J Am Soc Nephrol 10: 594–600, 1999

# Asymmetric Dimethylarginine Plasma Concentrations Differ in Patients with End-Stage Renal Disease: Relationship to Treatment Method and Atherosclerotic Disease

JAN T. KIELSTEIN,* RAINER H. BÖGER,† STEFANIE M. BODE-BÖGER,† JÜRGEN SCHÄFFER,* MARK BARBEY,‡ KARL M. KOCH,* and JÜRGEN C. FRÖLICH†

**Department of Nephrology, †Institute of Clinical Pharmacology, and ‡Department of Diagnostic Radiology, Hannover Medical School, Hannover, Germany.*

*Abstract.* Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide (NO) synthase. Its concentration is elevated in patients with end-stage renal disease (ESRD), in part because it is excreted via the kidneys. In this study, the plasma concentrations of ADMA, symmetric dimethylarginine, and L-arginine were determined in relation to plasma nitrate levels (as an index of NO formation) for a group of 80 patients with ESRD. The effects of two treatment methods, *i.e.*, hemodialysis (HD) and peritoneal dialysis (PD), and the role of the presence of atherosclerotic disease were evaluated. Forty-three patients receiving HD and 37 patients receiving PD were compared with healthy control subjects. Plasma L-arginine and dimethylarginine levels were determined by HPLC, using precolumn derivatization with *o*-phthaldialdehyde. Plasma nitrate levels were determined by gas chromatography-mass spectrometry. Predialysis ADMA concentrations in HD-treated patients were approximately sixfold higher than those in the control group (6.0 ± 0.5 *versus* 1.0 ± 0.1 μmol/L; $P < 0.05$). Plasma nitrate concentrations were significantly lower in HD-treated patients, which suggests that ADMA may inhibit NO synthase. In contrast, plasma ADMA levels and nitrate concentrations in PD-treated patients were similar to those in control subjects. Plasma L-arginine concentrations were not significantly decreased in patients with ESRD. ADMA concentrations were significantly decreased 5 h after HD, compared with baseline values. ADMA levels were significantly higher in HD-treated patients with manifest atherosclerotic disease than in HD-treated patients without atherosclerotic disease (7.31 ± 0.70 *versus* 3.95 ± 0.52 μmol/L; $P < 0.05$). This study confirms that ADMA is accumulated in ESRD. PD-treated patients exhibit significantly lower ADMA levels than do HD-treated patients. Accumulation of ADMA may be a risk factor for the development of endothelial dysfunction and cardiovascular disease in patients with ESRD.

Endothelium-derived nitric oxide (NO) plays an important role in the regulation of BP and platelet aggregation. It is synthesized by stereospecific oxidation of the terminal guanidino nitrogen of the amino acid L-arginine (1). NO is produced by the action of a family of NO synthases, with endothelial, neuronal, and macrophage isoforms (2).

The synthesis of NO can be selectively inhibited by guanidino-substituted analogs of L-arginine, such as *N*-monomethyl-L-arginine, which act as competitive antagonists at the active site of the enzyme (3). Asymmetric dimethylarginine (ADMA) has been recently characterized as an endogenous inhibitor of NO synthase (4). It is synthesized and metabolized by human endothelial cells (5). ADMA and its biologically inactive stereoisomer symmetric dimethylarginine (SDMA) are at least in part eliminated via urinary excretion (6). Vallance *et al.* (7) were the first to report elevated plasma levels of ADMA and SDMA in a small group of patients with end-stage renal disease (ESRD). In their study, dimethylarginine (DMA) levels were elevated approximately sixfold, compared with those for healthy control subjects. Those authors suggested that the high incidence of conditions such as hypertension, atherosclerosis, and immune dysfunction among patients with ESRD might be caused at least in part by dysfunction of the L-arginine/NO pathway secondary to accumulation of ADMA (7).

Data from several experimental studies suggest that ADMA concentrations in a pathophysiologically high range (3 to 15 μmol/L) significantly inhibit vascular NO formation (8,9). Moreover, it was recently found by our group that plasma ADMA levels are also elevated in atherosclerotic patients and in hypercholesteremic subjects with normal renal function, suggesting that mechanisms other than decreased renal elimination may contribute to the elevation of plasma ADMA levels in hypercholesterolemia and atherosclerosis (10). It is therefore unclear whether elevated ADMA concentrations in patients

Received July 7, 1998. Accepted September 17, 1998.
Portions of this work were presented orally at the 35th Annual Meeting of the European Dialysis and Transplantation Association (June 6 to 9, 1998, Rimini, Italy).
Correspondence to Dr. Rainer H. Böger, Institute of Clinical Pharmacology, Medical School Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. Phone: 49 511 532 4631; Fax: 49 511 532 5199; E-mail: boeger.rainer@mh-hannover.de
1046-6673/1003-0594$03.00/0
Journal of the American Society of Nephrology

with ESRD are a cause or a consequence of accompanying cardiovascular disease.

The aim of this study was to determine the plasma concentrations of ADMA, SDMA, and L-arginine and their relationship to NO formation (measured as plasma nitrate concentrations) in patients with ESRD, compared with age-matched control subjects with normal renal excretory function. The effects of two treatment methods, *i.e.*, hemodialysis (HD) and peritoneal dialysis (PD), on these parameters were also evaluated.

## Materials and Methods

### *Patients and Study Design*

Four groups of individuals were included in this study, after they had given informed consent for participation. The first group consisted of 43 patients receiving HD (20 female and 23 male patients; mean age, 64.9 ± 1.6 yr) who had been treated with HD for a median of 38 mo (range, 1 to 200 mo), with residual diuresis of 582 ± 95 ml of urine/24 h. Sixteen of the 43 (37%) HD-treated patients were anuric. The average diuresis among the remaining 27 patients was 776 ± 116 ml/24 h.

For these patients, a venous blood sample was drawn at midweek before dialysis. Patients were treated with HD three times each week and were in clinically stable conditions. The mean serum creatinine level was 707 ± 108 μmol/L, and the mean arterial BP was 141.0 ± 3.4/78 ± 1.7 mmHg. Average serum cholesterol and triglyceride levels were 5.29 ± 0.17 and 2.09 ± 0.16 mmol/L, respectively. The body mass index averaged 23.6 ± 0.6, and the mean serum albumin level was 37.6 ± 1.1 g/L. Patients underwent standard bicarbonate HD with biocompatible membranes (Hemophan® [Gambro Medizintechnik, Munich, Germany] and polyamide membranes, sterilized with steam). The average dialysis time was 270 ± 6 min, blood flow was 290 ± 8 ml/min, and dialysate flow was 500 ml/min. The delivered dialysis dose measured by urea reduction rate was 67.2 ± 1.1%, well above the suggested dialysis dose (by urea reduction rate) of 65% (11).

Subgroup analysis was performed for patients with ESRD with atherosclerotic vascular disease ($n = 22$), which was defined as the presence of clinically and angiographically or duplex-sonographically verified peripheral arterial occlusive disease (Fontaine stage IIb to IV) alone ($n = 2$) or in combination with a history of prior myocardial infarction ($n = 20$), compared with patients with ESRD without vascular disease ($n = 11$). For a subgroup of eight HD-treated patients, additional heparinized blood samples for the analysis of ADMA, SDMA, L-arginine, and nitrate were drawn 1, 5, and 18 h after the end of a dialysis session (duration, 4.5 h). These patients (five female and three male patients; mean age, 63.1 ± 4.5 yr) had been receiving HD for a median of 18 mo (range, 1 to 82 mo). The mean serum creatinine concentration was 760 ± 49 μmol/L, and the mean arterial BP was 133.8 ± 9.7/81.3 ± 3.7 mmHg. Average serum cholesterol and triglyceride levels were 5.33 ± 0.50 and 2.44 ± 0.42 mmol/L, respectively.

The second group consisted of 37 patients (16 female and 21 male patients) receiving PD (24 patients undergoing nightly intermittent PD, nine continuous ambulatory PD, three continuous cyclic PD, and one intermittent PD; mean age, 46.4 ± 2.5 yr), who had received PD for a median of 55 mo (range, 7 to 168 mo). These patients exhibited residual diuresis of 658 ± 129 ml of urine/24 h. Thirteen of the 37 (35%) patients receiving PD were anuric. The average diuresis among the remaining 24 patients was 1015 ± 151 ml/24 h ($P$ = not significant *versus* HD). The weekly $Kt/V_{urea}$ value for the patients receiving PD was 2.13 ± 0.09, well above the recommended weekly $Kt/V_{urea}$ value of 2.0 (12).

The mean serum creatinine concentration was 922 ± 47 μmol/L, and the mean BP was 137.2 ± 2.8/83.8 ± 1.6 mmHg. Average serum cholesterol and triglyceride levels were 6.16 ± 0.24 and 2.80 ± 0.26 mmol/L, respectively. The body mass index averaged 23.2 ± 0.6, and the mean serum albumin concentration was 36.7 ± 0.8 g/L. Heparinized blood samples from fasting subjects were obtained during routine morning visits in the PD clinic.

The control group consisted of 37 elderly, healthy, normotensive subjects with no apparent disease (13 female and 24 male patients; mean age, 68.3 ± 1.1 yr), who exhibited mean serum creatinine concentrations of 79.3 ± 3.5 μmol/L. Average cholesterol and triglyceride levels were 4.86 ± 0.11 and 1.61 ± 0.12 mmol/L, respectively. The presence of cardiovascular or metabolic disease was excluded by medical histories, physical examinations, and routine laboratory tests.

An additional control group consisted of 33 patients with manifest arteriosclerotic disease and normal renal function. These patients (13 female and 20 male patients; mean age, 61.4 ± 4.0 yr) had peripheral arterial occlusive disease, as verified by angiography or duplex-sonography. The serum creatinine concentration in this group was 96.0 ± 4.4 μmol/L; average cholesterol and triglyceride levels were 6.24 ± 0.22 and 1.81 ± 0.14 mmol/L, respectively, and BP was 152.1 ± 3.2/79.4 ± 2.0 mmHg.

### *Biochemical Analyses*

The plasma concentrations of L-arginine, $N^G$,$N^G$-DMA (ADMA), and $N^G$,$N'^G$-DMA (SDMA) were measured by HPLC with precolumn derivatization with *o*-phthaldialdehyde (OPA), using a modification of a previously published method (13). L-Homoarginine (10 μmol/L) was added to 0.5 ml of plasma as an internal standard. Plasma samples and standards were extracted on solid-phase extraction cartridges (CBA Bond Elut; Varian, Harbor City, CA). The recovery rates were 82.9 ± 3.8%. Eluates were dried under nitrogen and resuspended in double-distilled water for HPLC analysis. Samples and standards were incubated with OPA reagent (5.4 mg/ml OPA in borate buffer, pH 8.4, containing 0.4% 2-mercaptoethanol) for exactly 30 s before automatic injection into the HPLC system. The OPA derivatives of L-arginine, ADMA, and SDMA were separated on a 250- × 4.5-mm (inner diameter), 7-μm, Nucleosil phenyl column (Macherey and Nagel, Düren, Germany), with the fluorescence detector set for an excitation wavelength of 340 nm and an emission wavelength of 450 nm. Samples were eluted from the column with 0.96% citric acid/methanol (2:1, pH 6.8), at a flow rate of 1 ml/min. Standard curves generated for ADMA and SDMA in water and in pooled human plasma showed linearity over a concentration range from 0.1 to 16 μmol/L. The coefficients of variation of this method were 5.2% within assays and 5.5% between assays; the detection limit was 0.1 μmol/L.

The ADMA/creatinine ratio was calculated, as ADMA (micromolar)/creatinine (micromolar) × $10^{-3}$, for patients for whom multiple samples were drawn before and after HD sessions, to assess the different effects of HD treatment on methylarginine levels, as opposed to serum creatinine levels. Plasma nitrate was assayed as its pentafluorobenzyl derivative by gas chromatography-mass spectrometry, as described previously (14). The detection limit of the method was 20 fmol of nitrite or nitrate. Intra- and interassay variabilities were <3.8%. Serum creatinine concentrations were determined spectrophotometrically with the alkaline picric acid method, in an automatic analyzer (Beckman, Galway, Ireland). Protein concentrations were determined spectrophotometrically using the biuret method. All other

laboratory data were obtained from routine laboratory tests, using certified assay methods.

### *Statistical Analyses*

Data are presented as mean ± SEM unless otherwise stated. Statistical significance was tested using ANOVA, followed by the Fisher protected least significant difference test for comparisons between patient groups. Repeated measurements were tested for statistical significance using ANOVA and the Scheffé *F* test. Statistical significance was accepted for $P < 0.05$.

## Results

### *Plasma L-Arginine, ADMA, SDMA, and Nitrate Concentrations in ESRD*

Compared with control subjects, HD-treated patients exhibited significantly higher plasma ADMA concentrations ($P < 0.05$) (Figure 1). Plasma SDMA levels were also significantly higher, but L-arginine concentrations were not significantly different between the two groups (Table 1). Plasma nitrate concentrations were significantly lower in HD-treated patients than in control subjects (Table 1).

ADMA levels in PD-treated patients were not different from those in control subjects ($P > 0.05$) (Figure 1). However, SDMA concentrations in the PD-treated group were significantly higher than those in control subjects ($P < 0.05$) (Table 1). L-Arginine and nitrate concentrations and the L-arginine/ADMA ratio were not significantly different, compared with those for the control group ($P > 0.05$) (Table 1).

HD-treated patients exhibited significantly higher mean ADMA concentrations, compared with PD-treated patients. SDMA, L-arginine, and nitrate concentrations did not differ significantly between these groups. The L-arginine/ADMA ratio in the HD-treated patients was significantly lower than that in the PD-treated patients (Table 1).

### *Effects of HD on the Time Course of Plasma L-Arginine, DMA, and Nitrate Levels*

For eight HD-treated patients, the changes in the plasma levels of L-arginine, DMA, and nitrate were assessed before and after one HD session. One hour after the end of dialysis, no change in plasma ADMA and SDMA concentrations was observed. When the concentrations of these L-arginine analogs were expressed with respect to serum creatinine concentrations, there was a significant elevation 1 h after dialysis, compared with predialysis concentrations (ADMA, 0.86 ± 0.20 *versus* 2.02 ± 0.33; $P < 0.05$). Five hours after the end of the HD session, ADMA concentrations were significantly decreased, below values measured 1 h after dialysis. ADMA levels then continuously increased until 18 h after dialysis (Figure 2). A similar time course was observed for SDMA levels (Table 2). However, it seemed that the reduction in plasma SDMA levels at 5 h after dialysis was not as marked as that for ADMA. Plasma L-arginine concentrations remained unchanged after HD. Plasma nitrate levels and total protein concentrations did not change significantly.

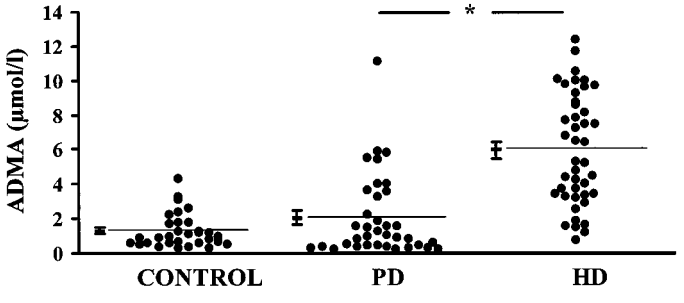


*Figure 1.* Plasma asymmetric dimethylarginine (ADMA) concentrations in healthy control subjects (1.0 ± 0.1 µmol/L, $n = 37$), peritoneal dialysis (PD)-treated patients (2.1 ± 0.4 µmol/L, $n = 37$), and hemodialysis (HD)-treated patients (6.0 ± 0.5 µmol/L, $n = 43$). Each point represents one individual. Horizontal bars indicate the mean ± SEM. *$P < 0.05$ by ANOVA.

*Table 1.* ADMA, SDMA, L-arginine, L-arginine/ADMA ratio, and nitrate concentration as determinants of nitric oxide synthesis in control subjects (control) compared with PD and HD patients

| Agent | Control ($n = 37$) | PD ($n = 37$) | HD ($n = 43$) |
|---|---|---|---|
| SDMA | 0.8 ± 0.1 | 5.1 ± 0.6[b] | 5.2 ± 0.4[b] |
| ADMA/SDMA | 1.31 ± 0.21 | 0.72 ± 0.11[b] | 1.21 ± 0.09[c] |
| L-arginine | 75.5 ± 3.9 | 84.0 ± 9.3 | 75.9 ± 7.2 |
| L-arg/ADMA | 79.2 ± 5.0 | 68.9 ± 11.3 | 13.7 ± 1.4[b,c] |
| Nitrate | 39.1 ± 1.9 | 36.0 ± 1.5 | 23.9 ± 1.7[b,c] |

[a] Data are mean ± SEM. Results are given as µmol/L. ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; PD, peritoneal dialysis; HD, hemodialysis.
[b] $P < 0.05$ *versus* control.
[c] $P < 0.05$ *versus* PD.

### *ADMA Concentrations with Respect to Renal Function and Atherosclerosis*

HD-treated patients with atherosclerosis exhibited significantly higher ADMA levels than did HD-treated patients without atherosclerosis (7.31 ± 0.70 µmol/L [$n = 22$] *versus* 3.95 ± 0.52 µmol/L [$n = 11$]; $P < 0.05$) (Figure 3). Similarly, patients with peripheral arterial occlusive disease with normal renal function exhibited significantly higher ADMA levels than did healthy control subjects (3.23 ± 0.33 µmol/L [$n = 33$] *versus* 1.01 ± 0.05 µmol/L [$n = 37$]; $P < 0.05$) (Figure 3). There was no significant difference in ADMA levels between HD-treated patients without atherosclerosis and patients with peripheral arterial occlusive disease with normal renal function. A similar pattern of SDMA concentrations was observed (Table 3). Plasma L-arginine concentrations did not differ among control patients, patients with peripheral arterial occlusive disease, and patients with ESRD with atherosclerosis. However, patients with ESRD without atherosclerosis exhibited lower plasma L-arginine concentrations than did patients with ESRD with atherosclerotic disease.

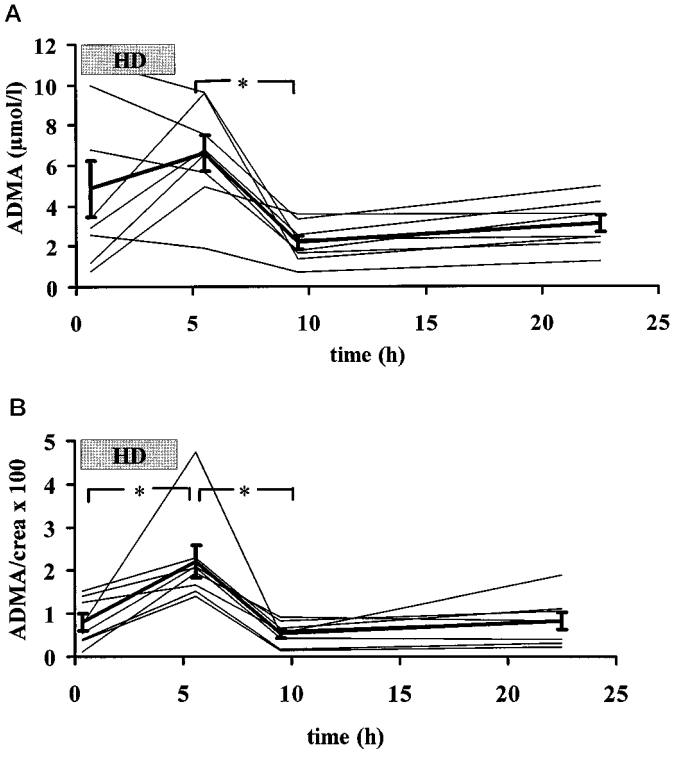


*Figure 2.* (A) Time course of plasma ADMA concentrations in eight HD-treated patients before and 1, 5, and 18 h after a 4.5-h dialysis session. Each line represents the time course of ADMA concentrations in one subject. The thick line indicates the mean ± SEM. *$P < 0.05$ by ANOVA. (B) Time course of plasma ADMA/creatinine ratios in eight HD-treated patients before and 1, 5, and 18 h after a 4.5-h dialysis session. Each line represents the time course of ADMA/creatinine ratios in one subject. The thick line indicates the mean ± SEM. *$P < 0.05$ by ANOVA.

## Discussion

The major findings of this study are that: (*1*) ADMA and SDMA are accumulated in patients with ESRD; (*2*) ADMA levels are significantly lower in patients with ESRD treated with PD than in those treated with HD; (*3*) elevated ADMA levels are accompanied by low plasma levels of nitrate, the oxidative metabolite of NO; and (*4*) regardless of renal function, patients with atherosclerosis have higher plasma levels of ADMA than those without atherosclerosis.

### *Accumulation of ADMA in ESRD*

Our finding that DMA levels are significantly higher in patients with ESRD than in healthy control subjects is consistent with the first description by Vallance *et al.* (7), who studied nine HD-treated patients. They reported a mean total DMA level of 8.7 ± 0.7 µmol/L. This concentration was six times higher than that measured for control subjects (1.2 ± 0.1 µmol/L) (7). In other studies, lower concentrations of DMA were reported. MacAllister *et al.* (15) found elevated ADMA and SDMA levels (0.9 ± 0.1 and 3.8 ± 0.4 µmol/L, respectively) in six HD-treated patients. Anderstam *et al.* (16) reported ADMA levels of 0.6 ± 0.2 µmol/L in 19 patients, and no difference between PD and HD. However, in these studies ADMA and SDMA levels in control subjects were also very low. The relative increase in DMA levels was 2.5- to sixfold in all of these studies. Comparing two treatment methods for patients with ESRD, *i.e.*, HD and PD, we found that PD-treated patients exhibited lower plasma ADMA concentrations than did HD-treated patients. This difference may be caused by differences in dialytic clearance of ADMA with the two treatment methods or the metabolism of ADMA.

### *Metabolism of ADMA*

The origin of ADMA in human plasma is currently unclear. Animal studies suggest that the relatively low levels of ADMA and SDMA present in plasma from healthy rabbits are derived mainly from the degradation of methylated proteins (6). Different methyltransferases seem to be responsible for L-arginine methylation in various tissues; the enzyme present in the vasculature mainly yields ADMA, as judged by the observation that cultured human endothelial cells release more ADMA than SDMA (17,18).

Urinary excretion is the main elimination route for SDMA in rabbits, whereas $N^G$-monomethyl-L-arginine and ADMA are partly eliminated by other metabolic pathways (6). In rats, Ogawa and coworkers (19) demonstrated that $^{14}$C-labeled ADMA is metabolized to citrulline by the enzyme DMA dimethylaminohydrolase (DDAH), which is present in various tissues of rats and human subjects (5,20,21). These tissues include the kidneys, the vasculature, and other tissues in which this enzyme is colocalized with isoforms of NO synthase and may affect NO-mediated cell function (20,22). DDAH presence and activity have also been demonstrated in human en-

*Table 2.* Time course of plasma L-arginine, SDMA, SDMA/creatinine ratio as determinants of nitric oxide synthesis in 10 HD patients before, 1, 5 and 18 h after a 4.5-h dialysis session[a]

| Agent | Pre-HD ($n$ = 8) | 1 h Post-HD ($n$ = 8) | 5 h Post-HD ($n$ = 8) | 18 h Post-HD ($n$ = 8) |
|---|---|---|---|---|
| L-arginine (µmol/L) | 69.44 ± 9.55 | 67.32 ± 12.78 | 51.50 ± 7.11 | 62.55 ± 7.60 |
| SDMA (µmol/L) | 3.97 ± 0.85 | 6.25 ± 0.63 | 4.55 ± 0.56 | 5.72 ± 0.62 |
| SDMA/creatinine ($\times 10^{-3}$) | 0.60 ± 0.14 | 1.70 ± 0.26 | 0.99 ± 0.11 | 1.14 ± 0.13 |
| Nitrate (µmol/L) | 32.41 ± 2.68 | 40.51 ± 5.48 | 39.89 ± 4.26 | 35.61 ± 2.56 |
| Protein (mg/ml) | 64.08 ± 1.74 | 69.2 ± 1.91 | 69.96 ± 3.29 | 64.99 ± 2.59 |

[a] Data are mean ± SEM. Abbreviations as in Table 1.

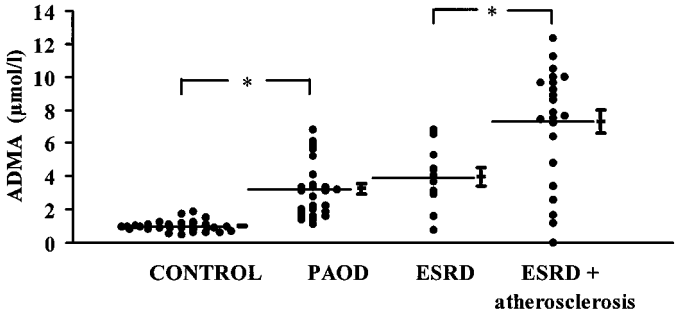


*Figure 3.* Plasma ADMA concentrations in healthy control subjects, patients with peripheral arterial occlusive disease (PAOD), and patients with end-stage renal disease (ESRD) treated with HD, without or with concomitant atherosclerosis. Each point represents one individual. Horizontal bars indicate the mean ± SEM. *$P < 0.05$ by ANOVA.

dothelial cells (5,23). In isolated rat aortic rings studied *ex vivo*, inhibition of DDAH activity causes vasoconstriction, which is reversed by L-arginine (23). Differences in the tissue origin of ADMA and SDMA and differences in the metabolism of these compounds may have contributed to the differences in plasma ADMA and SDMA levels in this study. Further investigation will be needed to identify the influence of ESRD and different treatment regimens on the metabolism of DMA.

### *Influence of HD on ADMA Levels*

The data presented here showed that 1 h after HD, there were slight increases in plasma ADMA and SDMA concentrations, which were significant when the concentrations of DMA forms were expressed with respect to serum creatinine concentrations. This suggests that dialytic clearance for creatinine is greater than for ADMA and SDMA. Part of the increase could also be the result of redistribution of tissue DMA into the plasma compartment during HD. Hemoconcentration did not play a role in this increase, because there was no significant change in total protein levels during HD. At 5 h after the HD session, plasma ADMA levels were decreased by 65%, compared with 1 h after dialysis; levels slowly increased again until 18 h after HD. This slow increase is probably attributable to accumulation of newly synthesized DMA released into plasma. Our data confirm the decrease in plasma ADMA concentrations of approximately 40% that was previously reported by Vallance *et al.* (7) and by Anderstam *et al.* (16). MacAllister *et al.* (15) found a smaller (approximately 20%) decrease in plasma ADMA levels after HD. However, none of those authors reported the exact time point of blood withdrawal, with respect to the end of dialysis session. The data presented here show that the timing of blood withdrawal is very important for assessing the effects of HD on DMA levels.

### *Effects of ADMA on NO Elaboration*

Several studies performed *in vitro* suggest that ADMA, at concentrations between 1 and 10 µmol/L, inhibits NO elaboration by NO synthase in the presence of L-arginine in isolated blood vessels, in cultured macrophages, and in cultured endothelial cells (8,9,17,18). Because intracellular ADMA levels have been shown to be approximately one order of magnitude greater than the levels in conditioned cell culture media, the accumulation of ADMA observed in patients with ESRD may be sufficient to cause clinically relevant inhibition of endothelial NO elaboration (17).

In 1992, Vallance *et al.* (7) hypothesized that inhibition of vascular NO formation caused by accumulated ADMA might be responsible for cardiovascular disorders such as hypertension and atherosclerosis, which are frequently observed in ESRD. Since then, the presence of dysfunctional endothelium-dependent vasodilation in this disease has been confirmed in several clinical investigations: Joannides *et al.* (24) showed that flow-induced, NO-dependent forearm vasodilation was impaired in adult patients receiving HD. Kari *et al.* (25) found impaired flow-induced, NO-dependent vasodilation in children with chronic renal failure. This was associated with elevated ADMA levels and reduced levels of nitrosothiols in plasma. Moreover, Hand *et al.* (26) demonstrated that the defect of endothelium-dependent vasodilation that was present in patients with ESRD before HD was reversed after HD sessions. Furthermore, L-arginine, but not D-arginine, restored endothelial function independently of HD. This study strongly supports the hypothesis that levels of ADMA present in the plasma of patients with ESRD induce clinically relevant inhibition of endothelial NO synthase activity, because competitive inhibition of NO synthase by ADMA can be overcome by excess L-arginine (17,27).

In this study we measured the plasma concentration of nitrate, a final oxidative metabolite of NO. Nitrate levels were significantly lower at baseline in HD-treated patients than in matched healthy control subjects. After HD, nitrate levels transiently increased and finally returned to baseline. These data are in accordance with studies performed by other investigators who also found evidence for reduced basal NO production in patients with ESRD (28) and increased NO forma-

*Table 3.* L-arginine and SDMA plasma concentrations in healthy controls, PAOD patients, and ESRD patients maintained on hemodialysis without or with concomitant atherosclerosis[a]

| Agent | Control ($n = 37$) | PAOD ($n = 33$) | ESRD ($n = 11$) | ESRD + Atheroclerosiss ($n = 22$) |
|---|---|---|---|---|
| L-arginine | 75.5 ± 3.9 | 78.6 ± 6.4 | 51.9 ± 8.6 | 98.9 ± 11.3 |
| SDMA | 0.8 ± 0.1 | 2.4 ± 0.5 | 3.8 ± 0.4 | 6.1 ± 0.5 |

[a] Data are mean ± SEM. Results are given as µmol/L. PAOD, peripheral arterial occlusive disease; ESRD, end-stage renal disease.

tion during HD (29,30). Using stable-isotope techniques, Rhodes *et al.* (31) recently found that in human subjects the major portion of circulating nitrite/nitrate is derived directly from the L-arginine/NO pathway. Although we cannot exclude the possibility that differences in dietary nitrate intake accounted at least in part for differences in plasma nitrate levels between patient groups in this study, accumulated evidence from these studies suggests that NO production is altered in ESRD. This is further supported by the recent finding of impaired endothelium-dependent, NO-mediated vasodilation in patients undergoing dialysis (24–26). Increased ADMA levels may be one important factor determining endothelial function in ESRD.

### *Consequences of Reduced NO Elaboration in ESRD*

Aside from volume changes during HD, acute alterations in NO formation during HD may contribute to peridialytic BP instability in HD-treated patients (32–34). Changes in ADMA-induced inhibition of endothelial NO production may contribute to this phenomenon. In the long term, chronic reduction of NO synthase activity secondary to accumulation of ADMA may contribute to the pathogenesis of hypertension and atherosclerosis. Chronic inhibition of NO synthesis has been shown to produce hypertension (35) and to accelerate atherosclerosis in animal models (13,36,37). Chronically elevated ADMA concentrations may induce similar proatherogenic effects, like those observed in these experimental models (17).

Our group previously reported that plasma ADMA concentrations were elevated two- to threefold in patients with generalized atherosclerosis and peripheral arterial occlusive disease (10). This increase, which was associated with reduced urinary excretion of nitrate and cGMP, was independent of the presence of impaired renal excretory function. Moreover, we found a twofold elevation of ADMA levels in the plasma of asymptomatic young hypercholesterolemic subjects with normal renal function (38). We therefore investigated, in this study, whether the presence of atherosclerosis was also associated with increased ADMA levels in patients with ESRD. We found a similar relative elevation of ADMA levels in patients with ESRD with atherosclerosis, compared with patients with ESRD without atherosclerosis, and in patients with peripheral arterial occlusive disease with normal renal function, compared with control subjects. From these data it remains unresolved whether patients with ESRD with atherosclerosis exhibited higher ADMA levels because of the presence of atherosclerosis or whether they suffered from atherosclerosis as a consequence of higher ADMA levels. However, the elevation of ADMA levels in patients with peripheral arterial occlusive disease with normal renal function, compared with control subjects, suggests that mechanisms other than reduced renal excretion may also contribute to the greater accumulation of ADMA in patients with ESRD with peripheral arterial occlusive disease, compared with patients with ESRD without atherosclerosis. The concept of accelerated atherosclerosis in patients chronically undergoing dialysis has been widely accepted since it was first published (39). However, in a recent review, London and Drüeke (40) argued that the high mortality rates resulting from cardiovascular disease are not proof of accelerated atherosclerosis in patients undergoing maintenance dialysis. Many patients undergoing dialysis have more or less marked vascular lesions at the start of dialysis treatment, and the risk factors present in the predialysis phase may be of primary importance for the manifestation of cardiovascular disease. Multiple known cardiovascular risk factors have been shown to be present in patients with ESRD (41–44). Elevated ADMA levels may be a newly identified, preexistent risk factor for atherosclerosis.

In conclusion, we have demonstrated that ADMA is accumulated during chronic renal failure. Different dialysis treatment strategies affect ADMA levels differently. The presence of atherosclerosis is associated with higher ADMA levels in patients with normal renal function, as well as in patients undergoing dialysis, but this phenomenon may be unrelated to renal handling of ADMA. Reduced NO elaboration secondary to accumulation of ADMA may be an important pathogenic factor for atherosclerosis in chronic renal failure.

## Acknowledgments

The authors gratefully acknowledge the excellent technical assistance of T. Suchy, F.-M. Gutzki, and B. Schubert. The authors are grateful to their colleagues Drs. Hilfenhaus, Jonassen, Lorenzen, Lüth, Riechers, and Zaunbauer for help in collecting the clinical data from the patients.

## References

1. Moncada S, Higgs A: The L-arginine-nitric oxide pathway. *N Engl J Med* 329: 2002–2012, 1993
2. Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H: Nitric oxide synthase isozymes: Characterization, purification, molecular cloning, and functions. *Hypertension (Dallas)* 23: 1121–1131, 1994
3. Rees DD, Palmer RM, Hodson HF, Moncada S: A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br J Pharmacol* 96: 418–424, 1989
4. Vallance P, Leone A, Calver A, Collier J, Moncada S: Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 20[Suppl 12]: S60–S62, 1992
5. MacAllister RJ, Fickling SA, Whitley GS, Vallance P: Metabolism of methylarginines by human vasculature: Implications for the regulation of nitric oxide synthesis. *Br J Pharmacol* 112: 43–48, 1994
6. McDermott JR: Studies on the catabolism of $N^G$-methylarginine, $N^G$,$N^G$-dimethylarginine and $N^G$,$N^G$-dimethylarginine in the rabbit. *Biochem J* 154: 179–184, 1976
7. Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339: 572–575, 1992
8. Kurose I, Wolf R, Grisham MB, Granger DN: Effects of an endogenous inhibitor of nitric oxide synthesis on postcapillary venules. *Am J Physiol* 268: H2224–H2231, 1995
9. Faraci FM, Brian JEJ, Heistad DD: Response of cerebral blood vessels to an endogenous inhibitor of nitric oxide synthase. *Am J Physiol* 269: H1522–H1527, 1995
10. Böger RH, Bode-Böger SM, Thiele W, Junker W, Alexander K, Frölich JC: Biochemical evidence for impaired nitric oxide syn-

thesis in patients with peripheral arterial occlusive disease. *Circulation* 95: 2068–2074, 1997
11. National Kidney Foundation DOQI: *Clinical Practice Guidelines for Hemodialysis Adequacy*, New York, National Kidney Foundation, 1997, pp 42–46
12. National Kidney Foundation DOQI: *Clinical Practice Guidelines for Peritoneal Dialysis Adequacy*, New York, National Kidney Foundation, 1997, pp 46–47
13. Bode-Böger SM, Böger RH, Kienke S, Junker W, Frölich JC: Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. *Biochem Biophys Res Commun* 219: 598–603, 1996
14. Tsikas D, Böger RH, Bode-Böger SM, Gutzki FM, Frölich JC: Quantification of nitrite and nitrate in human urine and plasma as pentafluorobenzyl derivatives by gas chromatography-mass spectrometry using their $^{15}N$-labelled analogs. *J Chromatogr B Biomed Appl* 661: 185–191, 1994
15. MacAllister RJ, Rambausek MH, Vallance P, Williams D, Hoffmann KH, Ritz E: Concentration of dimethyl-L-arginine in the plasma of patients with end- stage renal failure. *Nephrol Dial Transplant* 11: 2449–2452, 1996
16. Anderstam B, Katzarski K, Bergstrom J: Serum levels of $N^G$,$N^G$-dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. *J Am Soc Nephrol* 8: 1437–1442, 1997
17. Böger RH, Bode-Böger SM, Tsao PS, Lin Chan JR, Cooke JP: The endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) exerts pro-atherosclerotic effects in cultured human endothelial cells [Abstract]. *Circulation* 96: I-1588, 1997
18. Fickling SA, Leone A, Nussey SS, Vallance P, Whitley G: Synthesis of $N^G$,$N^G$-dimethylarginine by human endothelial cells. *Endothelium* 1: 137, 1998
19. Ogawa T, Kimoto M, Watanabe H, Sasaoka K: Metabolism of $N^G$,$N^G$- and $N^G$,$N'^G$-dimethylarginine in rats. *Arch Biochem Biophys* 252: 526, 1987
20. Kimoto M, Tsuji H, Ogawa T, Sasaoka K: Detection of $N^G$,$N^G$-dimethylarginine dimethylaminohydrolase in the nitric oxide-generating systems of rats using monoclonal antibody. *Arch Biochem Biophys* 300: 657–662, 1993
21. Kimoto M, Whitley GS, Tsuji H, Ogawa T: Detection of $N^G$,$N^G$-dimethylarginine dimethylaminohydrolase in human tissues using a monoclonal antibody. *J Biochem (Tokyo)* 117: 237–238, 1995
22. Tojo A, Welch WJ, Bremer V, Kimoto M, Kimura K, Omata M, Ogawa T, Vallance P, Wilcox CS: Colocalization of demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. *Kidney Int* 52: 1593–1601, 1997
23. MacAllister RJ, Parry H, Kimoto M, Ogawa T, Russell RJ, Hodson H, Whitley GS, Vallance P: Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. *Br J Pharmacol* 119: 1533–1540, 1996
24. Joannides R, Bakkali EH, Le RF, Rivault O, Godin M, Moore N, Fillastre JP, Thuillez C: Altered flow-dependent vasodilatation of conduit arteries in maintenance haemodialysis. *Nephrol Dial Transplant* 12: 2623–2628, 1997
25. Kari JA, Donald AE, Vallance DT, Bruckdorfer KR, Leone A, Mullen MJ, Bunce T, Dorado B, Deanfield JE, Rees L: Physiology and biochemistry of endothelial function in children with chronic renal failure. *Kidney Int* 52: 468–472, 1997
26. Hand MF, Haynes WG, Webb DJ: Hemodialysis and L-arginine, but not D-arginine, correct renal failure-associated endothelial dysfunction. *Kidney Int* 53: 1068–1077, 1998
27. Böger RH, Bode-Böger SM, Brandes RP, Phivthong-ngam L, Böhme M, Nafe R, Mügge A, Frölich JC: Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: Comparison with lovastatin. *Circulation* 96: 1282–1290, 1997
28. Blum M, Yachnin T, Wollman Y, Chernihovsky T, Peer G, Grosskopf I, Kaplan E, Silverberg D, Cabili S, Iania A: Low nitric oxide production in patients with chronic renal failure. *Nephron* 79: 265–268, 1998
29. Rysz J, Luciak M, Kedziora J, Blaszczyk J, Sibinska E: Nitric oxide release in the peripheral blood during hemodialysis. *Kidney Int* 51: 294–300, 1997
30. Yokokawa K, Mankus R, Saklayen MG, Kohno M, Yasunari K, Minami M, Kano H, Horio T, Takeda T, Mandel AK: Increased nitric oxide production in patients with hypotension during hemodialysis. *Ann Intern Med* 123: 35–37, 1995
31. Rhodes P, Leone A, Francis P, Struthers A, Moncada S: The L-arginine:nitric oxide pathway is the major source of plasma nitrite in fasted humans. *Biochem Biophys Res Commun* 209: 590–596, 1995
32. Maiorca R: Cardiovascular problems in the choice of dialysis therapy for the elderly. *Contrib Nephrol* 106: 74–83, 1994
33. Maiorca R, Cancarini GC, Zubani R, Movilli E, Brunori G: Differing dialysis treatment strategies and outcome [Review]. *Nephrol Dial Transplant* 11[Suppl 2]: 134–139, 1996
34. Maiorca R, Cancarini GC, Brunori G, Zubani R, Camerini C, Manili L, Movilli E: Comparison of long-term survival between hemodialysis and peritoneal dialysis [Review]. *Adv Perit Dial* 12: 79–88, 1996
35. Baylis C, Mitruka B, Deng A: Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* 90: 278–281, 1992
36. Cayatte AJ, Palacino JJ, Horten K, Cohen RA: Chronic inhibition of nitric oxide production accelerates neointima formation and impairs endothelial function in hypercholesterolemic rabbits. *Arterioscler Thromb* 14: 753–759, 1994
37. Naruse K, Shimizu K, Muramatsu M, Toki Y, Miyazaki Y, Okumura K, Hashimoto H, Ito T: Long-term inhibition of NO synthesis promotes atherosclerosis in the hypercholesterolemic rabbit thoracic aorta: $PGH_2$ does not contribute to impaired endothelium-dependent relaxation. *Arterioscler Thromb* 14: 746–752, 1994
38. Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Cooke JP: Asymmetric dimethylarginine (ADMA): A novel risk factor for endothelial dysfunction. Its role in hypercholesterolemia. *Circulation* 98: 1842–1847, 1998
39. Lindner A, Charra B, Sherrard DJ, Scribner BH: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290: 697–701, 1974
40. London GM, Drüeke TB: Atherosclerosis and arteriosclerosis in chronic renal failure. *Kidney Int* 51: 1678–1695, 1997
41. Attman PO, Samuelsson O, Alaupovic P: Progression of renal failure: Role of apolipoprotein B-containing lipoproteins. *Kidney Int Suppl* 63: S98–S101, 1997
42. Nishizawa Y, Shoji T, Kawagishi T, Morii H: Atherosclerosis in uremia: Possible roles of hyperparathyroidism and intermediate density lipoprotein accumulation. *Kidney Int Suppl* 62: S90–S92, 1997
43. Wanner C, Zimmermann J, Quaschning T, Galle J: Inflammation, dyslipidemia and vascular risk factors in hemodialysis patients. *Kidney Int Suppl* 62: S53–S55, 1997
44. Gupta A, Robinson K: Hyperhomocysteinaemia and end stage renal disease. *J Nephrol* 10: 77–84, 1997