The clinical significance of asymmetric dimethylarginine

Siroen, M.P.C.

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The human liver clears both asymmetric and symmetric dimethylarginine

M.P.C. Siroen[1]
J.R.M. van der Sijp[1]
T. Teerlink[2]
C. van Schaik[3]
R.J. Nijveldt[1]
P.A.M. van Leeuwen[1]

Departments of Surgery[1], Clinical Chemistry[2], and Radiology[3]
VU University Medical Center, Amsterdam, The Netherlands

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ABSTRACT

Asymmetric (ADMA) and symmetric dimethylarginine (SDMA) inhibit production of nitric oxide. The concentration of both dimethylarginines is regulated by urinary excretion, while ADMA, but not SDMA, is also subject to degradation by dimethylarginine dimethylaminohydrolase which is highly expressed in the liver but also present in the kidney. The exact roles of the human liver and kidney in the metabolism of dimethylarginines are currently unknown. Therefore, we aimed to investigate renal and hepatic handling of ADMA and SDMA in detail in 24 patients undergoing hepatic surgery. In order to calculate net organ fluxes and fractional extraction (FE) rates, blood was collected from an arterial line, the portal vein, hepatic vein, and renal vein and blood flow of the hepatic artery, portal vein, and renal vein was determined using Doppler ultrasound techniques. Results showed a significant net uptake (median (IQR)) of ADMA in both the liver (9.6 nmol/min (5.6-13.2)) and the kidney (12.1 nmol/min (1.3-17.1)). SDMA uptake was not only present in the kidney (12.7 nmol/min (3.5-25.4)), but also in the liver (7.7 nmol/min (2.8-16.4)). FE rates of ADMA for the liver and kidney were 5.0% (3.5-7.4) and 8.4% (1.3-13.9), respectively. For SDMA, hepatic and renal FE rates were 3.4% (2.1-7.5) and 12.5% (3.6-16.2), respectively. In conclusion, this study gives a detailed description of the hepatic and renal elimination of dimethylarginines and reveal that the clearing of SDMA is not only confined to the kidney, but the human liver also takes up substantial amounts of SDMA from the portosystemic circulation.

INTRODUCTION

In 1992 it was discovered that asymmetric dimethylarginine (ADMA) plays a regulatory role in the arginine-nitric oxide (NO) pathway by inhibiting the enzyme NO synthase.[1] In addition, ADMA is able to interfere with NO synthesis by competing with arginine and symmetric dimethylarginine (SDMA) for cellular transport across cationic amino acid transporters (CAT) of system y^+.[2] Since these observations, there has been growing interest in ADMA, especially with respect to cardiovascular disease and the role of ADMA as a novel cardiovascular risk factor is emerging.[3] Thus, it is of great importance to understand which organs are of relevance in the metabolism of dimethylarginines. Both ADMA and SDMA are removed from the body by urinary excretion and the role of the kidney in the metabolism of dimethylarginines has been extensively studied.[4-6] Several reports have shown elevated concentrations of ADMA and SDMA in patients with renal failure and ADMA has emerged as a predictor of mortality and cardiovascular outcome in these patients.[7-10] Although SDMA has no direct inhibitory activity towards the enzyme NO synthase, Fleck and co-workers[11] pointed out the potential importance of SDMA, and concluded in their study in a large population of renal failure patients, that not only ADMA levels but also SDMA levels were likely responsible for hypertension in these patients, possibly by competition for reabsorption between SDMA and arginine in the kidney.

Besides elimination of ADMA via the kidney, ADMA is also degraded by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) which is highly expressed in the liver, but also present in the kidney, the pancreas, and in endothelial cells.[12,13] Interestingly, the liver abundantly expresses CAT,[14] suggesting a high hepatic capacity for uptake of ADMA and SDMA from the circulation. Unfortunately, the potential role of the liver in the metabolism of dimethylarginines has been underexposed. Carnegie and coworkers[15] pointed out the potential role of the liver in the metabolism of ADMA by reporting an increased urinary excretion of ADMA in patients with liver disease. However, from this study no precise data on the hepatic metabolism of ADMA can be derived, as only urinary concentrations were measured. In an organ balance study in rats, we[16] found a very high uptake of ADMA by the liver, suggesting a crucial role for the liver in regulating systemic ADMA concentrations. In addition, critically ill patients with hepatic failure have seriously elevated levels of ADMA.[17] These results were confirmed in patients eligible for liver

transplantation who had highly elevated ADMA levels that decreased significantly after successful liver transplantation, indicating an important role of the liver in the metabolism of ADMA.[18] Recently, Lluch and coworkers[19] also reported elevated ADMA levels in cirrhotic patients and suggested that ADMA might oppose the peripheral vasodilation caused by excessive systemic NO production during liver cirrhosis. Dimethylarginines may play an important pathophysiological role in liver cirrhosis because this disease is characterised by endothelial dysfunction and NO deficiency in the intrahepatic circulation. Since the exact contribution of the human liver and kidney in the metabolism of dimethylarginines is unknown, we studied hepatic and renal handling of both ADMA and SDMA in detail in patients undergoing hepatic surgery.

PATIENTS and METHODS

The study was approved by the institutional review board and medical ethical review committee. Before study entry, patients were informed on the purpose of the study and informed consent was obtained.

Patients

The study population consisted of 24 patients undergoing partial liver resection because of colorectal metastases who were admitted to the department of surgery of the VU University Medical Center in Amsterdam, The Netherlands. Patients were included if: 1. less than 10% of total liver volume contained liver metastases, 2. metastases were limited to either the left (segment I-IV) or right (segment V-VIII) lobe of the liver, 3. extrahepatic disease was excluded by abdominal and thoracic computer tomography. Patients with conditions known to influence metabolism of dimethylarginines (hypercholesterolemia, hyperhomocysteinemia, diabetes mellitus, renal or hepatic dysfunction) were not included in the study. In addition, patients had no signs of infection prior to the operation.

Doppler ultrasound measurements

During the operation, all patients were examined by means of colour Doppler ultrasound (Aloka Prosound SSD 5000, Aloka Co., Ltd, Tokyo, Japan). All measurements were performed by one senior radiologist (CvS) with more than 10 years of experience in Doppler examination of deep abdominal vessels. After incision of the abdominal wall and exposure of the liver, but before start of the resection, a sterile transducer (Aloka 5-10 MHz linear UST-579T-7,5, Aloka Co., Ltd, Tokyo, Japan) was put on the hepatoduodenal ligament to trace the portal vein and hepatic artery. For accurate measurements, care was taken to keep the angle between the ultrasonic beam direction and the blood flow direction below 60°. The time-averaged mean velocity of the blood flow in the hepatic artery was measured where a straight stretch runs parallel to the portal vein. At least three cardiac cycles were obtained for the analysis. The cross-sectional area of the vessel was calculated automatically by drawing an area ellipse at the same point the velocity measurement was performed. For the portal vein, time-averaged mean velocity and cross-sectional area were measured before it branched into the left and right portal trunk. Via the same methods, time-averaged mean velocity and cross-sectional area of the right renal vein were measured. Hepatic arterial, portal venous, and renal venous blood flows were calculated from the product of the time-averaged mean velocity of blood in the vessel and the cross-sectional area of the lumen of the vessel. Plasma flow was computed by correction for hematocrit (plasma flow = blood flow x (1 - hematocrit)) which was determined during the Doppler measurements.

Blood sampling and analysis

Directly after these measurements, blood samples were collected from an indwelling arterial line, from the portal vein, from the hepatic vein, and from the right renal vein to avoid interference with blood from the testicular or ovarian vein. ADMA, SDMA, and arginine plasma concentrations were measured by high-performance liquid chromatography (HPLC) with fluorescence detection, as recently described.[20] Briefly, 0.1 mL of plasma was mixed with 0.1 mL of a 40 µmol/L solution of the internal standard MMA and 0.8 mL phosphate buffered saline (PBS). This mixture was applied to Oasis MCX solid-phase extraction columns (Waters, Milford, MA, USA) for extraction of basic amino acids. The columns were consecutively washed with 1.0 mL of 100 mM HCl and 1.0 mL methanol. Analytes were eluted with 1.0 mL of

concentrated ammonia/water/methanol (10/40/50). After evaporation of the solvent under nitrogen, the amino acids were derivatised with ortho-phthaldialdehyde reagent containing 3-mercaptopropionic acid. The derivatives were separated by isocratic reversed-phase chromatography on a Symmetry C18 column (3.9 x 150 mm; 5µm particle size; Waters). Potassium phosphate buffer (50 mM; pH 6.5), containing 8.7% acetonitrile was used as mobile phase at a flow-rate of 1.1 mL/min and a column temperature of 30°C. Fluorescence detection was performed at excitation and emission wavelengths of 340 and 455 nm, respectively. After elution of the last analyte, strongly retained compounds were quickly eluted by a strong solvent flush with 50% acetonitrile, resulting in a total analysis time of 30 min. Intra-assay coefficients of variation (CVs) were 1.2% for ADMA, 0.8% for SDMA, and 0.4% for arginine. Inter-assay CVs for ADMA, SDMA, and arginine were 2.0%, 2.6%, and 2.9%, respectively.
Laboratory parameters indicating renal and hepatic function were analysed by routine laboratory procedures.

Calculations
Load, net production, fractional extraction (FE) rates, and net organ fluxes of ADMA, SDMA, and arginine were calculated for the liver and the kidney. The corresponding quantities for the liver were determined as follows: Load = F_{ha}·[A] + F_{pv}·[PV]; Net production = $F_{(ha + pv)}$·[HV]; FE = (load - net production)/load; Net flux = F_{ha}·([A] - [HV]) + F_{pv}·([PV] - [HV]), where F_{ha} denotes plasma flow in the hepatic artery, F_{pv} denotes plasma flow in the portal vein, and [A], [PV], and [HV] denote arterial, portal vein, and hepatic vein concentrations, respectively.
Kidney fluxes and renal extraction for ADMA, SDMA, and arginine were calculated from the plasma flow and the arteriovenous concentration difference of the right kidney. Net kidney flux is calculated as F_{rv}·([A] - [RV]), where F_{rv} denotes plasma flow in the renal vein, and [A] and [RV] denote arterial and renal vein concentrations. Renal extraction is calculated as [A]-[RV]/[A].
A positive net organ flux and fractional extraction rate means net uptake of a substance, whereas a negative sign indicates net release. Each parameter was calculated for each individual patient using its individual substrate concentrations and plasma flows. FE and organ fluxes were considered significant if the interquartile range (IQR) did not include zero.

RESULTS

Patients

Patient characteristics are presented in **Table 1**. On the day before the operation procedure, all patients had normal renal function as determined by creatinine plasma level, creatinine clearance, and urea concentration and proper liver function as determined by bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (AP), prothrombin time (PT), and albumin.

Table 1: Demographic data

Number of patients	24	
Gender: male / female	17 / 7	
Age: median (range)	63	(37-79)
Biochemical markers of hepatic function		
Bilirubin (μmol/L)	8	(6-10)
AST (U/L)	21	(18-26)
ALT (U/L)	18	(15-28)
LD (U/L)	372	(320-455)
γ-GT (U/L)	37	(23-67)
AP (U/L)	96	(80-124)
PT (sec)	1.00	(0.98-1.05)
Albumin (g/L)	39	(36-40)
Biochemical markers of renal function		
Creatinine (μmol/L)	88	(77-95)
Creatinine clearance (mL/min)	78	(65-101)
Urea (mmol/L)	5	(4.1-5.7)

Data are presented as median and IQR unless otherwise stated.

Hepatic and renal plasma flow

Mean arterial blood pressure remained stable in all patients during the Doppler ultrasound measurements. Median (IQR) hematocrit level was 0.31 (0.29-0.33). In **Table 2**, hepatic and renal plasma flows are presented. From this table, it can be concluded that hepatic artery flow constitutes 17% and portal vein flow constitutes 83% of total liver plasma flow. These percentages are in concordance with previous findings.[21]

Table 2: Hepatic and renal plasma flow (ml/min)

Liver		
Portal vein	306	(179-417)
Hepatic artery	62	(39-188)
Total	359	(253-569)
Kidney		
Renal vein	300	(138-457)

Data are presented as median and IQR.

Plasma concentrations, fluxes, and fractional extraction rates of ADMA, SDMA, and arginine

Table 3 shows plasma concentrations of ADMA, SDMA, and arginine. Net organ fluxes of ADMA, SDMA, and arginine for the liver and the kidney are shown in **Figure 1^{A}** and **1^{B}**. It should be noted that a positive sign indicates net uptake of a substance, while a negative sign indicates net release. Fractional extraction rates of ADMA, SDMA, and arginine for the liver and the kidney are presented in **Table 4**.

Table 3: Plasma concentrations of ADMA, SDMA, and arginine (µmol/L)

	ADMA		SDMA		arginine	
Artery	0.45	(0.40-0.53)	0.52	(0.46-0.56)	58	(45-71)
Portal vein	0.47	(0.43-0.53)	0.54	(0.49-0.59)	61	(41-74)
Hepatic vein	0.44	(0.40-0.49)	0.49	(0.47-0.54)	49	(32-65)
Renal vein	0.40	(0.36-0.48)	0.46	(0.45-0.50)	59	(45-70)

Data are presented as median and IQR.

Figure 1^{A}: Net organ fluxes of ADMA and SDMA

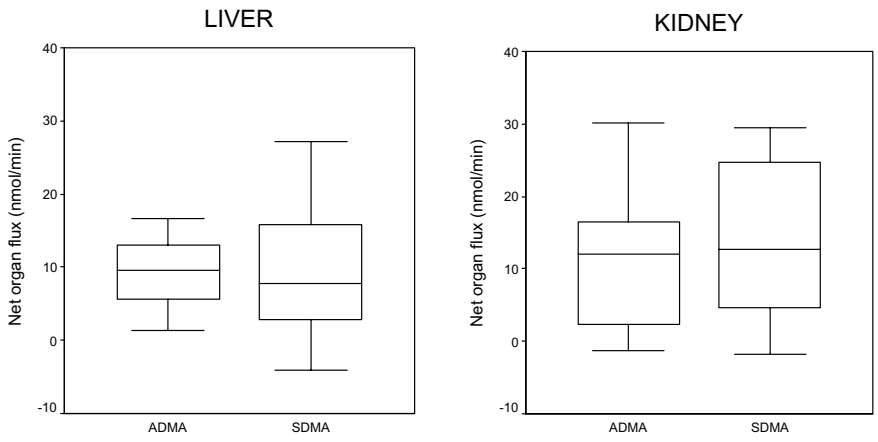

Figure 1[B]: Net organ flux of arginine

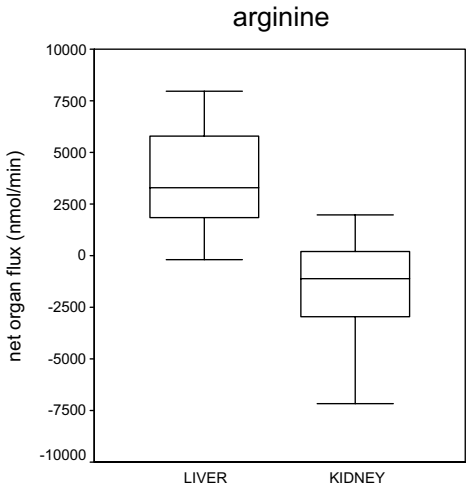

Table 4: Fractional extraction rates of ADMA, SDMA, and arginine (%)

	ADMA		SDMA		arginine	
Liver	5.0	(3.5-7.4)	3.4	(2.1-7.5)	8.6	(-3.7-25.8)
Kidney	8.4	(1.3-13.9)	12.5	(3.6-16.2)	-10.9	(-28.2-3.7)

Data are presented as median and IQR.

DISCUSSION

This study gives detailed insight into the hepatic and renal handling of dimethylarginines in humans. The combination of peri-operative plasma flow with arteriovenous concentration differences enabled us to calculate fractional extraction rates and fluxes of ADMA, SDMA, and arginine for the liver and the kidney; both important organs in the metabolism of arginine and dimethylarginines.

The most important and novel finding of this study was the hepatic extraction of SDMA which is in contrast with the results of an organ balance study in rats in which SDMA was not affected by the liver.[16] Therefore, this unexpected finding warrants further discussion. Urinary excretion is a generally accepted process modulating SDMA concentration in humans and, unarguably, SDMA cannot be degraded by DDAH which is highly expressed in the liver. Interestingly, Ogawa and coworkers[22] demonstrated the presence of other, relatively unknown metabolic routes for

dimethylarginines. In a rat model, they found that both dimethylarginines were metabolised by pathways forming the corresponding α-ketoacid analogues and the oxidatively decarboxylated products of the α-ketoacids in addition to N-acetyl conjugates. The amounts metabolised by these pathways were very small in comparison with the catabolism by DDAH. Possibly, the importance of these minor alternative metabolic routes is more significant in the human liver.

Since ADMA is regarded as the predominant endogenous inhibitor of NO production, most studies in the field of dimethylarginines focus solely on ADMA. Its role in cardiovascular disease is emerging and ADMA appears to be an important causal factor in dysfunction of the vascular system. Dimethylarginines are synthesised by post-translational modification, involving addition of methyl groups to arginine residues in proteins by enzymes called protein arginine methyltransferases. These methylated proteins are predominantly found in the nucleus and play a role in RNA processing and transcriptional control.[23] Methylarginines are released when these proteins are hydrolysed, thereby being an obligatory product of protein turnover. Several studies show that ADMA accumulates in renal failure, and ADMA has been held responsible for the cardiovascular complications accompanying renal failure. However, during renal insufficiency both dimethylarginines are elevated and several studies have shown that this increase is more pronounced for SDMA.[8,11,24,25] Interestingly, Fleck and coworkers[11] have shown that SDMA, and not only ADMA, is likely to be responsible for hypertension seen in patients with chronic renal failure. More recently, the same research group revealed that SDMA levels in a large group of patients with renal insufficiency were significantly higher in those who had coronary heart disease or left ventricular hypertrophy compared with those without cardiac co-morbidity.[24] In addition, SDMA concentration was increased additionally in all patients with hypertension in comparison with renal patients with normal blood pressure. Thus, although SDMA is not able to directly inhibit NO synthase, it may be of clinical significance by reducing substrate availability of NO synthase through competition with arginine for the y^+ pump.

Arginine, methylarginines, and other cationic amino acids such as ornithine and lysine are transported into endothelial cells by the y^+ transporter. Closs and coworkers[2] investigated transport of dimethylarginines by CAT, and found that both ADMA and SDMA were transported across this y^+ carrier. Theoretically, changes in the expression of CAT mRNA may influence dimethylarginine transport and the

abundant expression of CAT-2 mRNA in the liver points to a potentially high uptake of dimethylarginines in this organ.[14] This is confirmed in our study which showed a significant net hepatic extraction of both ADMA (5.0%) and SDMA (3.4%). Furthermore, metabolic data of the kidney revealed fractional extraction rates of 8.4% for ADMA and 12.5% for SDMA. Of interest is the smaller extraction of ADMA as compared with our previous findings in fasting humans undergoing elective cardiac catheterisation showing renal extraction rates of 16.2% for ADMA and 10.5% for SDMA.[5] Possibly, metabolic changes during general anaesthesia, a condition which was unavoidable in the investigation of hepatic and renal handling of dimethylarginines in humans, are responsible for the difference in ADMA clearance between these two studies. Compared with rats, our results in humans show quite lower extraction rates. In the rat kidney, fractional extraction rates of approximately 35% for ADMA and 30% for SDMA have been reported.[6,16] For the liver, fractional extraction rates of approximately 30% for ADMA were shown, while no significant hepatic uptake of SDMA was found in rats.[16,26] Studies performed in the seventies and eighties investigated urinary recovery of intravenously injected amino acids in the urine of rabbits and rats.[22,27] However, by solely determining urinary concentrations, metabolic degradation of dimethylarginines is neglected and therefore, fractional renal extraction rates could not be computed.

Based on data on dimethylamine excretion, Achan and coworkers[28] estimated the total body production of ADMA at 300 μmol/day. From the results of our study, it can be calculated that approximately 50 μmol/day is extracted from the systemic circulation by the liver and the kidney. This may seem a relatively small fraction of whole body ADMA production, but it is important to consider that both formation of free ADMA, i.e. by proteolysis of methylated proteins, and its degradation by DDAH are intracellular processes. Thus, a substantial amount of ADMA does not reach the circulation but is degraded intracellularly into dimethylamine which is transported to the kidney for urinary excretion. Unarguably, both the kidney (urinary excretion and degradation by DDAH) and the liver (degradation by DDAH) play a key role in the metabolism of ADMA, thereby regulating systemic plasma concentrations of ADMA.[1,16] The presence of DDAH at other locations, e.g. brain, endothelium, pancreas, and cells of the immune system, does not necessarily mean that these organs contain DDAH in order to regulate plasma ADMA levels but it is more likely that DDAH at these locations is present to regulate their intracellular NO production.

Blood samples from both the afferent vessel(s) and efferent vessel of the liver and kidney can only be collected during the operation. In order to accurately measure concentrations of dimethylarginines, we used our HPLC method which has a high precision and sensitivity and has proven to be a valuable tool in the research on the metabolism of ADMA and SDMA in different clinical conditions.[20] For calculation of fractional extraction rates and fluxes, organ blood flow must be determined by multiplying time averaged mean velocity and cross sectional area of the vessel of interest. These values were precisely obtained by an experienced radiologist who performed the Doppler ultrasound measurements during the operation with an intra-abdominal transducer.

Our finding that the liver contributes in the elimination of SDMA is of great importance. As mentioned, SDMA does not directly act on NO synthase but could reduce NO production indirectly via competition with arginine transport into the cells. Especially in clinical conditions with reduced arginine concentrations in combination with high levels of SDMA, synthesis of NO may decrease significantly and the hemodynamical consequences may be the same as reported for ADMA.[28,29] Nevertheless, the clinical relevance of our finding remains to be elucidated and additional studies focussing on the effect of liver failure on SDMA levels and in vitro studies investigating the effects of high SDMA levels on the cardiovascular system must be performed. Dimethylarginines are also potentially important in the pathophysiology of liver cirrhosis since this condition is characterised by endothelial dysfunction and NO deficiency in the intrahepatic circulation. Intracellular ADMA concentrations are higher than plasma concentrations[30] and an IC50 value of 1.5 µM for ADMA to inhibit NO synthase has been reported.[31] Thus, it is plausible that a slight elevation of plasma ADMA concentration may lead to inhibition of NO synthase activity. Reduced bioavailability of NO enhances microcirculatory vasoconstriction, thereby increasing vascular tone.[32] Paradoxically, the splanchnic vasculature exhibits endothelial NO overproduction, leading to vasodilation. Furthermore, the development of renal failure in patients with severe liver disease is also characterised by vasoconstriction and renal hypoperfusion. Therefore, we have suggested that ADMA plays a causal role in the development of the hepatorenal syndrome.[33] When the liver fails, hepatic degradation of ADMA becomes impaired, leading to accumulation of ADMA. Its dependence on basal NO synthesis for regulation of blood flow and glomerular filtration makes the kidney highly vulnerable to

accumulation of ADMA. Considering the differential expression of DDAH in different organs, it can be hypothesised that NO synthesis may be differentially regulated in different vascular beds in order to modulate vascular tone during various pathophysiological processes. In this light, it is of particular interest to notify that myocardial blood flow is regulated by local activity of DDAH.[34]

In conclusion, these results give detailed insight in the hepatic and renal handling of dimethylarginines in humans and show that the elimination of SDMA is not confined to the kidney, but the human liver is also capable of clearing SDMA from the portosystemic circulation. Future research investigating the pathophysiological role of dimethylarginine handling in the setting of liver disease must further unravel the importance of our novel finding.

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