

*Original Article*

## Handling of asymmetrical dimethylarginine and symmetrical dimethylarginine by the rat kidney under basal conditions and during endotoxaemia

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### Abstract

**Background.** Asymmetrical dimethylarginine (ADMA) is capable of inhibiting nitric oxide synthase enzymes, whereas symmetrical dimethylarginine (SDMA) competes with arginine transport. The potential role of inflammation in the metabolism of ADMA has been elucidated in an *in vitro* model using tumour necrosis factor- $\alpha$ , resulting in a decreased activity of the ADMA-degrading enzyme dimethylarginine dimethylamino-hydrolase (DDAH). The kidney probably plays a crucial role in the metabolism of ADMA by both urinary excretion and degradation by DDAH. We aimed to further elucidate the role of the kidney in a rat model under basal conditions and during endotoxaemia.

**Methods.** Twenty-five male Wistar rats weighing 275–300 g were used for this study. The combination of arteriovenous concentration differences and kidney blood flow allowed calculation of net organ fluxes. Blood flow was measured using radiolabelled microspheres according to the reference sample method. Concentrations of ADMA, SDMA and arginine were measured by high-performance liquid chromatography.

**Results.** The kidney showed net uptake of both ADMA and SDMA and fractional extraction rates were 35% and 31%, respectively. Endotoxaemia resulted in a lower systemic ADMA concentration ( $P=0.01$ ), which was not explained by an increased net renal uptake. Systemic SDMA concentrations increased during endotoxaemia ( $P=0.007$ ), which was accompanied by increased creatinine concentrations.

**Conclusions.** The rat kidney plays a crucial role in the regulation of concentrations of dimethylarginines,

as both ADMA and SDMA were eliminated from the systemic circulation in substantial amounts. Furthermore, evidence for the role of endotoxaemia in the metabolism of dimethylarginines was obtained as plasma levels of ADMA were significantly lower in endotoxaemic rats.

**Keywords:** L-arginine; kidney; nitric oxide; nitric oxide synthase

### Introduction

The arginine–nitric oxide (NO) pathway has been recognized to play critical roles during infection, inflammation, organ injury, transplant rejection and atherosclerosis. Recently, it was recognized that endogenous arginine analogues may play a regulatory role in the arginine–NO pathway. Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of all isoforms of nitric oxide synthase (NOS) [1,2]. Symmetrical dimethylarginine (SDMA) is not biologically active on NOS, but may interfere with NO synthesis by competing with arginine for transport across cell membranes [3–6].

In asymptomatic humans with hypercholesterolaemia, elevated levels of ADMA were found and ADMA levels were associated with impaired endothelium-dependent vasodilation and reduced nitrate excretion [7]. ADMA levels also increased in elderly patients with peripheral arterial disease and generalized atherosclerosis [8]. Miyazaki *et al.* [9] measured dimethylarginine levels in plasma of 116 human subjects who had no sign of coronary or peripheral artery disease. They found that ADMA levels were positively

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correlated with age, mean arterial pressure and glucose tolerance. Most intriguingly, ADMA levels were significantly correlated with carotid artery intima-media thickness in stepwise regression analysis. The study of Miyazaki *et al.* suggests that elevation of this endogenous inhibitor precedes the occurrence of vascular occlusive disease. Others proposed that ADMA might also play a regulatory role in the action of inducible NOS, inhibiting overwhelming NO synthesis [10,11].

In 1992, Vallance *et al.* [4] reported elevated levels of ADMA in patients with renal failure. Elevated levels may be responsible for the hypertension seen in patients with end-stage renal disease. In patients with chronic renal failure also, a sharp rise of SDMA, the stereoisomer of ADMA, has been reported [12]. Fleck *et al.* [13] 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, possibly by competition for reabsorption between SDMA and arginine in the kidney. Moreover, we [14] recently confirmed the role for the kidney in the regulation of plasma levels of dimethylarginines, since both dimethylarginines were significantly extracted from the arterial supply of the human kidney.

ADMA is thought to be eliminated from the body by both degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) and urinary excretion, while SDMA would only depend on urinary excretion. DDAH has been isolated from the rat kidney and is co-localized with the different NOS enzymes [6,15–17].

Theoretically, a reduced activity of DDAH may be responsible for elevated ADMA concentrations [18]. DDAH activity is influenced by factors such as oxidative stress and inflammation [19,20]. In an *in vitro* model of human umbilical vein endothelial cells, a reduced activity of DDAH was found after exposure to oxidized low-density lipoprotein (LDL) and tumour necrosis factor (TNF)- $\alpha$  [20]. *In vivo*, more confirmation on the potential role of oxidized LDL was obtained by the occurrence of high ADMA levels in hypercholesterolaemia, making ADMA a potential risk factor for atherosclerosis [7,20]. However, no *in vivo* data are present on the role of TNF- $\alpha$  and inflammation on the metabolism of dimethylarginines.

The aim of this study was to investigate dimethylarginine handling of the rat kidney in detail. Therefore, we designed a metabolic study in rats in which arteriovenous concentration differences were determined, together with blood flow measurement using radioactive microspheres. The combination of arteriovenous concentration differences and kidney blood flow allows calculation of net organ fluxes. Furthermore, we administered lipopolysaccharide (LPS), a component of the outer membrane of most Gram-negative bacteria that is used as a stimulator of the inflammatory response, to a second group of animals to study the role of the inflammatory state on the metabolism of ADMA and SDMA.

## Subjects and methods

### *Animal model and experimental design*

Twenty-five male Wistar rats (Harlan, Horst, The Netherlands) weighing 275–300 g were used for this study. After permission of the Institutional Animal Care and Use Committee, the animals were maintained in accordance with the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals' [21]. After admission, rats were allowed to adapt to the laboratory environment for at least 5 days. Animals had free access to food and water and were subjected to a 12:12 h light/dark cycle.

After overnight fasting, rats were anaesthetized using ketamine HCl (70 mg/kg i.m.) and Nembutal (60 mg/kg i.p.) and placed in the supine position on a heating pad that maintained rectal temperature at 37°C. Anaesthesia was maintained by Nembutal (10 mg/kg/h i.p.). The trachea was intubated with a small piece of polyethylene tubing (PE-240; Fisher, Scientific, Springfield, NY, USA) to facilitate breathing. The right carotid artery and right femoral artery were cannulated using PE-28 tubing; the right jugular vein and right femoral vein were cannulated using PE-50 tubing.

Through a small (0.5 cm) midline incision in the lower abdomen, the bladder was cannulated using PE-28 tubing. The cannula was secured with one stitch in the wall of the bladder. Hereafter, the incision was closed and a small clamp was placed on the end of the cannula. Following these procedures the rats were allowed to stabilize for 30 min.

Rats were randomly assigned to receive intravenous LPS ( $n = 13$ ) infusion [1.5 ml; dose: 8 mg/kg body weight (BW)] starting at  $t = 0$  min or to intravenous infusion of 1.5 ml 0.9% NaCl (control;  $n = 12$ ).

To determine urinary concentrations of ADMA, SDMA, arginine and creatinine, urine was collected during the last 30 min period of the experiment ( $t = 150$ – $180$  min) and the amount of urine was weighed to calculate urine production.

During the entire experiment, haemodynamical parameters were continuously recorded and at  $t = 180$  min kidney blood flow was measured using radiolabelled microspheres. Immediately hereafter, blood samples were taken and the experiment was terminated by exsanguination of the animals.

### *Blood flow measurement*

Blood flow measurement was performed at the end of the experiment ( $t = 180$  min). Blood flow was measured using radiolabelled microspheres according to the reference sample method [22]. This method was chosen for its high reproducibility. The catheters in the right common carotid artery and left femoral artery were connected to P23Db Statham pressure transducers. Pressure wave monitoring was used to place the carotid catheter into the left ventricle. Mean arterial pressure (MAP) and heart frequency (HF) were continuously recorded during the experiment.

At the end of the experiment, an intraventricular injection of  $^{103}\text{Ru}$ -labelled microspheres ( $\sim 1.3 \times 10^5$  microspheres dissolved in 0.3 ml saline) was performed. A reference blood sample was obtained from the right femoral artery at a rate of 0.4 ml/min over 120 s, starting 5 s before the microsphere injection.

Immediately after the microsphere procedure, the abdomen was opened and blood samples were drawn from

renal vein and abdominal aorta, after which the animal was exsanguinated.

Kidneys were removed and wrapped in tissue paper. The kidneys were weighed and placed in counting vials. Radioactivity was measured in a  $\gamma$  counter (CS 1282; Wallace Compugamma, Turku, Finland). Kidney blood flow was computed according to the reference sample technique using the equation  $F = F_a (Q_o / Q_a)$ , where  $F_a$  is the reference flow,  $Q_o$  is the count rate in the kidney tissue and  $Q_a$  is the count rate in the reference blood sample. Reference blood flow was computed from the weight of blood in the reference syringe and the duration of withdrawal assuming a whole-blood density of 1.055 g/ml. Kidney blood flow was expressed as ml/min/g tissue. Plasma flow was computed by correction for haematocrit (Microhaematocrit reader; Hawsley and Sons, London, UK) by the equation:

$$\text{plasma flow} = \text{blood flow} \times (1 - \text{haematocrit})$$

#### *Plasma analysis of AMDA, SDMA, arginine and chemical analysis*

Blood samples were immediately placed on ice and centrifuged at 3000 r.p.m. for 10 min at 4°C (Sorvall GLC 2 centrifuge; Sorvall Operations, DuPont, Newton, CT, USA). Plasma was pipetted and immediately put in liquid nitrogen and stored at -70°C before analysis.

Arginine, ADMA and SDMA were measured simultaneously by high-performance liquid chromatography with fluorescence detection as described previously [23]. Briefly, 0.1 ml plasma was mixed with 0.1 ml of a 40  $\mu\text{mol/l}$  solution of the internal standard L-NMMA and 0.8 ml phosphate-buffered saline. 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 100 mM HCl and 1.0 ml methanol. Analytes were eluted with 1.0 ml concentrated ammonia/water/methanol (10/40/50). After evaporation of the solvent under nitrogen, the amino acids were derivatized with *o*-phthalaldehyde reagent containing 3-mercaptopropionic acid. The derivatives were separated by isocratic reversed-phase chromatography on a Symmetry C18 column (3.9  $\times$  150 mm; 5  $\mu\text{m}$  particle size; Waters). Potassium phosphate buffer (50 mM; pH 6.5) containing 8.7% acetonitrile was used as the 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. The intra-assay coefficients of variation (CVs) for arginine, ADMA and SDMA were 0.4%, 1.2% and 0.8%, respectively. The interassay CVs for arginine, ADMA and SDMA were 2.9%, 2.0% and 2.6%, respectively.

Plasma creatinine was determined in arterial plasma samples using an automated analyser (H 737; Hitachi, Tokyo, Japan).

#### *Calculations*

Kidney uptake or release (flux) and fractional extraction rates for ADMA, SDMA and arginine were calculated from the plasma flow and the arteriovenous concentration

difference for the left kidney. The flux calculation of the left kidney was multiplied by 2 to obtain values for both kidneys and results are presented as such. Flux is presented as nmol/100 g BW/min. Fractional extraction is calculated as  $[A] - [RV] / [A]$ , where [A] and [RV] denote arterial and renal vein plasma concentrations, respectively. Each parameter was calculated for each individual animal using its individual substrate concentrations and renal plasma flow.

Urine volume was calculated by dividing urine weight by 1.020 to correct for specific gravity. Clearances of creatinine, ADMA, SDMA and arginine were calculated as  $[U] \times [V] / [P]$ , where [U] and [P] denote urinary and arterial plasma concentrations, respectively, and [V] denotes urine volume per min. Fractional excretion was calculated as clearance of ADMA, SDMA or arginine divided by the clearance of endogenous creatinine.

#### *Statistical methods*

Statistical analysis was performed using the SPSS 9.0 statistical package. Differences between the groups were tested using the non-parametric Wilcoxon ranked sum test. Haemodynamics were recorded every 30 min during the experiment and were evaluated using the general linear model for repeated measurements. When appropriate, intergroup differences on the individual time-points were further tested using the Wilcoxon ranked sum test, whereas the Wilcoxon paired test was used for testing the intragroup differences. Values are expressed as means  $\pm$  SEM and  $P < 0.05$  was considered statistically significant.

## **Results**

#### *General*

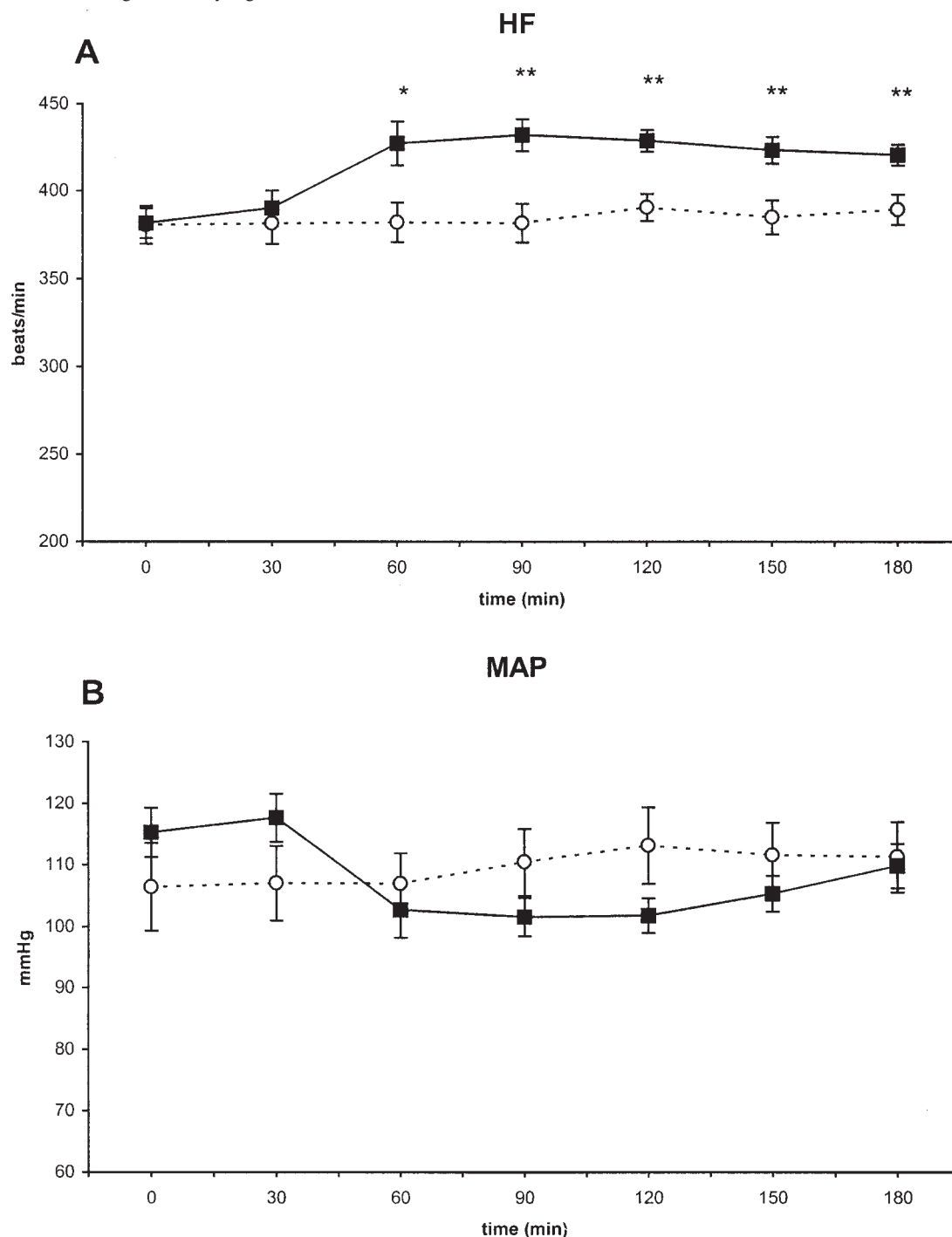
HF (Figure 1A) was significantly higher in LPS-treated rats from  $t = 60$  until  $t = 180$  min, when compared with control rats. MAP (Figure 1B) remained stable in both groups and no significant differences were observed between both experimental groups. Haematocrit levels were  $0.41 \pm 0.02$  and  $0.42 \pm 0.01$  in control and LPS-treated rats, respectively.

#### *Kidney parameters*

In Table 1, an overview of kidney parameters is displayed. A significantly lower renal plasma flow was found in LPS-treated rats, when compared with control rats. Plasma creatinine concentration was significantly higher in LPS-treated rats. Urine production and filtration fraction were not statistically different between both groups.

#### *Plasma concentrations of ADMA, SDMA and arginine*

In Table 2, arterial and renal vein plasma concentrations of ADMA, SDMA and arginine are given, together with calculated arteriovenous concentration differences and fractional extraction rates.



**Fig. 1.** HF (A) and MAP (B) in control (open circles) and LPS-treated (closed squares) rats. Values are displayed as means  $\pm$  SEM. \* $P=0.02$ , \*\* $P < 0.01$ .

In control rats, arterial and renal vein ADMA concentrations were  $0.754 \pm 0.022$  and  $0.491 \pm 0.028$   $\mu\text{mol/l}$ , respectively. LPS treatment resulted in significantly lower arterial plasma ADMA concentration, when compared with control rats, while renal vein ADMA concentration was not significantly different. Both arteriovenous concentration difference and fractional extraction rate of ADMA were significantly lower in LPS-treated rats.

In control rats, arterial and renal vein SDMA concentrations were  $0.452 \pm 0.016$  and  $0.317 \pm 0.024$   $\mu\text{mol/l}$ , respectively. In contrast to ADMA, arterial concentrations of SDMA were higher in LPS-treated rats. Also in renal vein plasma, SDMA concentration was higher in LPS-treated rats. Arteriovenous concentration difference was not significantly different between LPS and control groups. Fractional extraction rate was lower in LPS-treated rats.

**Table 1.** Renal plasma flow (ml/min), plasma creatinine ( $\mu\text{mol/l}$ ), urine production (ml/min) and filtration fraction at  $t = 180$  min in control and LPS rats

	Control	LPS	<i>P</i> -value
Renal plasma flow	$8.5 \pm 0.6$	$6.2 \pm 0.5$	0.02
Plasma creatinine	$49.8 \pm 0.4$	$63.6 \pm 1.7$	<0.001
Urine production	$0.019 \pm 0.003$	$0.015 \pm 0.002$	NS
Filtration fraction	$0.28 \pm 0.07$	$0.32 \pm 0.05$	NS

Values are expressed as means  $\pm$  SEM. NS, not significant.

**Table 2.** Plasma concentrations ( $\mu\text{mol/l}$ ) of ADMA, SDMA and arginine in aorta (A) and renal vein (RV), arteriovenous (A-RV) and fractional extraction (A-RV/A) in control and LPS rats

	Control	LPS	<i>P</i> -value
<b>ADMA</b>			
A	$0.754 \pm 0.022$	$0.670 \pm 0.021$	0.01
RV	$0.491 \pm 0.028$	$0.515 \pm 0.020$	NS
A-RV	$0.263 \pm 0.021$	$0.154 \pm 0.017$	0.001
A-RV/A	$0.35 \pm 0.03$	$0.23 \pm 0.02$	0.002
<b>SDMA</b>			
A	$0.452 \pm 0.016$	$0.515 \pm 0.015$	0.007
RV	$0.317 \pm 0.024$	$0.405 \pm 0.013$	0.005
A-RV	$0.135 \pm 0.014$	$0.110 \pm 0.012$	NS
A-RV/A	$0.31 \pm 0.03$	$0.21 \pm 0.03$	0.01
<b>Arginine</b>			
A	$123.2 \pm 5.2$	$78.6 \pm 4.4$	<0.001
RV	$142.3 \pm 6.2$	$119.8 \pm 2.6$	0.005
A-RV	$-19.2 \pm 0.04$	$-41.2 \pm 0.04$	0.001
A-RV/A	$-0.16 \pm 0.04$	$-0.57 \pm 0.07$	<0.001

Values are expressed as means  $\pm$  SEM. NS, not significant.

In control rats, arterial and renal vein arginine concentrations were  $123.2 \pm 5.2$  and  $142.3 \pm 6.2 \mu\text{mol/l}$ , respectively. Arterial and renal vein arginine concentrations were significantly lower in LPS-treated rats. In control rats, renal arteriovenous concentration and fractional extraction indicated release of arginine, which was more pronounced in the LPS group.

#### Net renal fluxes of ADMA, SDMA and arginine

In Figure 2 net renal fluxes of ADMA (A), SDMA (B) and arginine (C) are displayed.

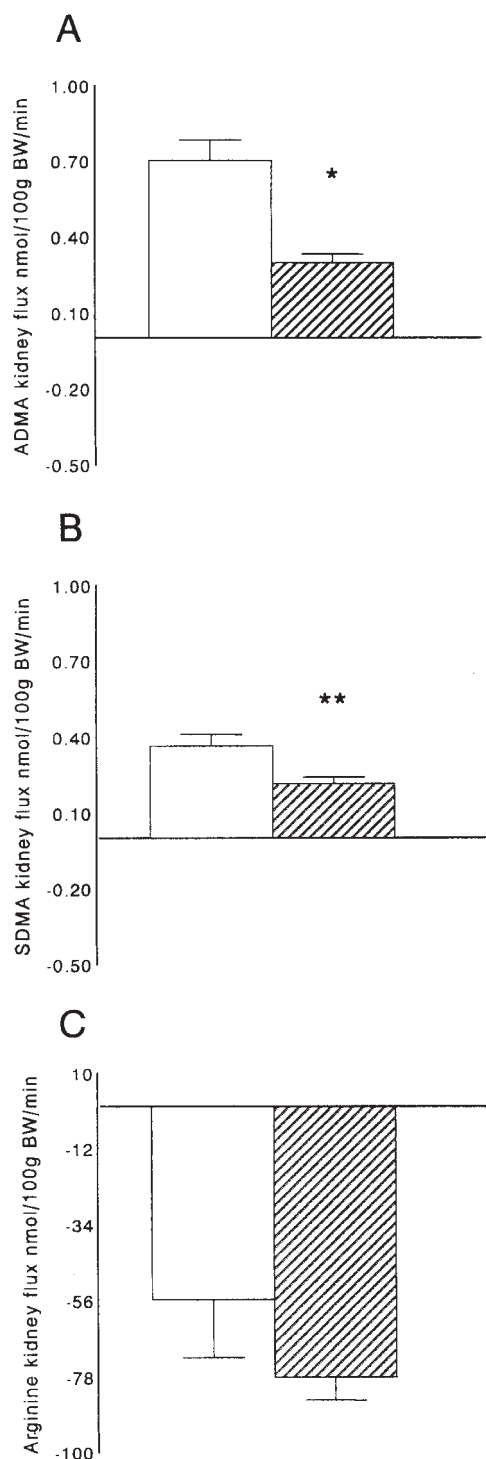
In control rats net uptake of ADMA and SDMA was observed, which was significantly lower in LPS-treated rats.

In control rats, net release of arginine was observed, which was (not significantly) higher in LPS treated-rats.

#### Urinary excretion and clearance of ADMA, SDMA and arginine

In Table 3, urine concentration, clearance and fractional excretion of ADMA, SDMA and arginine are given. Urinary ADMA excretion was negligible in both control and LPS-treated animals, as evidenced by low ADMA concentrations and a low clearance rate.

In contrast to ADMA, urinary excretion of unchanged SDMA was found in both experimental groups.



**Fig. 2.** Net fluxes of ADMA (A), SDMA (B) and arginine (C) in nmol/100 g BW/min. Open bars indicate control (0.9% NaCl) rats; closed bars indicate LPS-treated rats. Values are displayed as means  $\pm$  SEM. \* $P < 0.001$ , \*\* $P = 0.003$ .

Clearance of SDMA was significantly higher in control rats, when compared with LPS-treated rats, as was fractional excretion. Urinary SDMA excretion accounted for 30.1% and 25.0% of net renal flux of SDMA in control and LPS treated animals, respectively.

**Table 3.** Urine concentration ( $\mu\text{mol/l}$ ), clearance ( $\text{ml/min}$ ) and fractional excretion [clearance ( $\text{ml/min}$ ) / creatinine clearance ( $\text{ml/min}$ )] of ADMA, SDMA and arginine in control and LPS rats

	Control	LPS	<i>P</i> -value
<b>ADMA</b>			
Urine concentration	$0.11 \pm 0.02$	$0.05 \pm 0.02$	NS
Clearance	$0.003 \pm 0.0004$	$0.001 \pm 0.0004$	NS
Fractional excretion	$0.002 \pm 0.0005$	$0.001 \pm 0.0004$	NS
<b>SDMA</b>			
Urine concentration	$19.9 \pm 3.8$	$10.8 \pm 0.7$	NS
Clearance	$0.74 \pm 0.07$	$0.33 \pm 0.06$	0.001
Fractional excretion	$0.57 \pm 0.06$	$0.26 \pm 0.03$	0.003
<b>Arginine</b>			
Urine concentration	$332.6 \pm 67.8$	$292.7 \pm 25.0$	NS
Clearance	$0.05 \pm 0.005$	$0.05 \pm 0.007$	NS
Fractional excretion	$0.04 \pm 0.004$	$0.05 \pm 0.004$	NS

Values are expressed as means  $\pm$  SEM. NS, not significant.

Urinary arginine concentration was similar in both groups. Clearance and fractional excretion of arginine were low in both groups.

## Discussion

Our study provides detailed insight into the kidney handling of dimethylarginines in an *in vivo* experimental design by measurement of arteriovenous concentration differences together with determination of blood flow, allowing calculation of net fluxes. One group of animals was used to study kidney handling of dimethylarginines during normal conditions and a second group of animals to study the changed dimethylarginine metabolism and renal handling during endotoxaemia. For the endotoxaemia rat model we chose the same dose of LPS (8 mg/kg) as was used by our group in previous studies, as this model proved to be a valuable tool in studying changes in renal haemodynamics and metabolism during endotoxaemia, closely mimicking critically ill intensive-care patients [24]. By measuring haemodynamical parameters the clinical condition of the animals was monitored closely. From  $t = 60$  min, HF was significantly higher in LPS-treated rats, a characteristic phenomenon for advanced endotoxaemia [24]. Renal perfusion was significantly lower in LPS-treated rats, as was reported previously following LPS infusion [24]. Creatinine levels increased significantly in LPS-treated rats, which may be indicative for impairment of renal function.

To adequately measure arteriovenous concentration differences in small sample volumes, we developed a high-performance liquid chromatography (HPLC) method with a high precision and sensitivity. This method allowed simultaneous measurement of ADMA, SDMA and arginine and proved to be a valuable tool in the research on the metabolism of dimethylarginines and their role in the arginine-NO pathway.

Recently, we pointed to the kidney as an important contributor in the regulation of plasma levels of

dimethylarginines, by showing significant extraction of both ADMA and SDMA from the arterial supply of the human kidney [14]. These results are further substantiated in the present study in rats, providing detailed insight into the renal handling of dimethylarginines. We found a significant net uptake of both ADMA and SDMA by the rat kidney, with fractional extraction rates of 35% and 31%, respectively. Furthermore, strong evidence was obtained for a differential renal handling of the two dimethylarginines. In our model we found that the elimination of ADMA by the rat kidney could not be explained by urinary excretion, because urinary concentration of unchanged ADMA was negligible. This finding points to a high metabolic turnover of ADMA in the kidney, which is fully responsible for the observed net renal uptake of ADMA. In contrast to the rat kidney, human kidneys are capable of excreting unchanged ADMA [14,25], so there also seems to be a difference in the handling of dimethylarginines between humans and rats. Ogawa *et al.* [26] investigated the metabolic fate of ADMA and SDMA in the rat and, interestingly, they found that only 4.6% of injected ADMA was found in the first 12 h urine as unchanged ADMA, compared with 17.8% for SDMA. Furthermore, they found that both dimethylarginines are metabolized by a pathway forming the corresponding  $\alpha$ -ketoacid analogues and the oxidatively decarboxylated products of the  $\alpha$ -ketoacids in addition to *N*-acetyl conjugates, and that these metabolites were mainly found in urine. Interestingly, especially the acetyl conjugation reaction has been recognized as typical for the rat species, as has been reported for the acetylation of the  $\alpha$ -amino group of 3-methylhistidine [27]. However, with our HPLC method we were only able to measure unchanged ADMA and SDMA and related metabolites of these dimethylarginines would not have been detected. If the formation of  $\alpha$ -ketoacids or *N*-acetyl conjugates is confined to the rat species, this metabolic pathway could explain the controversy between the data obtained from rat and human experiments. In the experiment of Ogawa *et al.* [26], only for ADMA an additional pathway was found, leading to the formation of citrulline and related amino acids. This pathway seemed to be the main route for ADMA elimination, as most ADMA derived radioactivity was found in tissues instead of urine. Later, this catalytic pathway was recognized both in rats and humans and proven to be degradation by the enzyme DDAH [16,17]. In our study, the rat kidney proved to be capable of excretion of SDMA into urine and  $\sim 30\%$  of net renal uptake could be explained by excretion of unchanged SDMA in the urine. According to the study of Ogawa *et al.* [26], and because SDMA cannot be degraded by DDAH, it could be speculated that the remainder of the renal uptake of SDMA could be explained by urinary excretion of  $\alpha$ -ketoacids and *N*-acetyl conjugates.

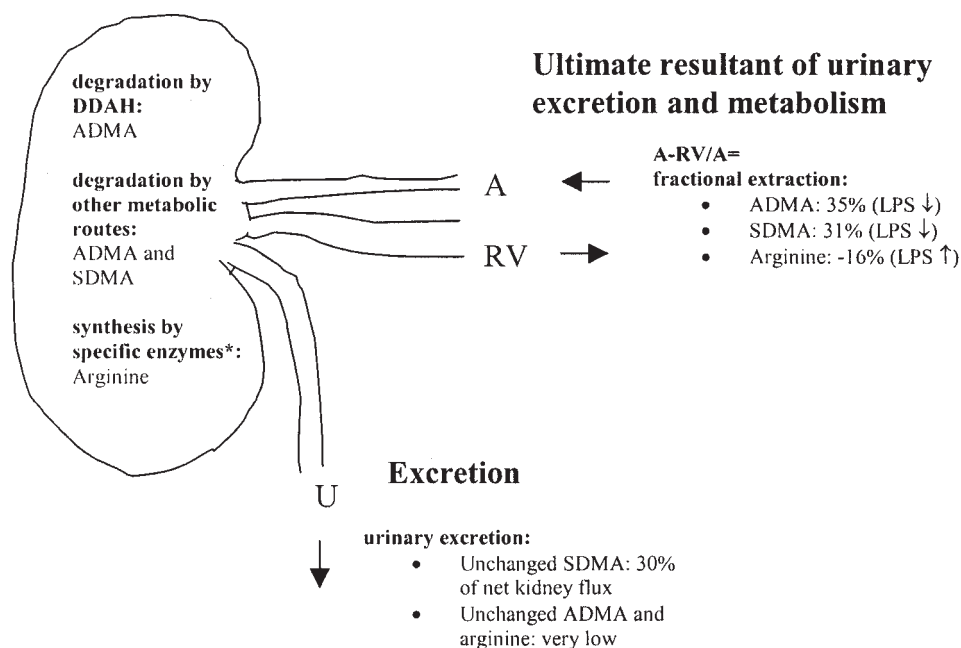
In contrast to our expectations, in rats treated with endotoxin a significantly lower ADMA concentration was found. To our knowledge no *in vivo* studies have been performed on the role of endotoxaemia in the

metabolism of dimethylarginines. In the present study we deliberately chose to use LPS infusion instead of TNF- $\alpha$ , because we aimed to mimic a clinical condition, driven by the naturally complex combination of inflammatory mediators, and not to study only the response to TNF- $\alpha$ . Although we cannot designate a single mediator for the observed effect, in the present experiment strong evidence was obtained for increased metabolic turnover of ADMA during endotoxaemia. Interestingly, the increased metabolic turnover of ADMA was not accompanied by an increased renal elimination of ADMA, as both renal fractional extraction rate and net renal uptake were significantly lower in LPS-treated rats. Therefore, the kidney seems not to be responsible for this ADMA-lowering effect of endotoxaemia. A potential explanation for the lower plasma concentration of ADMA might be increased uptake by the  $y^+$  transporter during endotoxaemia. Cationic amino acids, such as arginine, ornithine and lysine, are transported into endothelial cells by the cationic amino acid transporters (CAT) of system  $y^+$ . Closs *et al.* [5] investigated transport of dimethylarginines by CAT and found that both ADMA and SDMA were transported across this  $y^+$  carrier. In rats it has been shown that the expression of CAT transporters was significantly increased in lung, heart and kidney by LPS injection [28]. Clinical conditions associated with severe endotoxaemia include sepsis and septic shock and these conditions are characterized by overproduction of NO

due to inducible NOS (iNOS) activity. The activity of iNOS appears to be mainly regulated at the transcriptional level. However, regulation of arginine availability can also determine the cellular rate of NO production, since arginine is the only substrate for NOS. It has been suggested that the immunostimulant-elicited increase in arginine transport activity plays a key role in NO formation and that arginine transport is stimulated by endotoxin during sepsis. In the present study we found decreased concentrations of both arginine and ADMA during experimental endotoxaemia, which may have resulted from increased transport by the  $y^+$  carrier. One biological question that has to be answered is what the potential role of ADMA in inflammation and infection is. It has been speculated that ADMA could possibly serve as a 'brake' on the action of iNOS and inhibit overwhelming NO synthesis [11]. In contrast to the decreased ADMA levels, SDMA levels were higher in endotoxin-treated rats and the increase of SDMA was accompanied by a reduced renal fractional extraction and a reduced net uptake by the kidney. As creatinine levels were also significantly higher in LPS-treated rats, an impaired renal clearance of SDMA could underlie the rise in SDMA levels.

Thus, LPS infusion resulted in a reduced renal elimination of both ADMA and SDMA, as reflected by reduced fractional extraction and fluxes. However, in contrast to SDMA, systemic ADMA levels were significantly lower in LPS-infused rats. Therefore, while

### Processes within the kidney



**Fig. 3.** Hypothetical overview of the renal handling of ADMA, SDMA and arginine based on the findings of the present study. The ultimate resultant of urinary excretion and metabolism is presented as fractional excretion. Urinary excretion is presented as the amount of unchanged substance as percentage of net kidney flux. Processes within the kidney include synthesis and degradation by the action of specific enzymes. DDAH is known to degrade ADMA and not SDMA. Other potential metabolic pathways include the formation of ketoacids and *N*-acetyl conjugates. \*Arginine is known to be synthesized from citrulline by the enzymes argininosuccinate synthetase and argininosuccinate lyase. Note that the processes within the kidney could not be further quantified, because metabolites of ADMA, SDMA and arginine were not measured. A, supply via the renal artery; RV, drainage via renal vein; U, urinary excretion.

changes in systemic SDMA levels may be logically explained by reduced renal elimination, the systemic effect of LPS on ADMA leading to a lower plasma concentration needs further study.

In the current study we also presented data on arginine handling of the kidney, as arginine is the key amino acid in the arginine–NO pathway and its molecular structure closely resembles that of dimethylarginines. In the past, our group and others studied arginine handling of the kidney during normal conditions and during endotoxaemia [29,30]. The results of the present study are in agreement with those reports and the important role of the kidney in maintaining arginine levels was demonstrated.

In conclusion, in the present study the handling of dimethylarginines by the rat kidney was studied in detail and novel facets were elucidated. Based on our findings we propose the scheme presented in Figure 3. Both dimethylarginines are eliminated from the systemic circulation in substantial amounts. There seems to be a differential metabolism for both dimethylarginines, at least in the rat kidney, as a negligible amount of unchanged ADMA was excreted, whereas substantial amounts of unchanged SDMA were recovered in urine. Furthermore, evidence for the role of endotoxaemia on the metabolism of dimethylarginines was obtained as plasma levels of ADMA were significantly lower in endotoxaemic rats, whereas renal elimination of ADMA was decreased. In endotoxaemia, SDMA levels were elevated, which was accompanied by a decreased renal function as was measured by creatinine levels and a diminished renal elimination of SDMA. The mechanisms underlying the decreased ADMA levels in endotoxaemia were not elucidated in this study, but warrant further study.

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**Conflict of interest statement.** None declared.

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