# Accumulation of Symmetric Dimethylarginine in Hepatorenal Syndrome

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In patients with cirrhosis, nitric oxide (NO), asymmetric dimethylarginine (ADMA), and possibly symmetric dimethylarginine (SDMA) have been linked to the severity of the disease. We investigated whether plasma levels of dimethylarginines and NO are elevated in patients with hepatorenal syndrome (HRS), compared with patients with cirrhosis without renal failure (no-HRS). Plasma levels of NO, ADMA, SDMA, and L-arginine were measured in 11 patients with HRS, seven patients with no-HRS, and six healthy volunteers. SDMA concentration in HRS was higher than in no-HRS and healthy subjects (1.47 ± 0.25 vs. 0.38 ± 0.06 and 0.29 ± 0.04 $\mu M$, respectively; $P < 0.05$). ADMA and $NO_x$ concentrations were higher in HRS and no-HRS patients than in healthy subjects (ADMA, 1.20 ± 0.26, 1.11 ± 0.1, and 0.53 ± 0.06 $\mu M$, respectively; $P < 0.05$; $NO_x$, 94 ± 9.1, 95.5 ± 9.54, and 37.67 ± 4.62 $\mu M$, respectively; $P < 0.05$). In patients with HRS there was a positive correlation between serum creatinine and plasma SDMA ($r^2 = 0.765$, $P < 0.001$) but not between serum creatinine and ADMA or $NO_x$. The results suggest that renal dysfunction is a main determinant of elevated SDMA concentration in HRS. Accumulation of ADMA as a result of impaired hepatic removal may be the causative factor initiating renal vasoconstriction and SDMA retention in the kidney. Exp Biol Med 231:70–75, 2006

Key words: nitric oxide; methylarginines; cirrhosis; renal failure

## Introduction

Hepatorenal syndrome (HRS), a major complication of end-stage cirrhosis, is characterized by functional renal failure and severe alterations in the systemic circulation (for review see Ref. 1). Impairment of kidney function is a consequence of marked reduction of renal blood flow probably caused by activation of specific vasoconstrictor systems including sympathetic nerves, renin–angiotensin, and arginine-vasopressin to counteract the vasodilatation of splanchnic circulation (1). Endothelial nitric oxide (NO) seems to play a decisive role in determining the decrease in splanchnic vascular resistance observed in decompensated cirrhosis (2). Asymmetric dimethylarginine (ADMA), a guanidino-substituted analogue of L-arginine, is synthetized endogenously and can act as inhibitor of NO synthase (3), the enzyme responsible for the formation of NO from L-arginine. Symmetric dimethylarginine (SDMA), a stereoisomer of ADMA, has no inhibitory effect on NO synthase but may interfere with NO synthesis by competing with L-arginine for transport across cell membrane (3). ADMA is excreted in part by the kidneys, but the main metabolic pathway is degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to L-citrulline (4). Nijveldt *et al.* (5) hypothesized a causal role for ADMA in the development of HRS. They proposed that accumulation of ADMA, probably caused by impaired hepatic removal, may have detrimental effects on renal function by inhibiting NO synthesis, thereby interfering with renal blood flow and glomerular filtration (5). In contrast to ADMA, SDMA is eliminated by renal clearance and cannot be degraded by DDAH (4). Recently, Siroen and co-workers (6) have shown that the human liver takes up substantial amounts of SDMA from the portal and systemic circulation and suggested that high plasma levels of SDMA may have hemodynamic consequences similar to those reported for ADMA. To date, increased ADMA blood levels have been documented in patients with end-stage liver disease before liver transplantation (7) and in alcoholic cirrhosis (8). In patients with alcoholic cirrhosis, increased ADMA and NO levels correlate strongly with severity of the disease. However, plasma levels of SDMA are within normal values in alcoholic cirrhosis (8).

Plasma levels of dimethylarginines and NO in HRS are

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not known. We hypothesized that the renal failure that accompanies cirrhosis might trigger changes in plasma levels of NO and dimethylarginines different from those observed in human alcoholic cirrhosis with normal renal function. Accordingly, the aim of the present study was to evaluate plasma levels of dimethylarginines (ADMA and SDMA) and NO, measured as plasma nitrite plus nitrate ($NO_x$ concentration), in patients with HRS and in patients with cirrhosis without renal failure.

## Materials and Methods

Eleven patients with HRS (eight men, three women; mean age 54 ± 3 years) were included in the study. Seven patients had Type 1 HRS and four Type 2 HRS. The HRS was diagnosed according to the criteria proposed by the International Ascites Club (9): a low glomerular filtration rate as assessed by serum creatinine concentration greater than 1.5 mg/100 ml, proteinuria less than 500 mg/day, absence of shock, bacterial infection and fluid losses, no improvement of renal function (as based on serum creatinine levels) after oral diuretic withdrawal and plasma volume expansion, and no ultrasonographic evidence of renal disease or uninary tract obstruction. The diagnosis of cirrhosis was based on clinical, biochemical, and ultrasonographic findings in all patients. In eight patients, the diagnosis was confirmed by liver biopsy. All patients had moderate to severe ascites. The presence of ascites was confirmed by diagnostic paracentesis. According to the Pugh classification (10), eight patients belonged to class C, and three patients to class B, with a Child-Pugh score above 8. The cause of cirrhosis was alcoholic in six, hepatitis C virus in three, and hepatitis B virus in two. None of the studied subjects had heart failure, hypertension, or diabetes mellitus.

The results obtained in the HRS were compared with those from a group of patients with cirrhosis without renal failure. This group comprised seven patients (five men, two women; mean age 59 ± 3 years) with alcoholic (4 patients) or virus-related cirrhosis (three patients). All the patients in this group had moderate to severe ascites. These patients belonged to class B–C with a Child-Pugh score above 8 and had normal renal function. In addition, another group consisted of six healthy subjects (four men, two women; mean age 58 ± 3 years) with normal laboratory findings. The study was undertaken with the approval of the local ethics committee and after obtaining informed consent from each patient.

**Determination of L-Arginine and Dimethylarginines.** Plasma concentrations of L-arginine, ADMA, and SDMA were accomplished by high-performance liquid chromatography using a modification of a previously described method (3). In brief, dimethylarginines from 1 ml of plasma were purified with Bond Elut SCX columns and eluted with 4 ml of methanol containing 30% distilled water and 2% triethylamine. The eluent was then evaporated to dryness at 60°C, and the dried extract was redissolved in running buffer. High-pressure liquid chromatography was carried out on a Shimadzu chromatography system (Shimadzu Corporation, Kyoto, Japan). Separation of dimethylarginines was achieved with a 250 × 4.6-mm (inner diameter), 5-μm Kromasil C18 analytic column using 25 m*M* phosphoric acid containing 10 m*M* hexane sulfonic acid and 1% (v/v) acetonitrile, pH 5.0. The analysis was carried out at a flow rate of 1.3 ml/min, and the absorbance monitored at 200 nm. Concentrations of L-arginine, ADMA, and SDMA in the samples were determined by comparison with standards. The variability of the method was below 7%, and the detection limit of the assay was 0.1 μ*M*.

**Determination of Nitrite Plus Nitrate ($NO_x$).** NO is rapidly converted to nitrite and nitrate in human plasma. In our study, plasma $NO_x$ levels were measured in triplicate after conversion of nitrate to nitrite by nitrate reductase and nitrite measured by using the Griess reaction, as described previously (11). The intra- and interassay coefficients of variation were 3% and 7%, respectively. Recoveries of both nitrites and nitrates in our samples were greater than 95%.

**Statistical Analysis.** Results are expressed as mean ± SE. Data were analyzed by ANOVA and Fisher's multiple comparison tests. The unpaired *t* test was applied for comparisons of means of study groups. Statistical significance was accepted when *P* values were less than 0.05. Linear regression analyses and correlation coefficients were calculated according to the least-squares methods. The statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) version 12 for Windows.

## Results

Table 1 shows the main clinical and laboratory data of healthy subjects, patients with HRS, and cirrhotic patients without HRS. Both groups of patients were similar with respect to the severity of liver failure. Moreover, there were no significant differences between the two groups with respect to the other baseline variables except for serum creatinine.

The plasma concentrations of ADMA, SDMA, $NO_x$, and L-arginine are presented in Figure 1. In the HRS group, SDMA concentration was about fourfold higher compared to healthy subjects and patients with cirrhosis with normal kidney function (1.47 ± 0.25 vs. 0.29 ± 0.04 and 0.38 ± 0.06 μ*M*, respectively; $P < 0.05$). ADMA concentrations were higher in both groups of patients when compared to controls (1.20 ± 0.26 in HRS, 1.11 ± 0.10 in cirrhosis without HRS, and 0.53 ± 0.06 μ*M* in healthy subjects; $P < 0.05$ between patients and controls). No significant differences in ADMA concentrations were observed between HRS and cirrhosis with normal renal function ($P = 0.33$).

$NO_x$ concentrations were higher in both groups of patients than in controls (94.00 ± 9.15 μ*M* in HRS, 95.50 ± 9.54 μ*M* in cirrhosis with no HRS, and 37.67 ± 4.62 μ*M* in healthy subjects; $P < 0.05$). No significant differ-

**Table 1.** Clinical and Laboratory Findings

| | Healthy controls | Cirrhosis with HRS | Cirrhosis without HRS |
|---|---|---|---|
| Number | 6 | 11 | 7 |
| Men/women | 4/2 | 8/3 | 5/2 |
| Age (years) | 58 ± 3 | 54 ± 3 | 59 ± 3 |
| Child-Pugh score | — | 10.5 ± 0.5 | 10.0 ± 0.4 |
| Mean arterial pressure (mm Hg) | 85 ± 4 | 76 ± 4 | 80 ± 3 |
| Albumin (g/dL) | 4.2 ± 0.1 | 2.7 ± 0.2* | 3.0 ± 0.2* |
| Bilirrubin (mg/dL) | 0.6 ± 0.1 | 5.1 ± 1* | 4.0 ± 0.6* |
| Prothrombin time (%) | 93.2 ± 0.6 | 50.7 ± 4.1* | 54.0 ± 4.0* |
| Serum creatinine (mg/dL) | 0.9 ± 0.1 | 2.6 ± 0.4*,** | 1.1 ± 0.1 |

* $P < 0.05$ *vs.* control; ** $P < 0.05$ *vs.* cirrhosis without HRS.

ences were observed between patients with or without HRS ($P = 0.49$). L-Arginine levels were not significantly different among the three groups.

In patients with HRS, a significant positive correlation was observed between SDMA and serum creatinine ($r^2 = 0.765$, $P < 0.001$) but not between ADMA and creatinine ($r^2 = 0.038$, $P = 0.564$) (Fig. 2). No correlation was shown between $NO_x$ and creatinine ($r^2 = 0.024$, $P = 0.648$).

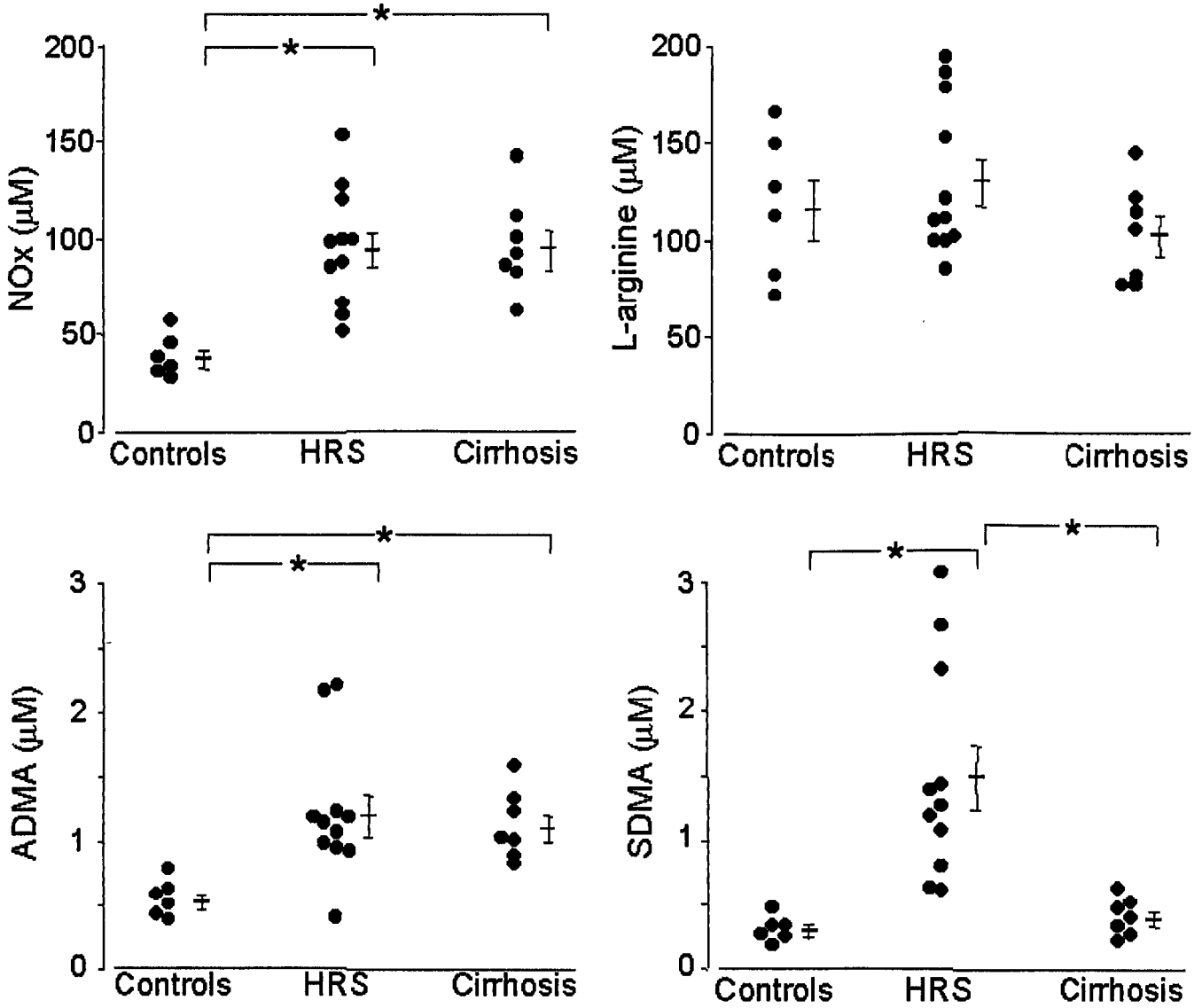


**Figure 1.** Individual plasma concentration of $NO_x$, L-arginine, ADMA, and SDMA in healthy subjects (n = 6), patients with hepatorenal syndrome (HRS, n = 11), and patients with cirrhosis without renal dysfunction (n = 7). Horizontal bars indicate mean ± SE. *$P < 0.05$.

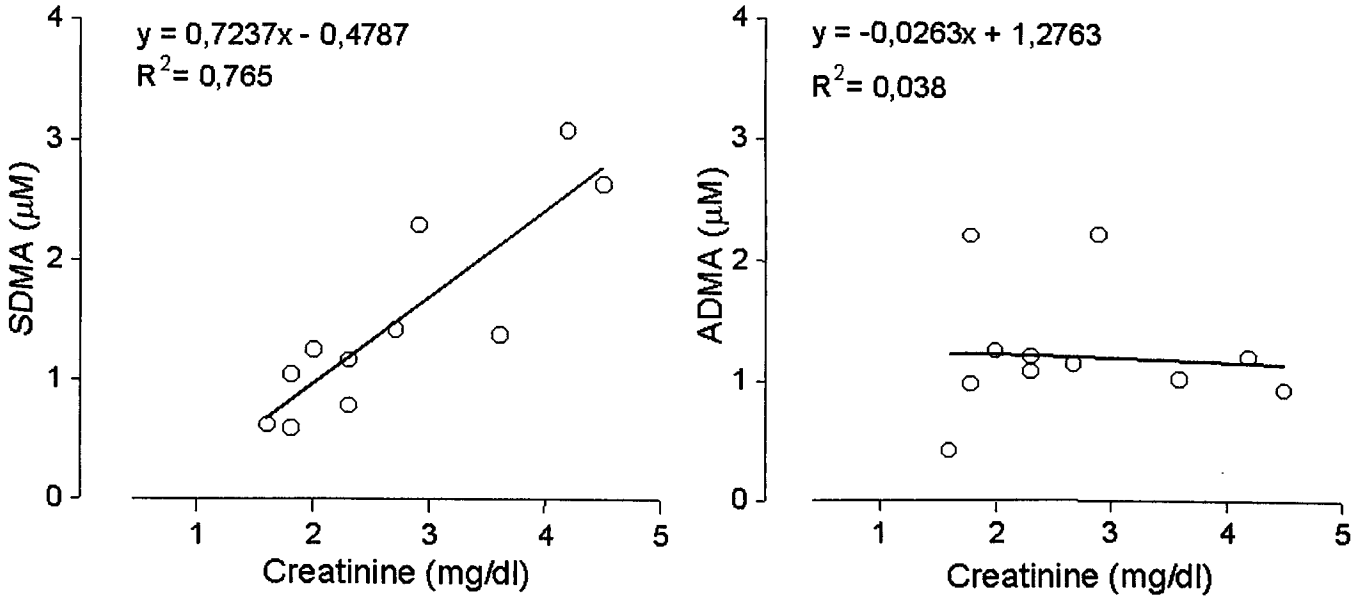


**Figure 2.** Correlation analyses between serum creatinine SDMA and ADMA in patients with hepatorenal syndrome.

The molar ratios between L-arginine and ADMA and SDMA concentrations are shown in Figure 3. Although the ratio between L-arginine and ADMA was significantly lower in the two groups of patients when compared with healthy subjects, the L-arginine/SDMA molar ratio was markedly decreased only in patients with HRS.

## Discussion

The main finding of this study is that plasma concentrations of $NO_x$, ADMA, and SDMA are elevated in patients with HRS compared with control subjects. SDMA, but not ADMA, is positively correlated with serum creatinine in patients with HRS. $NO_x$ and ADMA were increased in all patients compared with healthy subjects. A recent study in alcoholic cirrhosis has shown that plasma concentrations of both $NO_x$ and ADMA are positively correlated with the degree of liver failure (8). Because in the present report the two groups of patients revealed similar liver impairment, as indicated by the Child-Pugh score, no differences in $NO_x$ and ADMA plasma levels were observed. The rise in ADMA levels coinciding with an increase in $NO_x$ in cirrhotic patients has been interpreted as a compensatory mechanism to counterbalance excessive peripheral vasodilatation in the splanchnic vascular bed in response to increased synthesis of NO (8).

SDMA plasma levels do not correlate with the clinical score and remain within normal values in alcoholic cirrhosis with normal renal function (8) and in patients with end-stage liver failure before liver transplantation (7). This strongly suggests that the high levels of SDMA observed in our group of patients with HRS are caused by impairment of renal function. As a consequence, SDMA was not correlated with $NO_x$, but it was significantly correlated with serum creatinine. A positive correlation between plasma SDMA concentrations and serum creatinine has been previously demonstrated in patients with chronic renal failure from primary renal disease in the absence of liver dysfunction (3, 13, 15). After kidney transplantation, the concentrations of SDMA returned to baseline values (15). On the other hand, we found no correlation between plasma levels of ADMA and serum creatinine, which indicates that the increase in plasma ADMA in patients with HRS was not caused by a decrease in renal clearance of ADMA.

The liver plays an important role in the metabolism of ADMA by taking up large amounts from the systemic circulation (5). The enzyme dimethylarginine dimethylaminohydrolase (DDAH) is highly expressed in the liver (12) and removes ADMA by metabolizing it to L-citrulline and dimethylamine (4). A decrease in the activity of DDAH in the liver would lead to an increase in ADMA and a decrease in the local production of NO. A decrease in NO production by sinusoidal endothelial cells in the cirrhotic liver is an important factor in the development and maintenance of portal hypertension (2). In contrast to ADMA, SDMA is eliminated by renal clearance and cannot be degraded by DDAH (4). The significance of high plasma levels of SDMA in HRS is uncertain because there is no evidence that it may inhibit NO synthase (3). However, SDMA at high concentrations may interfere with NO production by blocking cellular L-arginine uptake (3, 14). Indeed, in primary chronic renal failure, the high plasma levels of ADMA and SDMA seem to be responsible for the increase in systemic arterial pressure (15). Therefore, SDMA, and not only ADMA, is likely to be one factor responsible for the increased intrarenal vascular resistance observed in HRS.

The pathogenesis of renal vasoconstriction in cirrhosis is multifactorial (1). Several lines of evidence suggest that

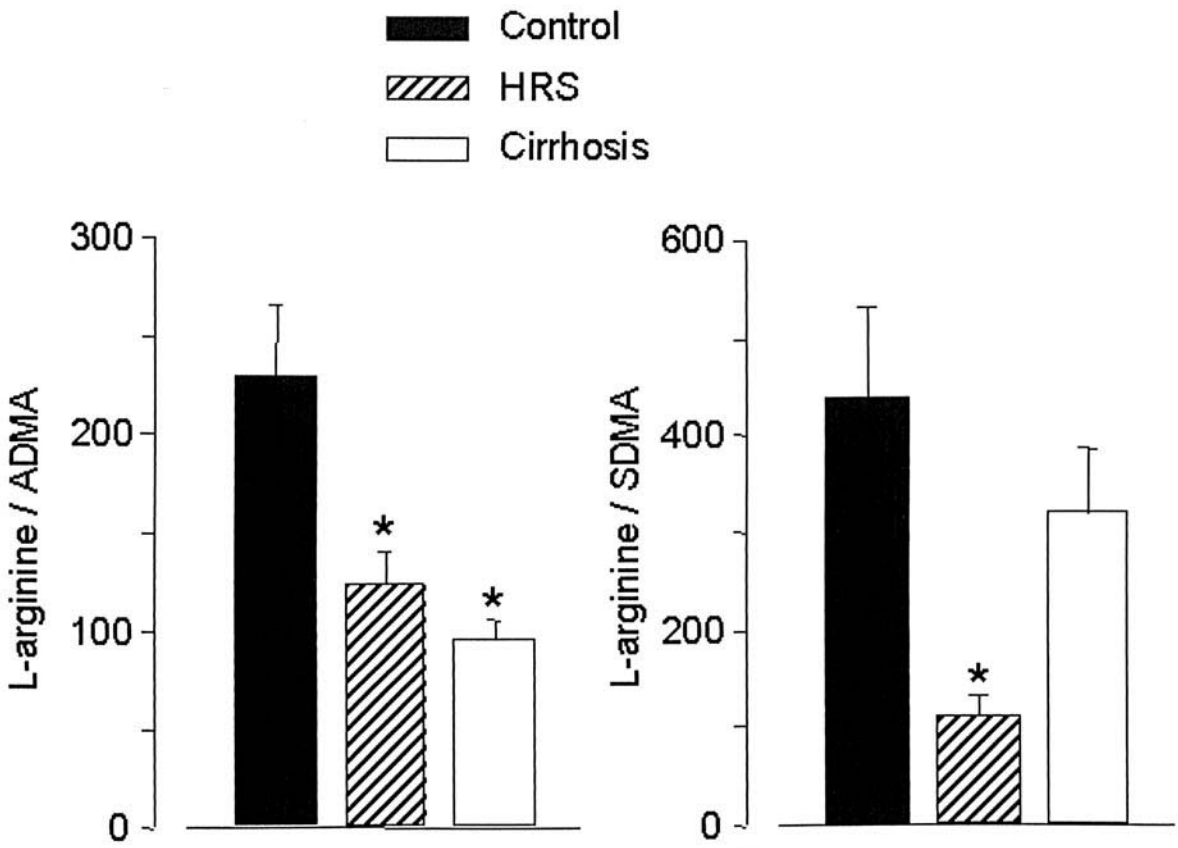


**Figure 3.** Plasma L-arginine/ADMA molar ratio and L-arginine/SDMA molar ratio in healthy subjects (n = 6), patients with hepatorenal syndrome (HRS, n = 11) and patients with cirrhosis with normal renal function (n = 7). Data are mean ± SE. *$P < 0.05$.

splanchnic arterial vasodilatation caused by an increased synthesis of NO plays a main role in the initiation of reduced renal perfusion. Intravenous administration of NO synthase inhibitors to cirrhotic patients increases renal blood flow and glomerular filtration rate, probably through an increase in renal perfusion caused by an increment in systemic arterial pressure in these patients (16). These findings led to the suggestion that NO synthase inhibitors might be useful in the treatment of ascites in cirrhosis (17). However, experiments in cirrhotic rats raise the possibility that NO blockade may have deleterious effects by increasing intrahepatic vascular resistance (18). Moreover, it is possible that the plasma concentrations of ADMA achieved in cirrhosis could be biologically effective in renal vessels. With regard to this, it has been proposed that increased ADMA in hepatic dysfunction plays an important role in the development of renal failure in patients with cirrhosis (5). Indeed, ADMA elicits contractile effects on human renal (19) and cerebral (20) arteries. In addition, NO synthase inhibitors enhance vascular contractile responses to adrenergic agonists and sympathetic stimulation of human arteries (21, 22). Thus, elevated ADMA levels could promote renal vasoconstriction by blocking NO synthesis in the endothelium of renal vessels as well as potentiating the effects of perivascular sympathetic nerves. This would lead to impairment of renal function and SDMA retention.

In conclusion, the present study demonstrated an elevation of $NO_x$, ADMA, and SDMA in patients with HRS. Only SDMA correlates positively with serum creatinine. These findings have two important implications. First, they suggest that SDMA may be a marker of renal dysfunction in cirrhosis. Second, accumulation of ADMA, caused by impaired hepatic removal, may be a factor determining not only the elevated intrahepatic vascular resistance but also the renal vasoconstriction and SDMA retention by the kidney.

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