetic Resonance Imaging**

MRI was performed with a neurovascularily optimized 1.5-T whole-body scanner (Signa NV/i, GE) and a head coil. All volunteers were examined with (1) axial fluid-attenuated inversion recovery MRI (repetition time [TR], 8000 ms; echo time [TE], 145ms; TI, 2100 ms; matrix, 256×192; slice thickness, 6 mm, with an interlace gap of 1.5 mm) and (2) an axial spin-echo echoplanar imaging diffusion-weighted MRI (TR, 8000 ms; TE, 88.4 ms; b=1000 s/mm²; matrix, 128×128) with contiguous slices (5 mm thick). In addition, a 3-dimensional time-of-flight study (TR, 39 ms; TE, 6.9 ms; flip angle, 20°) was obtained. The matrix, 256×160 (effective slice thickness, 1.6 mm) to image the circle of Willis from the middle of the basilar artery to the proximal part of the M2 segments of both middle cerebral arteries was performed to exclude gross pathology. Perfusion-weighted imaging consisted of T2-weighted spin-echo echoplanar imaging (TR, 2000 ms; TE, 60 ms; matrix, 128×128, with 12 contiguous slices with a thickness of 10 mm). The brain was imaged from the cerebellar horizontal fissure up to the supratentorial space above the Sylvian fissure. Spin-echo echoplanar imaging was chosen to obtain optimal sensitivity for small vessels.17 Dynamic susceptibility contrast imaging was performed with an injection of 15 mL gadobutrol (1 mol/L; Schering, Berlin, Germany) at a flow rate of 5 mL/s followed by a saline solution flush at the same flow rate. Gadobutrol at a concentration of 1 mol/L, was chosen because it produces a better signal-to-noise ratio in dynamic susceptibility contrast MR perfusion.18 The perfusion-weighted imaging sequence had a temporal resolution with a frame rate of 1.94 seconds per whole brain scan. All MR sequences were oriented parallel to the bicommissural line.

Regional cerebral perfusion was calculated by use of a modified version of commercially available software (Functool version 1.9x, GE) with a deconvolution algorithm. Parenchymal enhancement in the brain tissue was deconvolved pixel by pixel from the arterial input in the middle cerebral artery. The deconvolution algorithm is based on the method described by Eastwood et al.,19 which measures cerebral blood volume and cerebral blood flow and calculates mean transit time (MTT) (=CBV/CBF) from the central volume principle.

Total cerebral perfusion was obtained by averaging blood flow from 4 standardized regions of interest (ROIs) in each hemisphere (300 mm² in the region of the anterior cerebral artery, posterior cerebral artery in the basal ganglia) and an ROI of 1000 mm² in the region of the middle cerebral artery on parametric maps. For regional perfusion in the basal ganglia, ROIs of 350 mm² were selected in the caudate nucleus, putamen, and globus pallidus bilaterally on parametric maps. This anatomic region is supplied by the middle cerebral artery as well as the anterior cerebral artery. The hand-drawn ROI in the region of the basal ganglia predominantly consisted of gray matter, which did not contain large vessels.19 Reference values for dynamic susceptibility contrast MR perfusion imaging have been described by Helenius et al.20

**ADMA Measurements**

ADMA was measured by liquid chromatography–mass spectrometry.21 The coefficient of variation is 4.7%. All other measurements were completed with routine laboratory tests and certified assay methods.

**Statistical Analysis**

We used SPSS for statistical analysis (SPSS 11.51 for Windows, SPSS Inc, Chicago, Ill). Normality of the data distribution was confirmed with the Shapiro-Wilk test. Comparison between groups as well as between preinfusion and postinfusion data was performed with a paired t test for random data. Data are presented as mean±SD unless indicated otherwise. The level of significance was set at P<.05.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

Clinical and laboratory data for subjects in the experimental and control groups are presented in Table 1. Exogenous ADMA significantly decreased heart rate, whereas infusion of vehicle had no effect. Neither ADMA nor vehicle affected blood pressure when measured immediately before and after the infusion (Table 1). Infusion of ADMA had no effect on venous blood PCO₂ (in subjects who received ADMA, PCO₂ before and after the infusion was 40.6±1.9 and 39.3±2.3 mm Hg, respectively).

**TABLE 1. Clinical and Laboratory Characteristics of the Study Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Group</th>
<th>ADMA Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age, y</td>
<td>28±3</td>
<td>26±4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23±2</td>
<td>23±2</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>176±33</td>
<td>177±51</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>59±8</td>
<td>53±15</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>95±33</td>
<td>99±37</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>107±34</td>
<td>114±40</td>
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<tr>
<td>Mean arterial blood pressure, mm Hg, preinfusion</td>
<td>92±10</td>
<td>89±9</td>
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<tr>
<td>Mean arterial blood pressure, mm Hg, postinfusion</td>
<td>90±7</td>
<td>89±6</td>
</tr>
<tr>
<td>Heart rate, beats/min, preinfusion</td>
<td>63±3</td>
<td>61±9</td>
</tr>
<tr>
<td>Heart rate, beats/min, postinfusion</td>
<td>60±4</td>
<td>56±9*</td>
</tr>
<tr>
<td>ADMA, µmol/L, preinfusion</td>
<td>0.44±0.09</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td>ADMA, µmol/L, postinfusion</td>
<td>0.46±0.03</td>
<td>15.41±1.39*</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. *P<0.05, comparison between preinfusion and postinfusion values.
respectively, n=4, whereas in subjects who received vehicle, 
\(P_{CO_2}\) before and after the infusion was 41.3±3.1 and 
38.9±2.9 mm Hg, respectively, n=2).

Infusion of ADMA significantly decreased total cerebral perfusion by 15.1±4.5% \((P=0.007)\), whereas blood flow in the 
vehicle group increased by 7.7±2.8% \((P=0.02)\) (Figure 
1). This was also true for local blood flow in the basal 
ganglia, in which cerebral blood flow decreased by 15.5±5.6% 
\((P=0.036)\) in the subjects who received ADMA, whereas 
blood flow in the vehicle group tended to increase by 
4.8±6.3% \((P=NS; \text{Table } 2)\). For this region, there was also 
a trend toward an increase in cerebral blood volume in the 
vehicle group \((16.2±12.3\%; \text{NS})\) and a trend toward a 
decline in cerebral blood volume in the ADMA infusion 
group \((-14.2±19.3\%; \text{NS})\). Neither vehicle nor ADMA 
significantly affected the MTT (Table 2). Typical MR images 
before and after infusion of ADMA are shown in Figures 2 
and 3. Infusion of ADMA significantly increased the AIX 
\((-12.6±1.9 \text{ to } -9.6±1.5; \text{P}=0.0007)\), whereas vehicle did 
not (Figure 4).

### Table 2. Cerebral Blood Volume (CBV), Cerebral Blood Flow 
(CBF), and Mean Transit Time (MTT) in the Basal Ganglia 
Before and After Infusion of ADMA or Vehicle

<table>
<thead>
<tr>
<th></th>
<th>CBV (mL/L 100 g brain/min)</th>
<th>CBF (mL/L 100 g brain/min)</th>
<th>MTT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before vehicle</strong></td>
<td>7.67±2.58</td>
<td>115.19±17.24</td>
<td>3.92±0.98</td>
</tr>
<tr>
<td><strong>After vehicle</strong></td>
<td>8.46±2.02</td>
<td>120.20±7.48</td>
<td>4.21±0.90</td>
</tr>
<tr>
<td><strong>Before ADMA</strong></td>
<td>9.98±4.39</td>
<td>115.89±15.46</td>
<td>5.07±1.79</td>
</tr>
<tr>
<td><strong>After ADMA</strong></td>
<td>8.56±4.94</td>
<td>97.25±14.63*</td>
<td>5.07±2.13</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. 
*\(P<0.05\), comparing preinfusion and postinfusion data.

### Discussion

Our results document for the first time that systemic admin-
istration of exogenous ADMA decreases arterial compliance 
and concurrently decreases cerebral blood flow in healthy 
young men. Notably, these effects were obtained with sub-
pressor doses of ADMA. Accordingly, the changes in arterial 
compliance and cerebral blood flow cannot be attributed to 
changes in systemic arterial pressure.

### ADMA and Arterial Compliance

Arterial stiffness is associated with increased cardiovascular 
disease risk, including stroke, myocardial infarction, heart 
failure, and impaired renal function, and appears to be an 
independent predictor of all-cause and cardiovascular death. 
A large population-based study recently showed that this 
holds true even for apparently healthy subjects. 
Previous studies have indicated that pharmacological antagonists of 
NOS increase arterial stiffness in vivo. However, those 
studies are difficult to interpret because increases in mean

![Figure 1. Total cerebral blood flow (average of the 4 ROIs in each hemisphere) before and after infusion of ADMA or vehicle. Data are presented as mean±SEM. *\(P=0.007\), #\(P=0.02\) comparing preinfusion and postinfusion data.](http://stroke.ahajournals.org/)

![Figure 2. Pre-ADMA dynamic susceptibility contrast MRI in the region of the basal ganglia. A, Source image of caudate head (curved arrow), putamen, and globus pallidus (arrowhead). The pink lines (straight arrow) depict the ROIs where cerebral blood volume, cerebral blood flow, and MTT were measured. Paramet-
ric images of cerebral blood volume (B), cerebral blood flow (C), and MTT (D).](http://stroke.ahajournals.org/)
Arterial pressure may have contributed substantially to the rise in arterial stiffness. An increase in mean arterial pressure could passively distend vessels and thereby indirectly increase arterial stiffness. Accordingly, the previous studies did not show that NOS inhibition could alter the inherent vascular determinants of compliance. In contrast, we were able to document that even a subpressor dose of ADMA increases arterial stiffness. These observations suggest that ADMA has a direct effect on vascular compliance.

Arterial stiffness has been shown to be a good prognostic marker of vascular events in populations with hypertensive and end-stage renal disease, both groups that are known to have markedly elevated levels of ADMA. The most obvious consequences of arterial stiffening are higher systolic blood pressure and lower diastolic blood pressure. These hemodynamic changes increase left ventricular afterload, reduce coronary perfusion, and are associated with left ventricular hypertrophy. In end-stage renal disease patients, plasma ADMA levels are correlated with left ventricular hypertrophy. Chronic inhibition of NOS can cause adverse changes in vascular structure. Suda and coworkers observed that long-term ADMA infusion causes arteriosclerotic lesions in mice in vivo. Based on these data, it seems possible that long-term elevation of ADMA leads to increased arterial stiffness, atherosclerosis, and left ventricular hypertrophy.

**ADMA and Cerebral Blood Flow**

The central nervous system has the highest oxygen consumption and the second highest blood flow of all body organs. Stringent autoregulation of cerebral blood flow is necessary to match the metabolic demand. NO is arguably the most important endogenous vasodilator in regulating the perfusion of the brain, significantly influencing the tone of conductive and resistance arteries as well as venous vessels. A large number of preclinical studies have documented that vascular NO synthesis plays a critical role in cerebral vessel tone and blood flow (for a review, see Iadecola et al). Exogenous ADMA causes concentration- and endothelium-dependent contractions of the human middle cerebral artery. Topical application of ADMA through cranial windows of anesthetized rats constricted the basilar artery. Macrae and coworkers provided evidence that the effect of NOS inhibition is primarily due to a decrease in cerebral blood flow rather than a decrease in metabolic activity. Furthermore, there is an association of ADMA levels in the cerebrospinal fluid and cerebral vasospasm in a primate model of subarachnoid hemorrhage. Accordingly, ADMA may contribute to cerebral vasospasm. Our investigation provides evidence for an intriguing difference between the NOS inhibitors N-n-monomethyl-L-arginine (L-NMMA) and ADMA. Two previous studies examined the effect of the NOS inhibitor L-NMMA on basal global cerebral blood flow in humans. White et al found that high doses of L-NMMA reduced cerebral blood flow in healthy volunteers, but these high doses also increased mean arterial pressure from 84.0±8.2 to 103.6±12.3 mm Hg. In contrast to our study, subpressor doses of L-NMMA had no effect on cerebral blood flow. In another study of healthy volunteers, Kamper et al infused lower doses of L-NMMA, which increased mean arterial

![Figure 3. After ADMA (same parameters as in Figure 2).](image)

![Figure 4. AIx (corrected for heart rate) before and after infusion of ADMA or vehicle. Data are presented as mean±SEM. *P=0.007, comparing preinfusion and postinfusion data.](image)
pressure without any effect on cerebral blood flow. These findings are in contrast to our study, in which a small, subpressor dose of ADMA reduced global cerebral blood flow.

One limitation of our study is that neither arterial blood gas analysis nor end-expiratory CO₂ measurements were performed. Therefore we cannot exclude the possibility that the effect of ADMA on cerebral blood flow is not mediated via changes in Pco₂. However, there is no evidence in the literature that respiration or blood gases are affected by subpressor doses of ADMA.³³ It could be that the effects of ADMA on cerebral blood flow are related to changes in systemic hemodynamics, as described previously.¹⁴ However, previous data indicate that even dynamic exercise, markedly increasing blood pressure, and heart rate did not significantly change cerebral blood flow in healthy subjects.³⁴ Therefore, we suggest that the effect of ADMA on cerebral blood flow is due to a direct effect on the brain vasculature.

Pathophysiological Relevance and Therapeutic Implications
Although the ADMA blood levels in this study were supraphysiological, this does not invalidate the conclusion that ADMA regulates cerebrovascular compliance and flow. Indeed, our work is reminiscent of investigations with angiotensin II, wherein the dose of exogenous angiotensin II needed to exert vasoconstrictor effects was greater than observed in patients with hypertension and/or cardiovascular disease.³⁵ The discrepancies between plasma levels observed in pathophysiological states and the plasma levels required to induce pathophysiological states are likely related to differences in the chronicity of exposure, compartmentalization of the agent, the recruitment of countervailing neurohormonal adjustments, and/or other adaptations.

Is the ADMA/NO System a Potential Therapeutic Target in Cerebrovascular Disease?
Although various pharmacological agents have been shown to reduce plasma ADMA levels, as recently reviewed,³ there is yet no specific drug available that would increase the crucial metabolism of ADMA by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), thereby lowering ADMA. Therefore, current strategies have focused on the use of L-ARGININE, a potent NO donor. It is tempting to speculate that raising the plasma L-ARGININE concentration might attenuate the detrimental effects of ADMA in patients with cerebrovascular disease, as has been shown for atherosclerotic disease (for a review, see Boger and Ron³⁶). Indeed, exogenous nitrosodilators reduced arterial stiffness in humans independently of their effect on blood pressure.³⁷ Moreover, as recently reviewed by Willmott et al.,³⁸ there are several experimental studies in which L-ARGININE and NO donors reduced total cerebral infarct volume in permanent and transient models of ischemia. There is indirect evidence for the relevance of L-ARGININE availability in patients with cerebrovascular disease. In response to an intravenous infusion of L-ARGININE, patients with a history of previous stroke or transient ischemic attack manifested a greater increase in cerebral blood flow than did patients with cardiovascular risk factors but no clinical evidence of cerebrovascular disease.³⁹ In another study, patients undergoing carotid endarterectomy were randomized to an intravenous infusion of L-ARGININE, S-nitrosoglutathione, or vehicle 30 minutes after surgery.⁴⁰ There were highly significant reductions in the number of Doppler embolic signals in the L-ARGININE and S-nitrosoglutathione groups in comparison with vehicle, which persisted for 24 hours after surgery.

In conclusion, our data demonstrate for the first time that ADMA modulates vascular compliance and decreases cerebral blood flow independent of blood pressure changes in humans. ADMA may be involved in the pathogenesis of cerebrovascular disease. Because there is increasing evidence that plasma ADMA levels can be reduced by pharmacotherapy, the clinical significance of this in the context of cerebral vascular disease has to be proven.

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Disclosures
None.

References


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