Marked Increase of Asymmetric Dimethylarginine in Patients with Incipient Primary Chronic Renal Disease

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Abstract. In patients with uremia, increased blood concentrations of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) have been linked to the severity of atherosclerosis and to excess cardiovascular mortality. The ADMA levels and several traditional cardiovascular risk factors were assessed in 44 untreated nonsmoking patients with confirmed primary chronic renal disease at different stages of renal disease. True GFR was assessed by means of the inulin-clearance technique. For comparison, nonsmoking subjects matched with respect to age, gender, and body-mass index were examined. Mean plasma ADMA concentration was markedly higher ($P < 0.0001$) in all patients combined (4.2 ± 0.9 μmol/L) than in control subjects ($n = 16$; age 45 ± 10 yr; serum creatinine 1.0 ± 0.1 mg/dl; ADMA 1.4 ± 0.7 μmol/L). However, mean ADMA levels were similar in patients with normal renal function ($n = 16$; age 41 ± 9 yr; serum creatinine 1.1 ± 0.1 mg/dl; GFR 120 ± 14 ml·min^{-1}·1.73 m^2; ADMA 4.0 ± 0.7 μmol/L), in patients with moderate renal failure ($n = 15$; 47 ± 7 yr; 1.8 ± 0.3 mg/dl; 65 ± 10 ml·min^{-1}·1.73 m^2; 3.8 ± 0.6 μmol/L) and in patients with advanced renal failure ($n = 13$; 46 ± 9 yr; 4.2 ± 0.9 mg/dl; 25 ± 4 ml·min^{-1}·1.73 m^2; 4.7 ± 1.2 μmol/L). Furthermore, ADMA levels were increased to the same extent in normotensive ($n = 17$; 4.0 ± 0.8 μmol/L) and in hypertensive ($n = 27$; 4.2 ± 0.9 μmol/L) patients. In contrast to ADMA, mean total plasma homocysteine concentration were similar in control subjects (10.6 ± 2.9 μmol/L) and in patients with normal GFR (11.0 ± 2.9 μmol/L), but were significantly higher in patients with moderate renal failure (17.7 ± 4.1 μmol/L) and particularly in patients with advanced renal failure (28.2 ± 10.6 μmol/L). Finally, mean total serum cholesterol concentrations were comparable in the control group and in the three groups of patients with renal disease. In contrast to several traditional cardiovascular risk factors, markedly increased blood concentrations of ADMA, a putative biochemical marker of atherosclerosis, are present even in nonsmoking patients without diabetes with incipient primary renal disease. Thus, the early increase of ADMA levels may be of relevance for the excess cardiovascular morbidity and mortality due to arterio- and atherosclerotic complications in patients with renal disease.

Patients with nondiabetic renal diseases are characterized by high cardiovascular morbidity and mortality due to complications of premature atherosclerosis such as coronary heart disease (1–3), and many of them die before they reach the stage of terminal renal failure. The pathophysiologic background of this excessive cardiovascular burden in patients with renal disease without diabetes is not completely understood. Several traditional risk factors have been proposed as playing a certain role, such as hypertension (2), hyperlipidemia (including increased Lp(a) blood concentrations) (1,4,5), and hyperhomocystinemia (6).

In the last decade, a growing body of evidence has accumulated pointing out the role of reduced bioavailability of nitric oxide (NO), a potent antiatherosclerotic molecule, in the development of endothelial dysfunction, which is the first step in the process of atherosclerosis (7). In 1992, Vallance *et al.* (8) first reported elevated plasma levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of the NO synthase, in patients with terminal renal failure. This observation boosted further research on the contribution of a dysfunction in the NO pathway secondary to accumulation of ADMA, leading to the high incidence of atherosclerosis in patients with end-stage renal disease (8,9). Indeed, in patients with renal disease as well as in patients without renal disease, increased ADMA levels correlate strongly with severity of the atherosclerotic disease (10–13). Thus, ADMA is thought to be a novel biochemical marker for atherosclerosis (14). Furthermore, in a prospective study in 225 patients receiving maintenance hemodialysis, plasma ADMA concentrations were not only related to the severity of carotid atherosclerosis, but also were the strongest predictor of cardiovascular mortality among several risk factors assessed (15).

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To date, increased ADMA blood levels have been documented only in patients with advanced and terminal renal failure (8–10,15–18). It has been argued that this is the consequence of diminished renal excretion of dimethylarginines such as ADMA and its biologically inactive regioisomer symmetric dimethylarginine (SDMA) because both substances are excreted renally to some extent (9,18). Past and more recent studies have revealed that some of the potential atherogenetic risk factors (such as lipoprotein abnormalities and insulin resistance) are present very early in the course of renal disease (1,5,6,19). To explore at what stage of renal disease elevated plasma ADMA concentrations can be found, we examined nonsmoking patients with confirmed primary chronic renal disease and different degrees of renal function in a cross-sectional study. True GFR was measured by use of the inulin clearance technique. Importantly, with the exception of high BP, cardiovascular risk factors had not been treated specifically in the patients under study. The results were compared with data that were obtained from a group of nonsmoking subjects matched for age, gender, and body-mass index (BMI).

Materials and Methods

Participants and Protocol

The protocol was approved by the local ethics committee. All participants gave informed consent. Forty-four nonsmoking white adults aged 20 to 60 yr with confirmed primary chronic renal disease were examined; 24 had biopsy-proven IgA glomerulonephritis (IgA GN), and 20 had adult polycystic kidney disease (ADPKD) confirmed by family history and ultrasound examination. In all patients, a detailed history and a thorough physical examination, including electrocardiogram, were obtained to exclude clinically relevant peripheral vascular disease and coronary heart disease. None of the patients showed clinical signs of heart failure. Patients with known diabetes mellitus of any type, liver disease, malignancy, and a history of alcohol abuse were excluded from the study. All patients examined were assigned to 1 of 3 groups with respect to their serum creatinine concentration, as follows: (*1*) below 1.3 mg/dl (*i.e.*, normal renal function); (*2*) between 1.3 and 3.0 mg/dl (*i.e.*, moderate impairment of renal function); and (*3*) above 3.0 mg/dl (*i.e.*, advanced renal failure).

The three groups of patients were matched with respect to age, gender, and BMI (Table 1). All patients studied had been seen as outpatients in the Department of Nephrology. Patients had a stable renal function for at least 6 mo before the study. Until enrollment into the study, none of them had been treated with vitamin B_{12}, folate, antioxidant vitamins, vitamin D, erythropoietin, fish oil, or immunosuppressive agents, and none of them had a low-protein diet. Higher-grade proteinuria (*i.e.*, more than 1 g/d) was present in 3 of 15 patients with moderate renal failure and in 5 of 13 patients with advanced renal failure. Hypertension according to World Health Organization criteria—that is, mean arterial BP greater than 105 mmHg, antihypertensive therapy, or both—was present in 7 of 16 patients with normal renal function, in 10 of 15 patients with moderate renal failure, and in 11 of 13 patients with advanced renal failure. Antihypertensive drugs, if present, were washed out for time periods depending on their half-life of action—that is, short-acting drugs were withdrawn for at

Table 1. Clinical data of control subjects and of patients with renal disease[a]

Parameter	Control Subjects	Patients with Renal Disease		
		Serum Creatinine Concentration (mg/dl)		
		<1.3	>1.3 to <3.0	>3.0
No. patients	16	16	15	13
Gender (M/F)	15/1	15/1	14/1	12/1
IgA GN/ADPKD		10/6	7/8	7/6
Age (yr)	45 ± 10A	41 ± 9A	47 ± 7A	46 ± 9A
S-Creat. (mg/dl)	1.0 ± 0.1A	1.1 ± 0.1A	1.8 ± 0.3	4.2 ± 0.9
C_{in} (ml · min · 1.73 m^2)		120 ± 14	65 ± 10	25 ± 4
BMI (kg/m^2)	26.8 ± 3.2A	26.3 ± 3.8A	26.7 ± 2.2A	26.2 ± 3.6A
MAP (mmHg)	94 ± 8	104 ± 10A	108 ± 9A	112 ± 8
L-Arg. (μmol/L)	59.0 ± 9.4A	51.1 ± 5.2A	50.6 ± 8.8A	54.9 ± 11.7A
ADMA (μmol/L)	1.4 ± 0.7	4.0 ± 0.7AB	3.8 ± 0.6B	4.7 ± 1.2A
SDMA (μmol/L)	0.64 ± 0.41A	0.39 ± 0.13A	0.72 ± 0.35A	1.51 ± 0.46
L-Arg./ADMA	54.3 ± 32.0	13.0 ± 2.3A	13.6 ± 2.9A	12.7 ± 6.3A
Hcy (μmol/L)	10.6 ± 2.9A	11.0 ± 2.9A	17.7 ± 4.1	28.2 ± 10.6
Chol. (mg/dl)	227 ± 40A	195 ± 36A	202 ± 25A	208 ± 28A
Trigl. (mg/dl)	145 ± 54A	128 ± 49A	147 ± 53A	170 ± 64A
iPTH (pmol/L)		4.7 ± 1.1	8.6 ± 5.0	41.5 ± 24.7

[a] IgA GN, IgA glomerulonephritis; ADPKD, adult polycystic kidney disease; S-creat., serum creatinine concentration; C_{in}, glomerular filtration rate by inulin clearance; BMI, body mass index; MAP, mean arterial blood pressure; L-Arg., plasma L-arginine concentration; ADMA, plasma asymmetric dimethylarginine concentration; SDMA, plasma symmetric dimethylarginine concentration; L-Arg./ADMA, L-arginine/ADMA ratio; Hcy, total plasma homocysteine concentration; Chol., total serum cholesterol concentration; Trigl., serum triglyceride concentration; iPTH, plasma intact parathyroid hormone concentration. The statistical differences are given at a *P* level of 0.01 only; shared letters are not significantly different (differences in the number of participants and the proportion of gender and renal disease per group were not statistically tested).

least 3 d preceding examination, whereas long-acting drugs were washed out for at least 1 wk before examination.

For comparison, 16 normotensive nonsmoking subjects without diabetes between 20 and 60 yr of age were recruited from a population participating in a field study on the relationship between NO, ADMA, and vascular disease. Renal and cardiovascular diseases were excluded in control subjects, and they were matched with respect to age, gender, and BMI to the groups of patients (Table 1).

Both patients and control subjects adhered to a standardized diet for 3 d before the laboratory tests were carried out. Repeated verbal inquiries ascertained compliance. Blood samples for measurement of creatinine, L-arginine, ADMA, SDMA, total homocysteine (Hcy), and total cholesterol concentrations were taken in the morning after at least 12 h of fasting. In addition, mean arterial BP was measured oscillometrically with the patient in the supine position in a quiet environment with an automated device (Dinamap, Critikon Co., Tampa, FL). The mean of three consecutive measurements 5 min apart after a rest of at least 20 min was taken for analysis. In patients with renal disease, additional blood samples for measurement of intact parathyroid hormone concentrations were taken, and true GFR was assessed while the patient was supine by means of the steady-state inulin (C_{in}) infusion clearance technique, as described in detail elsewhere (20).

Biochemical Analyses

Plasma L-arginine and dimethylarginine levels were determined by HPLC by use of precolumn derivatization with o-phthalaldehyde (OPA) as described previously (21). Plasma samples and internal standards were extracted on CBA solid phase extraction cartridges (Varian, Harbor City, CA). The eluates were dried over nitrogen and dissolved in bidistilled water for HPLC analysis. Samples and standards were incubated for exactly 30 s 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 L-arginine, ADMA, and SDMA were separated on a C6H5 column (Macherey and Nagel, Düren, Germany) with the fluorescence monitor set at an excitation wavelength of 340 nm and an emission wavelength of 455 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. The coefficients of variation of this method is 5.2% within assay and 5.5% between assay; the detection limit of the assay is 0.1 μmol/L. Plasma inulin concentration was measured enzymatically by use of inulinase as described by Kuehnle *et al.* (22), and inulin clearance was calculated as described elsewhere (20). Intact parathyroid hormone was measured with an immunoradiometric assay (normal range, 1.2 to 6.0 pmol/L) and total plasma Hcy with a fluorescence-polarization immunoassay (normal range, 5.0 to 15.0 μmol/L). All other measurements were performed with routine laboratory tests that used certified assay methods.

Statistical Analyses

The SPSS software package (SPSS 10.0.7 for Windows; SPSS, Inc., Chicago, IL) was used for statistical analysis. After confirming approximate normality of the data distribution, control subjects and the three groups of patients with renal disease were compared by a two-tailed ANOVA. When this procedure gave significant results, between-group comparison was carried out by *t* test for random data. Pearson's correlation analysis between GFR on the one hand and L-arginine, ADMA, SDMA, and Hcy on the other hand was performed in pooled patients' data only. Correlations between ADMA and age, mean arterial BP, proteinuria, serum cholesterol, and Hcy levels were analyzed as well. All data are presented as mean ± SD. Differences were considered as significant at $P < 0.05$, and Bonferroni or Bonferroni-Holm corrections for multiple comparison were applied.

Results

Table 1 shows clinical data of patients with renal disease and of control subjects. Mean plasma ADMA concentration was markedly higher in patients than in control subjects. There was almost no overlap of ADMA values between both groups, so that the difference between the combined results of all patients (4.2 ± 0.9 μmol/L) and control subjects (1.4 ± 0.7 μmol/L) was highly significant ($P < 0.0001$) (Figure 1). In contrast, plasma ADMA levels were similar in the three groups of patients with renal disease, with a tendency for ADMA concentrations to be higher in the group with advanced renal failure. Consequently, the correlation between plasma ADMA concentrations and GFR in patients was weak and NS ($r = -0.26$, $P = 0.09$) (Figure 2), and so were the correlations between ADMA levels and age ($r = 0.03$, $P = 0.87$), mean arterial BP ($r = 0.14$, $P = 0.38$), serum cholesterol levels ($r = 0.21$, $P = 0.17$), proteinuria ($r = 0.24$, $P = 0.15$), and Hcy levels ($r = 0.32$, $P = 0.04$). Furthermore, ADMA levels were not significantly ($P = 0.52$) different in normotensive ($n = 17$;

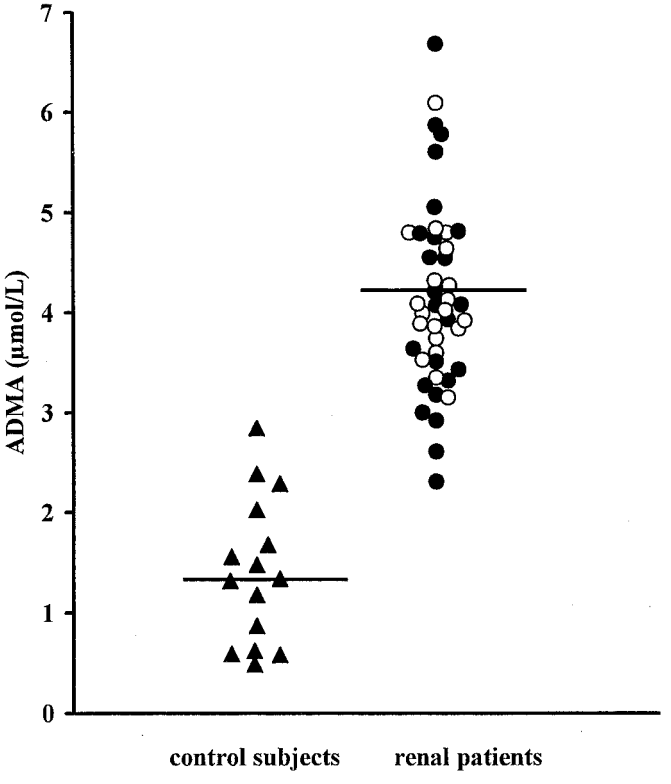

Figure 1. Individual data on plasma asymmetric dimethylarginine (ADMA) concentrations in control subjects ($n = 16$) and in patients with renal disease ($n = 44$). Because ADMA values barely overlapped between groups, the statistical difference between patients with renal disease and control subjects was highly significant ($P < 0.0001$). Patients with IgA glomerulonephritis (●); patients with adult polycystic kidney disease (○).

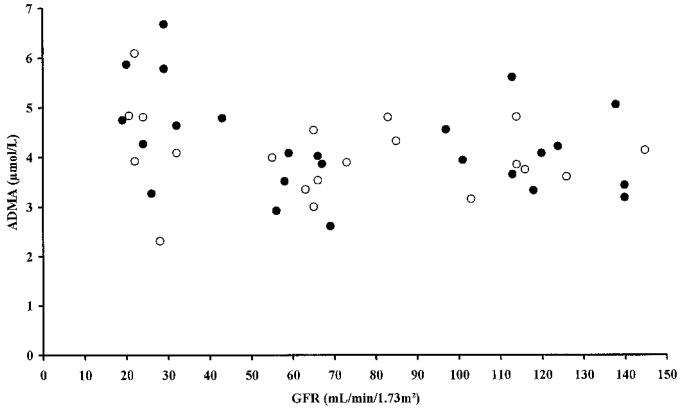

Figure 2. Correlation analysis between GFR (GFR by inulin clearance) and plasma asymmetric dimethylarginine (ADMA) concentrations in 24 patients with IgA glomerulonephritis (●) and in 20 patients with adult polycystic kidney disease (○). The correlation was NS ($r = -0.26$, $P = 0.09$).

97 ± 5 mmHg; 4.0 ± 0.8 μmol/L) and hypertensive ($n = 27$; 114 ± 6 mmHg; 4.2 ± 0.9 μmol/L) patients with renal disease, irrespective of renal function. Consequently, mean plasma ADMA concentration was significantly higher in normotensive patients as compared with normotensive controls ($P < 0.001$).

The separate descriptive analysis of patients with immune origin of renal disease (IgA GN) and patients with nonimmune renal disease (ADPKD) showed no differences between both groups with respect to plasma ADMA levels (Table 2). Mean ADMA concentrations were increased in both groups of patients irrespective of renal function. Similarly, as for the combined patients data, there was a tendency for plasma ADMA concentrations to be higher in the groups with advanced renal failure, both in patients with IgA GN and patients with ADPKD.

In contrast to ADMA, plasma L-arginine concentrations were similar in control subjects and in all three groups of patients with renal disease, whereas SDMA levels gradually increased in patients with renal failure (Table 1). Thus, the correlation between GFR and plasma SDMA levels in patients was highly significant ($r = -0.78$, $P < 0.0001$; Figure 3), whereas that between GFR and plasma L-arginine was not ($r = -0.18$, $P = 0.25$). Mean fasted total plasma Hcy concentrations were comparable in control subjects and in patients with normal GFR, but they were significantly higher in patients with moderate renal failure, particularly in patients with advanced renal failure (Table 1). Consequently, the correlation between GFR and total plasma Hcy levels in patients with renal disease was highly significant ($r = -0.73$, $P < 0.0001$) (Figure 4). Again, the separate descriptive analysis of patients with IgA GN and patients with ADPKD showed no differences between the two groups with respect to plasma L-arginine, SDMA, and Hcy concentrations (Table 2). Finally, the analysis of mean total serum cholesterol and triglyceride concentrations revealed no significant differences between the three groups of patients and the group of control subjects (Table 1).

Table 2. Cardiovascular risk factors in 24 patients with IgA glomerulonephritis (IgA GN) and in 20 patients with adult polycystic kidney disease (ADPKD)[a]

Parameter	Serum Creatinine Concentration (mg/dl)		
	<1.3	>1.3 to <3.0	>3.0
IgA GN (*n*)	10	7	7
C_{in} (ml · min · 1.73 m^2)	120 ± 15	59 ± 8	25 ± 4
age (yr)	45 ± 8	47 ± 7	43 ± 8
BMI (kg/m^2)	26.2 ± 3.9	26.3 ± 1.6	26.4 ± 4.0
MAP (mm Hg)	104 ± 12	108 ± 7	107 ± 9
ADMA (μmol/L)	4.1 ± 0.7	3.6 ± 0.8	4.8 ± 1.4
SDMA (μmol/L)	0.36 ± 0.08	0.88 ± 0.27	1.60 ± 0.55
Hcy (μmol/L)	10.4 ± 2.2	16.8 ± 2.5	23.7 ± 6.7
Chol. (mg/dl)	210 ± 26	202 ± 27	219 ± 29
ADPKD (*n*)	6	8	6
C_{in} (ml · min · 1.73 m^2)	120 ± 13	70 ± 9	25 ± 5
age (yr)	35 ± 6	47 ± 8	49 ± 8
BMI (kg/m^2)	26.5 ± 3.8	27.0 ± 2.6	26.0 ± 2.9
MAP (mm Hg)	104 ± 4	108 ± 11	117 ± 2
ADMA (μmol/L)	3.9 ± 0.5	4.0 ± 0.4	4.6 ± 0.7
SDMA (μmol/L)	0.43 ± 0.18	0.58 ± 0.35	1.42 ± 0.30
Hcy (μmol/L)	11.9 ± 3.5	18.5 ± 5.0	33.5 ± 11.9
Chol. (mg/dl)	171 ± 37	203 ± 22	190 ± 29

[a] C_{in}, glomerular filtration rate by inulin clearance; BMI, body mass index; MAP, mean arterial blood pressure; ADMA, plasma asymmetric dimethylarginine concentration; SDMA, plasma symmetric dimethylarginine concentration; Hcy, total plasma homocysteine concentration; Chol., total serum cholesterol concentration.

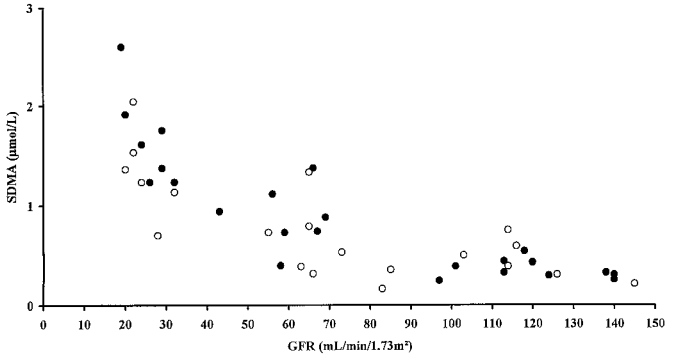

Figure 3. Correlation analysis between GFR (GFR by inulin clearance) and plasma symmetric dimethylarginine (SDMA) concentrations in 24 patients with IgA glomerulonephritis (●) and 20 patients with adult polycystic kidney disease (○). The correlation was highly significant ($r = -0.78$, $P < 0.0001$).

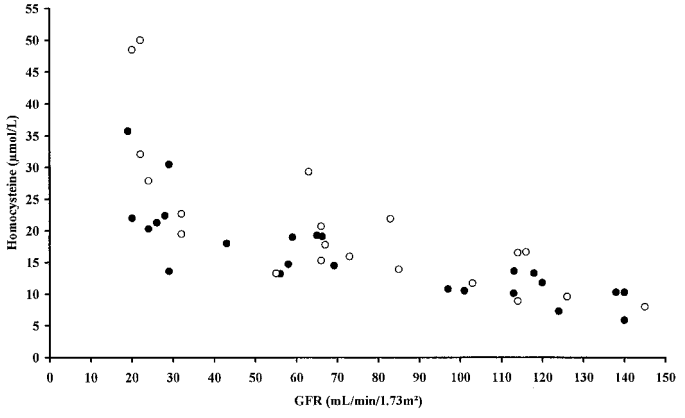

Figure 4. Correlation analysis between GFR (GFR by inulin clearance) and total plasma homocysteine (Hcy) concentrations in 24 patients with IgA glomerulonephritis (●) and 20 patients with adult polycystic kidney disease (○). The correlation was highly significant ($r = -0.73$, $P < 0.0001$).

Discussion

The most striking finding of this study is the demonstration that in nonsmoking patients without diabetes and with chronic renal disease, blood concentrations of the endogenous NO synthase inhibitor ADMA, a putative biochemical marker of atherosclerosis (14), are markedly increased at an early stage, even when GFR is still within the normal range. ADMA concentrations were significantly higher in patients with renal disease compared with matched controls, and there was almost no overlap between the two groups examined. For traditional cardiovascular risk factors such as serum lipids or insulin resistance, a wide dispersion of values is found even in the normal population, and in epidemiologic studies, the values in "normal" and "abnormal" populations under study show considerable overlap. This was clearly not the case with ADMA blood levels in this study.

Irrespective of its potential pathophysiologic role in the development of atherosclerosis, increased plasma ADMA levels obviously characterize patients with renal disease as a separate population when compared with normotensive subjects without renal and cardiac disease. Accordingly, blood ADMA levels were found to be increased to the same extent in both patients with immune renal disease (*i.e.*, IgA GN) and in patients with nonimmune renal disease (*i.e.*, ADPKD). Thus, ADMA may be an early indicator of—and potentially even a causal agent in the genesis of—excess cardiovascular morbidity and mortality due to arterio- and atherosclerotic complications in patients with primary renal disease (10).

In this context, it is of interest that a study documented impaired acetylcholine-induced endothelium-dependent vasorelaxation in patients with ADPKD and normal renal function (23). Although the mean age of these patients was somewhat higher than that of patients with ADPKD with normal renal function in our study, all of them had BP values still within the normal range, as documented by 24-h ambulatory BP measurement. Thus, impaired endothelium-dependent vasorelaxation could not be the consequence of high BP. This observation is in line with our finding that ADMA blood concentrations in normotensive and hypertensive patients with renal disease were increased to the same extent. Endothelium-dependent vasorelaxation is induced by the NO synthase activator acetylcholine and is a fundamental function of the healthy endothelium. Its impairment is thought to be a hallmark of incipient atherosclerosis at the level of small arteries (14), and two large, prospective, controlled studies have demonstrated a significant relationship between endothelial dysfunction and the later development of manifest atherosclerotic disease—that is, cardiovascular events (24,25).

ADMA is a potent inhibitor of the NO synthase, and increased levels could thus be responsible for the impaired acetylcholine-induced endothelium-dependent vasorelaxation (26), at least in patients with renal disease. Furthermore, normal elasticity of large arterial vessels is in part NO dependent, and in patients with end-stage renal disease, vascular remodeling coupled with endothelial dysfunction is a strong predictor of cardiovascular mortality (27). To obtain relatively homogeneous and comparable cohorts of controls subjects and patients, we excluded subjects with manifest cardiovascular disease in our population under study on the basis of noninvasive evaluation. This may explain why increased plasma ADMA concentrations in our patients were not associated with manifest cardiovascular problems. Further studies exploring the association between increased ADMA levels and cardiovascular pathology in patients with renal disease by more sensitive (invasive) techniques (*e.g.*, acetylcholine-induced, endothelium-dependent vasorelaxation) are therefore warranted.

It appears paradoxical that in patients with renal disease, ADMA should be increased even though GFR values are still within the normal range. We caution, however, that apparently normal renal function, as documented by normal GFR, does not necessarily exclude reduction of functional parenchyma by the disease process. Adaptive changes in glomerular filtration dynamics and single-nephron GFR may keep whole-kidney GFR within the normal range despite considerable reduction of tubular cell mass. Other cardiovascular risk factors, such as

increased Lp(a) concentrations (5) or insulin resistance (19), are found to be present in patients with incipient renal disease as well, and a recent post hoc analysis of the HOPE study revealed that the presence of even mild renal insufficiency is a potent predictor of cardiovascular mortality independent of known cardiovascular risk factors such as microalbuminuria (28). Increased ADMA levels in patients with renal disease could be the result of reduced activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that metabolizes ADMA (but not SDMA) to citrulline (29,30). The enzyme is present in abundance in renal tissue—that is, in endothelial cells within the glomerulus and in renal vessels, and particularly in renal tubular cells (30,31). It regulates (intra)cellular methylarginine levels, thereby governing cell-specific L-arginine uptake and NO generation in tubular cells (31). We propose the hypothesis that destruction of DDAH-rich renal tissue impairs renal degradation of ADMA, eventually leading to increased plasma levels. A complementary explanation may be that salt retention accompanying impaired renal function might affect the activity of DDAH and thus increase ADMA levels (32).

Plasma concentrations of L-arginine were similar in the group of control subjects and in patients with renal disease so that the L-arginine/ADMA ratio was markedly decreased in patients. A decrease of the L-arginine/ADMA ratio was associated with clinically manifest atherosclerosis in several studies (11,14). The ratio of both substances is thought to govern cell-specific L-arginine uptake and NO generation, not only in renal tubular cells but also in endothelial cells. In contrast to ADMA, plasma concentrations of SDMA were comparable in control subjects and patients with renal disease with normal GFR, but steadily increased in patients with progressive loss of renal function. As a consequence, SDMA but not ADMA levels were significantly correlated with GFR, probably reflecting a progressive loss of renal excretion capacity for this substance. This observation confirms earlier results of MacAllister *et al.* (9), who found markedly increased plasma SDMA levels in patients with terminal renal failure despite only moderately increased ADMA levels. The correlation between GFR and ADMA levels in our patients was weak, at least across a range of GFR between 140 to 20 $ml \cdot min^{-1} \cdot 1.73\ m^2$, suggesting that pronounced accumulation of ADMA due to impaired renal excretory function may indeed occur only with severe impairment of renal function. The biologic significance of SDMA is still uncertain because no direct inhibitory effect on NO synthase was documented (14). At high concentrations, however, it may indirectly interfere with NO metabolism by blocking cellular L-arginine uptake (31). The results of the study we present here warrant further research to elucidate the role of methylarginines in renal disease.

By using the inulin clearance for the measurement of true GFR, we could clearly document that total plasma Hcy concentrations increase with progressive renal failure (6). The correlation between GFR and plasma Hcy concentrations was remarkable, pointing to a very close relationship between renal function and Hcy metabolism (6). In this respect, Hcy resembles intact parathyroid hormone—its plasma concentrations were strongly associated with GFR as well ($r = -0.64$, $P < 0.0001$). Regardless of the role of increased Hcy levels as a cardiovascular risk factor in patients with renal disease (6), it is of interest that the metabolic pathways generating Hcy and ADMA are closely coupled. ADMA is generated via post-translational methylation of arginine residues of nucleolar proteins, and some authors argue that these methyl groups may come from the demethylation process of methionine to Hcy (33). In our patients with normal renal function, Hcy concentrations were comparable to those in control subjects, suggesting that factors other than hyperhomocystinemia are responsible for increased ADMA levels. Last, but not least, total serum cholesterol concentrations were comparable in the group of patients with renal disease and normotensive control subjects matched with respect to age, gender, and BMI. This observation is of importance because hypercholesterinemia was shown to be associated with increased plasma ADMA concentrations *in vivo*, and an inhibitory effect of LDL cholesterol on DDAH activity was documented *in vitro* (34).

In conclusion, increased plasma concentrations of ADMA—that is, the only biologically active endogenous NO synthase inhibitor (26,35)—are found at a very early stage of renal disease, even when GFR is still within the normal range. Because of the marked increase with almost no overlap between control subjects and patients with renal disease, ADMA by itself may be an indicator of incipient renal disease. This observation has several implications for further research and for the management of patients with renal disease. Numerous recent studies have documented a potential role of ADMA in the process of atherosclerosis (14). It remains to be seen whether interventions such as supplementation with L-arginine or administration of NO donors, preferably early on in the course of renal disease, are able to modulate the atherogenic profile of patients with renal disease and interfere with progression of renal failure (36,37).

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