Left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine (ADMA) in hemodialysis patients

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Background. The endogenous inhibitor of nitric oxide (NO), asymmetric dimethylarginine (ADMA), is a strong predictor of adverse cardiovascular outcomes in patients with end-stage renal disease (ESRD).

Methods. Since arterial and cardiac remodeling is associated with altered endothelial microcirculatory responses to forearm ischemia (a NO-dependent response), interference of ADMA with the NO system may be important for the pathogenesis of left ventricular hypertrophy (LVH) in these patients. This study sought to identify the relationship between plasma ADMA and LV geometry and function in a cohort of 198 hemodialysis patients.

Results. Plasma ADMA was significantly higher ($P = 0.008$) in patients with LVH (median 3.00 μmol/L, inter-quartile range 1.73 to 3.97 μmol/L) than in those without this alteration (1.88 μmol/L, 1.15 to 3.56 μmol/L) and was significantly related to left ventricular (LV) mass ($r = 0.26$, $P < 0.001$). Interestingly, ADMA was much higher ($P < 0.001$) in patients with concentric LVH (3.60 μmol/L, 2.90 to 4.33 μmol/L) than in patients with eccentric LVH (2.17 μmol/L, 1.47 to 3.24 μmol/L) or normal LV mass (1.76 μmol/L, 1.13 to 2.65 μmol/L). Furthermore, plasma ADMA was higher ($P = 0.02$) in patients with systolic dysfunction (3.52 μmol/L, 2.08 to 5.87 μmol/L) than in those with normal LV function (2.58 μmol/L, 1.53 to 3.84 μmol/L) and inversely related to ejection fraction (EF; $r = -0.25$, $P < 0.001$). The link between ADMA and LV mass and EF was confirmed by multivariate analysis (ADMA vs. LVMI, β = 0.17, $P = 0.006$; ADMA vs. EF, β = -0.24, $P < 0.001$).

Conclusions. Overall, this study indicates that raised plasma concentration of ADMA is associated to concentric LVH and LV dysfunction. Intervention studies are needed to see whether the link between ADMA and concentric LVH remodeling and LV dysfunction is a causal one.

Left ventricular hypertrophy (LVH) is a major risk factor for overall and cardiovascular mortality in patients with end-stage renal disease (ESRD) [1]. The pathogenesis of LVH in these patients is multifactorial [2] and several causative factors have been identified including hypertension, anemia, hypoalbuminemia, hyperparathyroidism, chronic volume expansion and, possibly, hyperhomocysteinemia [3]. A new potential risk factor for LVH in these patients is the accumulation of the endogenous inhibitor of nitric oxide synthase (NOS), asymmetric dimethylarginine (ADMA). ADMA is related to the severity of atherosclerosis independently of other vasculopathic factors [4]. We have recently found that this substance is a powerful predictor of overall and cardiovascular mortality in hemodialysis patients [5].

The interference of ADMA with the nitric oxide (NO) system may be important for the pathogenesis of LVH because NO modulates the growth of the myocardium [6]. In experimental models inhibition of NOS generates a NO-deficient hypertension that is associated with the development of concentric LVH [7]. Of note, arterial and cardiac remodeling in ESRD have recently been associated with altered endothelial microcirculatory responses to forearm ischemia [8]. The multiple links between the NO system and cardiovascular remodeling prompted us to study the relationship between the plasma concentra-

tion of ADMA, left ventricular (LV) mass, LV geometry and function in a large group of patients with ESRD.

METHODS

Protocol

The protocol was in conformity to the ethical guidelines of our Institutions and informed consent was obtained from each participant. All studies were performed during a mid-week non-dialysis day, in the morning hours between 8 AM and 1 PM.

Study cohort

One hundred and ninety-eight hemodialysis patients (110 males and 88 females) with ESRD who had been on regular dialysis treatment (RDT) for at least six months (median duration of RDT 43 months, inter-quartile range 20 to 110 months) with an ejection fraction >35% and without clinical evidence of heart failure (defined as dyspnea in addition to two of the following conditions: raised jugular pressure, bibasilar crackles, pulmonary venous hypertension or interstitial edema on chest x-ray requiring hospitalization or extra ultrafiltration) [9] were considered eligible for the study. These patients represented about 70% of the whole dialysis population of the four participating dialysis units.

Patients were being treated three times weekly with standard bicarbonate dialysis (Na 138 mmol/L, HCO_3 35 mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) or by high flux hemodialysis using 1.1 to to 1.7 m² dialyzers either with cuprophan or semi-synthetic membranes. The average Kt/V in these patients was 1.21 ± 0.26. Dry weight was targeted in each case to achieve a normotensive edema-free state. All these patients were all virtually anuric (24 hour diuresis <200 mL). The demographic, anthropometric and biochemical characteristics of the patients are detailed in Table 1.

Laboratory measurements

Blood sampling was performed during a mid-week non-dialysis day after 20 to 30 minutes of quiet resting in semirecumbent position. Samples were taken into pre-chilled ethylenediaminetetraacetic acid (EDTA) vacutainers, placed immediately on ice, centrifuged within 30 minutes at 4°C and the plasma stored at −80°C until analysis.

Serum lipids, albumin, calcium, phosphate and hemoglobin measurements were made using standard methods in the routine clinical laboratory. C-reactive protein (CRP) levels were measured by a commercially available kit (Behring, Scoppito, L'Aquila, Italy). Plasma homocysteine was quantified by a high-pressure liquid chromatography (HPLC) method [10].

Table 1. Somatometric, clinical and biochemical data of the study population

Somatometric data	
Age *years*	58.8 ± 15.0
BMI *kg/m²*	24.5 ± 4.4
Cardiovascular risk factors	
Systolic pressure *mm Hg*	131.8 ± 23.3
Diastolic pressure *mm Hg*	73.9 ± 12.3
Diabetics	14%
Smokers	38%
Hypercholesterolemia	
(serum cholesterol >5.2 mmol/L)	30%
On treatment with erythropoietin	56%
Biochemical data	
Hemoglobin *g/L*	106 ± 19
Plasma homocysteine *μmol/L*	26.9 (19.3–42.8)
Plasma fibrinogen *mg/dL*	384.0 (271.0–542.0)
Serum total cholesterol *mmol/L*	5.35 ± 1.46
Serum calcium *mmol/L*	2.3 ± 0.2
Serum phosphate *mmol/L*	2.0 ± 0.5
Serum PTH *pg/mL*	166 (69–374)
Serum CRP *mg/L*	7.8 (3.4–16.4)
Serum albumin *g/L*	42 ± 5
Plasma L-Arginine *μmol/L*	70.6 (57.9–78.8)

Data are mean ± SD, median (interquartile range), or percent frequency as appropriate.

Quantification of L-arginine and dimethylarginine concentrations

Concentrations of L-arginine and dimethylarginines in plasma were determined by HPLC using pre-column derivatization with o-phthalaldehyde (OPA) using a previously described method after extraction of plasma samples on CBA solid phase extraction cartridges (Varian, Harbor City, CA, USA) [11]. The coefficients of variation of this method were 5.2% within-assay and 5.5% between-assay; the detection limit of the assay was 0.1 μmol/L. The plasma concentration of ADMA in normal subjects has a positively skewed distribution with a median value of 0.95 μmol/L and the 90th percentile is 2.2 μmol/L.

Blood pressure measurements

Blood pressure was estimated by averaging all predialysis arterial pressure recordings during the month before the study (total of 12 measurements; that is, 3/week) [12].

Echocardiography

These studies were performed during a mid-week non-dialysis day, within two hours after blood sampling. Left ventricular mass (LVM) was calculated according to the Devereux cube formula and indexed to height²·⁷ (LVMI) [13]. LVH was defined by a LVMI of over 47 g/m²·⁷ in women or over 50 g/m²·⁷ in men [13]. The height-based indexing of LVM was specifically chosen to minimize any potential distortion attributable to extracellular volume expansion (surface area indexing being weight-sensitive). The relative wall thickness [RWT; 2*posterior

wall thickness/left ventricular end diastolic diameter (LVEDD)] was also calculated as an index of the left ventricular geometric pattern. Values indicative of concentric and eccentric left ventricular geometry were established on the basis of age-specific reference standards [14]. Systolic dysfunction was defined as a LV ejection fraction <45%. Mean wall thickness (MWT) was calculated by the standard formula [(inter-ventricular septum thickness + posterior wall thickness)]/2.

Statistical analyses

Data are reported as mean ± SD or as median and inter-quartile range, as appropriate. Variables that did not show a Gaussian distribution (Kolmogorov-Smirnov test) were log transformed before further analyses ($\log_{10}$). Relationships between paired parameters were analyzed by the least squares method.

To perform multiple linear regression analysis of LVMI, MWT and RWT, a set of independent variables were identified including plasma ADMA as well as a series of traditional and non-traditional cardiovascular risk factors in dialysis patients [age, sex, diabetes, previous cardiovascular events, duration of RDT, number of antihypertensive drugs, smoking, systolic pressure, cholesterol, fibrinogen, homocysteine, parathyroid hormone (PTH), calcium, phosphate, CRP, albumin, hemoglobin and Kt/V]. To test the independent relationship between plasma ADMA and left ventricular ejection fraction we constructed a multivariate model based on age, heart rate, diastolic pressure and left ventricular end diastolic volume. Independent variables were selected by a stepwise approach. Significant independent variables were ordered according to their standardized effect, defined as regression coefficient/standard error of the regression. By this approach we constructed models of adequate statistical power (at least 28 subjects for each variable in the final model).

The link between plasma ADMA and concentric LVH was tested by analysis of variance (ANOVA) and by the analysis of receiver operating characteristic (ROC) curve [15]. The diagnostic threshold of plasma ADMA for the identification of concentric LVH in dialysis patients was defined as the ADMA value combining better sensitivity and specificity, that is, as the optimal value in the ROC curve and the confidence intervals of sensitivity, specificity, positive and negative prediction values were calculated by a standard formula [16]. All calculations were done using a standard statistical package (SPSS for Windows Version 9.0.1, 11 Mar 1999; SPSS Inc., Chicago, IL, USA).

RESULTS

Plasma ADMA concentration (median 2.61 μmol/L, inter-quartile range 1.58-3.96 μmol/L) was above the

Table 2. Echocardiographic parameters of heart geometry and function in the study population

Left ventricular end diastolic diameter *cm*	5.04 ± 0.67
Posterior wall thickness *cm*	1.10 ± 0.20
Inter-ventricular septum *cm*	1.17 ± 0.20
Mean wall thickness *cm*	1.13 ± 0.19
Relative wall thickness	0.45 ± 0.11
Left ventricular mass index *g/m$^{2.7}$*	60.9 ± 18.4
Ejection fraction *%*	58.7 ± 9.9

Data are expressed as mean ± SD.

90th percentile of the normal range (>2.2 μmol/L) in the majority of dialysis patients (118 of 198; 60%).

One hundred forty-seven patients displayed LVH on echocardiography (eccentric LVH, $N = 75$, 38%; concentric LVH, $N = 72$, 36%; normal LVM, $N = 51$, 26%). Systolic dysfunction was present in 22 patients (11%). Echocardiographic parameters of heart geometry are detailed in Table 2.

Left ventricular hypertrophy, systolic dysfunction, and plasma ADMA

Plasma ADMA was significantly higher ($P = 0.008$) in patients with LVH (median 3.00 μmol/L, inter-quartile range 1.73 to 3.97 μmol/L) than in those without this alteration (1.88 μmol/L, 1.15 to 3.56 μmol/L). Similarly, plasma ADMA was significantly higher ($P = 0.02$) in patients with systolic dysfunction (3.52 μmol/L, 2.08 to 5.87 μmol/L) than in those with normal left ventricular function (2.58 μmol/L, 1.53 to 3.84 μmol/L).

Univariate and multivariate analysis of echocardiographic parameters

On univariate analysis plasma ADMA was directly related to MWT, RWT and LVMI (Fig. 1 A–C), and inversely related to ejection fraction (Fig. 1D). There was also a tendency for ADMA to be inversely related with LVEDD but this correlation was of marginal statistical significance ($r = -0.12$, $P = 0.098$). On multivariate analysis plasma ADMA was confirmed to be an independent correlate of LVMI as well as of the muscular component (MWT and RWT) of the left ventricle (Table 3). Similarly, multivariate analysis confirmed that plasma ADMA was an independent correlate of left ventricular ejection fraction ($\beta = -0.24$, $P < 0.001$) in a model including left ventricular end diastolic volume ($\beta = -0.46$, $P < 0.001$) and heart rate ($\beta = -0.24$, $P < 0.001$; multiple R = 0.56, $P < 0.001$). In this model age ($P = 0.06$) and diastolic pressure ($P = 0.17$) failed to be significantly related to left ventricular ejection fraction.

Discriminant power of plasma ADMA for the identification of concentric LVH

Patients with concentric LVH had markedly increased plasma ADMA in comparison to patients with eccentric LVH or normal LV mass (Fig. 2).
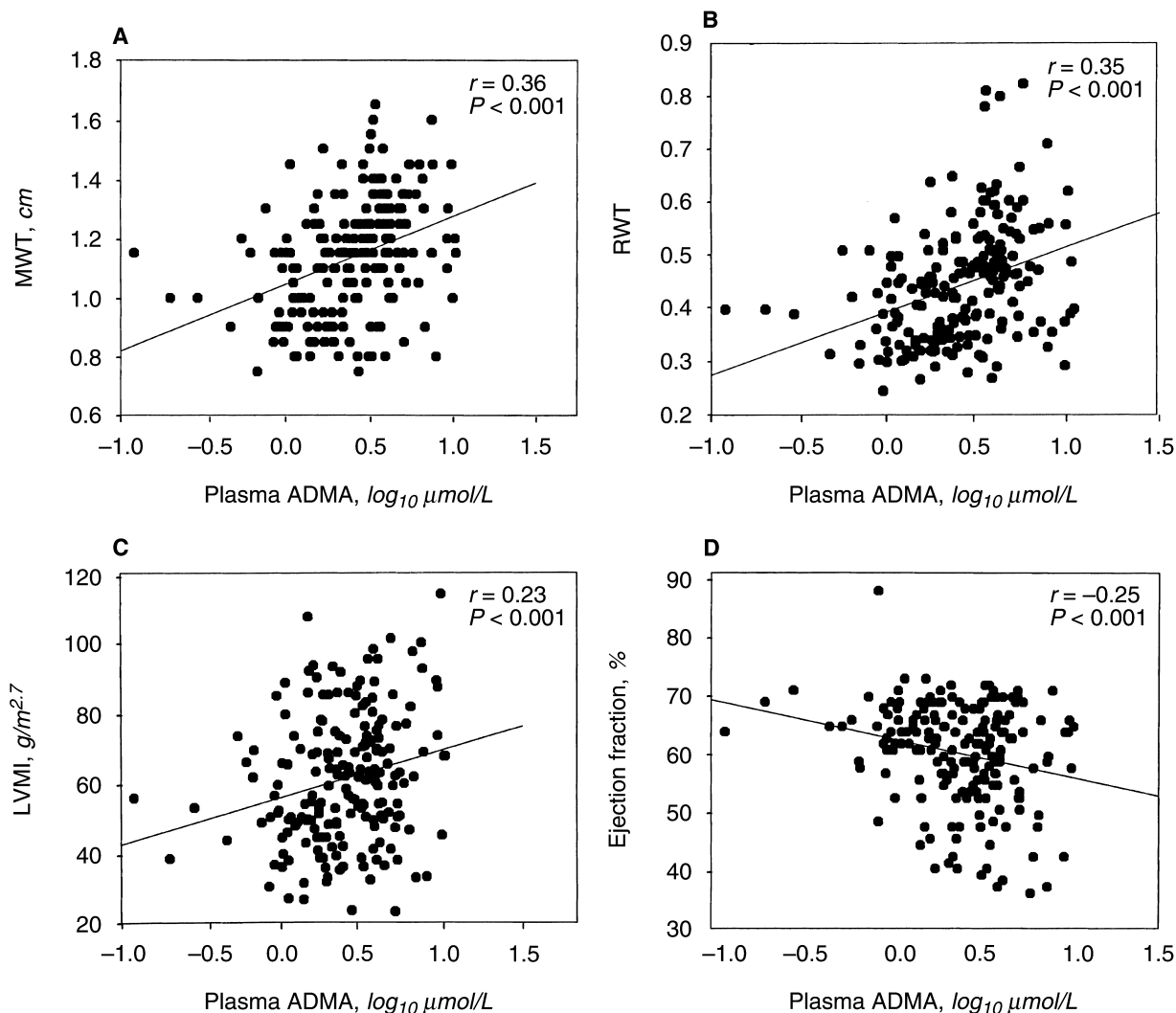
Fig. 1. Relationships between plasma asymmetric dimethylarginine (ADMA) and mean wall thickness (MWT; *A*), relative wall thickness (RWT; *B*), left ventricular mass index (LVMI; *C*) and left ventricular ejection fraction (*D*).

Notably, plasma ADMA showed a potential diagnostic value for the identification of concentric LVH because the area under the corresponding ROC curve (Fig. 3) was significantly ($P < 0.001$) greater than the threshold of diagnostic indifference (50%). When data were analyzed on the basis of the "best cut-off" derived from the ROC curve (2.76 μmol/L), the sensitivity was 79% (95% CI, 70 to 88%) and the specificity was 70% (95% CI, 62 to 78%). The positive predictive value was 60% (95% CI, 50 to 70%) while the negative predictive value was 85% (95% CI, 78 to 92%).

DISCUSSION

In a large group of hemodialysis patients the endogenous inhibitor of nitric oxide synthase (NOS), ADMA, was strongly associated to LVM, particularly to concentric LVH, and was inversely related to LV function.

Nitric oxide generation and endothelium-dependent vasodilation in ESRD

Total body NO production, either estimated from ^{15}N-citrulline accumulation [17] or by whole body NO balance [18] is reduced in patients with moderate to severe renal failure. Whether the NO synthesis rate is reduced in patients with ESRD is still unclear, because a recent study reported an increased whole body NO synthesis in these patients, but this increase appeared largely independent of dialysis treatment [19]. At least four studies have documented impaired endothelial vasodilatory responses to ischemia [8, 20] or acetylcholine [21, 22] in hemodialysis patients or in normotensive children with chronic renal disease. Thus, although it remains still to be established whether whole body NO generation is reduced in ESRD, it is unquestionable that renal failure has a direct adverse effect on endothelial

Table 3. Multiple regression models of LVMI, MWT and RWT

	β	*P*
Dependent variable: LVMI[a] multiple R = 0.57, *P* < 0.001		
Independent variable		
Systolic pressure	0.27	<0.001
Serum albumin	−0.25	<0.001
Previous cardiovascular events	0.22	0.001
Plasma ADMA	0.17	0.006
Male sex	0.14	0.02
Age	0.13	0.05
Dependent variable: MWT[b] multiple R = 0.61, *P* < 0.001		
Independent variable		
Systolic pressure	0.27	<0.001
Serum albumin	−0.26	<0.001
Plasma ADMA	0.25	<0.001
Smoking	0.19	0.002
Plasma fibrinogen	0.18	0.004
Hemoglobin	−0.15	0.01
Dependent variable: RWT[c] multiple R = 0.54, *P* < 0.001		
Independent variable		
Serum albumin	−0.22	0.002
Plasma ADMA	0.19	0.005
Plasma fibrinogen	0.18	0.008
Duration of RDT	0.17	0.01
Systolic pressure	0.17	0.01
Serum cholesterol	0.14	0.04
Hemoglobin	−0.13	0.04

Data are expressed as standardized regression coefficients (β) and *P* values.
[a] Out of the model: PTH ($P = 0.07$), hemoglobin ($P = 0.11$), number of antihypertensive drugs ($P = 0.11$), smoking ($P = 0.21$), phosphate ($P = 0.23$), diabetes ($P = 0.25$), fibrinogen ($P = 0.38$), Kt/V ($P = 0.46$), duration of RDT ($P = 0.71$), homocysteine ($P = 0.92$), CRP ($P = 0.92$), calcium ($P = 0.94$), and cholesterol ($P = 0.97$).
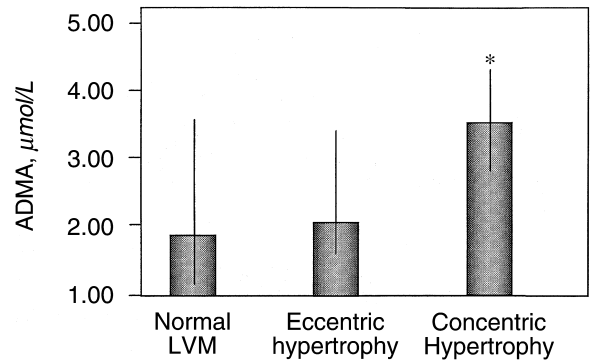[b] Out of the model: diabetes ($P = 0.11$), age ($P = 0.19$), cholesterol ($P = 0.20$), previous cardiovascular events ($P = 0.25$), number of antihypertensive drugs ($P = 0.29$), Kt/V ($P = 0.31$), duration of RDT ($P = 0.31$), calcium ($P = 0.43$), phosphate ($P = 0.46$), male sex ($P = 0.63$), CRP ($P = 0.78$), PTH ($P = 0.92$), and homocysteine ($P = 0.97$).
[c] Out of the model: smoking ($P = 0.28$), homocysteine ($P = 0.36$), diabetes ($P = 0.39$), male sex ($P = 0.43$), PTH ($P = 0.46$), CRP ($P = 0.60$), Kt/V ($P = 0.63$), number of antihypertensive drugs ($P = 0.63$), age ($P = 0.74$), previous cardiovascular events ($P = 0.82$), phosphate ($P = 0.97$), and calcium ($P = 0.97$).

Fig. 2. Relationship between plasma ADMA and geometric pattern of the left ventricle. Data are median and inter-quartile range. *$P < 0.001$ vs. patients with eccentric hypertrophy or with normal LVM.

Fig. 3. Receiver operating characteristic (ROC) curves for plasma ADMA concentration in predicting concentric left ventricular hypertrophy (see Methods section; area, 0.73; 95% CI, 0.66 to 0.80; *P* < 0.001).
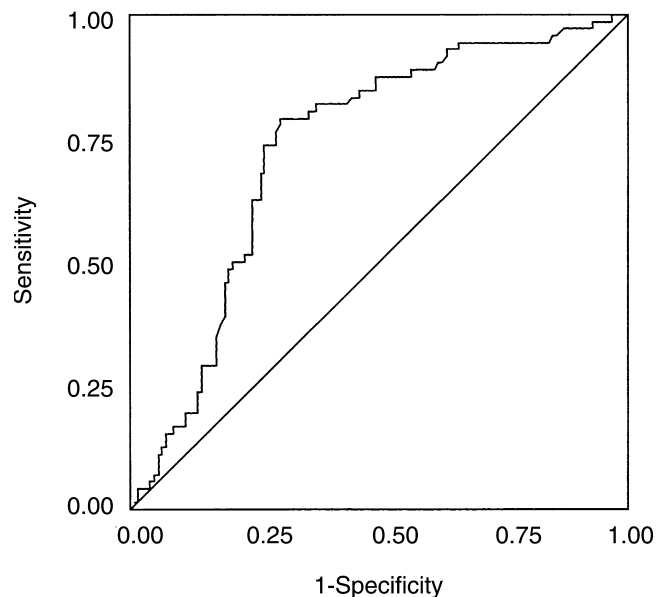
function and that this effect most likely depends on defective NO generation or bioavailability. This may influence later morbidity and mortality from large vessel atherosclerotic disease and LV hypertrophy.

ADMA in ESRD

After the article by Vallance et al [23] plasma ADMA was measured in at least 14 studies in patients with ESRD [4, 25–36] and always found to be elevated as compared to healthy subjects. It has been argued that it is unlikely that ADMA exerts meaningful biological effects at the plasma concentration measured in vivo in humans [32]. Although there is no conclusive proof that ADMA interferes with the cardiovascular system in uremic patients in vivo, most hemodynamic studies performed so far are compatible with the hypothesis that NOS inhibition is involved in the endothelial dysfunction of dialysis patients [8, 18–21]. Furthermore, cross-sectional [4] and prospective studies [5] have shown that plasma ADMA concentration is strongly associated with atherosclerotic compli-

cations, and that it predicts incident cardiovascular events in these patients.

ADMA and left ventricular mass and function in renal failure

Uremic patients are a population at very high cardiovascular risk. LVH represents a main component of such a high risk [2]. The link between endothelial function and vascular hypertrophy is well demonstrated in hypertensive humans [37]. Interestingly, in patients with essential hypertension LV mass is inversely related to forearm blood flow response to the endothelium–dependent vasodilating agent acetylcholine, and the endothelial dysfunction in these patients is particularly pronounced in hypertensives with concentric hypertrophy. The heart and

the arterial system form an integrated unit that coherently responds to hemodynamic stimuli and the endothelium plays a pivotal role in regulating cardiovascular remodeling. London et al demonstrated that cardiac and arterial remodeling proceed in parallel in uremic patients [38], and we recently confirmed these findings [39]. The relationship between endothelial dysfunction and cardiovascular remodeling in ESRD has been tested only in one study [8]. In this study the reactive hyperemic response of forearm blood flow (a parameter strongly influenced by endothelial function) was associated to common carotid artery intima media thickness and LV mass, suggesting that endothelial dysfunction may promote structural changes in the cardiovascular system in dialysis patients.

Our study is the first, to our knowledge, to establish a link between ADMA and left ventricular mass and geometry. ADMA emerged as an independent, high ranking correlate of LV mass in a statistical model including a series of well established determinants of this parameter in dialysis patients like systolic pressure, serum albumin, smoking and age. Interestingly, ADMA also was directly related to relative wall thickness, suggesting that this substance promotes concentric LVH. Accordingly, ADMA was two times higher in patients with concentric LVH than in those with eccentric LVH or normal LV mass. The link between ADMA and concentric LVH was so strong that post-hoc analysis of the ROC curve showed that plasma ADMA may have diagnostic potential for the identification of this LV geometric pattern. This intriguing possibility needs to be tested prospectively on the basis of *a priori* determined diagnostic thresholds established in large healthy populations. The strong link between ADMA and concentric LVH likely underlies a causal relationship because concentric LVH occurs in experimental models of chronic NO inhibition [7], and severe concentric hypertrophy and vascular damage is a hallmark in the knockout rat lacking the NOS and the ApoE genes [40]. As discussed, these observations are also in keeping with the strong association between endothelial dysfunction and concentric LVH in essential hypertensives [37]. Thus, ADMA may be an important factor mediating a process that may progress to important clinical complications ranging from heart failure to coronary and cerebrovascular involvement.

Nitric oxide production by the human myocardium is coupled with the inotropic state [41] and contributes to the modulation of LV function [42]. We speculate that the inverse association between ADMA and ejection fraction reported in this study reflects an interference with the functional role of NO on cardiac inotropism. In the aggregate the strong association between ADMA and concentric LVH and the inverse relationship between this substance and ejection fraction seem clinically relevant, because this LV geometric pattern and systolic dysfunction identify dialysis patients with the worst prognosis [43]. Intervention studies are needed to see whether the link between ADMA and concentric LVH remodeling and LV dysfunction is a causal one.

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