The importance of the arginine/ADMA ratio on the bioavailability of nitric oxide and organ function in critically ill patients

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Chapter 4

Low arginine/ADMA ratio deteriorates systemic hemodynamics and organ blood flow in a rat model

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Abstract

Introduction
Both arginine and asymmetric dimethylarginine (ADMA) play a crucial role in the arginine-nitric oxide pathway. Low arginine and high ADMA levels can be found in critically ill patients after major surgery. The aim of this study was to evaluate the effects of low arginine plasma concentrations in combination with high ADMA plasma concentrations on hemodynamics and organ blood flow.

Methods
A randomized, placebo-controlled animal laboratory investigation was performed. Twenty –one rats were randomly assigned to three groups: a control group, an ADMA group or an arginase (ASE)/ADMA group. In the control group, rats received (at t=0) an intravenous infusion of 1.5 mL 0.9% NaCl over a 20-min period. After 60 minutes (t=60), rats received an intravenous bolus of 1.0 mL 0.9% NaCl. In the ADMA group, rats received an intravenous infusion of 1.5 mL 0.9% NaCl over a 20-min period and at t=60 an intravenous bolus of 1.0 mL ADMA (20 mg/kg). In the ASE/ADMA group, rats received an intravenous infusion of 1.5 mL arginase (3200 IU) solution over a 20-min period and at t=60 an intravenous bolus of 1.0 mL ADMA (20 mg/kg).

Results
Infusion of ADMA (20mg/kg) and arginase (3200 IU) resulted in increased plasma ADMA levels and decreased arginine levels. During the whole experiment, systemic hemodynamics (heart rate, mean arterial pressure, cardiac output) were measured. In addition, organ blood flow was measured at t=90 and t=180 minutes, using fluorescent microspheres. Compared to the control group, mean arterial pressure and systemic vascular resistance was increased after infusion of ADMA. Infusion of arginase in combination with ADMA significantly deteriorated systemic hemodynamics (mean arterial pressure, cardiac output, stroke volume, systemic vascular resistance) and organ blood flow through the kidney and spleen. In addition, an initial decrease in arterial flow, followed by a later major increase, and panlobular apoptosis and necrosis of the liver was observed.

Conclusions
The present study shows that low arginine plasma levels in combination with high ADMA plasma levels deteriorates systemic hemodynamics and reduces blood flow through the kidney and spleen and liver. These data suggest that a diminished nitric oxide production may be involved in the onset of organ failure.

Introduction

Arginine is one of the most versatile amino acids, with multiple biological functions in mammalian cells. Arginine enhances wound healing, improves immune function and T-cell anti-tumour immunity, has anti-catabolic effects and is the precursor of nitric oxide (NO) (1-3). Although arginine is a non-essential amino acid in healthy humans, there are clinical conditions where arginine may become an essential amino acid. Arginine concentration is reduced after thoracoabdominal aortic surgery (4), surgery of oesophageal and lung cancer (5), after trauma (6), during sepsis (7), and after severe burns (8). Therefore, during these occasions arginine may become essential.

Arginine is converted into NO and citrulline by the action of NO synthase (NOS), a family of enzymes with endothelial, neuronal, and inducible isoforms (9). NO plays an important role in regulating vascular tone which is, especially during stress, of significant importance for regulating perfusion of important organs such as the kidney, liver and the heart (10). Other important functions of NO are inhibition of platelet aggregation, inhibition of adhesion of leucocytes to endothelial cells and cytotoxicity against micro-organisms (11-13).

Recent insights into NO metabolism have shown an important role of endogenously produced inhibitors of the enzyme NOS, in particular asymmetric dimethylarginine (ADMA) (14).

Elevated ADMA concentrations have been reported in patients with conditions characterized by endothelial dysfunction, including chronic kidney disease, peripheral arterial disease, diabetes mellitus, hypercholesterolemia, and hyperhomocysteinemia (15-20). In addition, we recently showed highly elevated concentrations of ADMA in critically ill patients with clinical evidence of organ dysfunction (21). In these patients, ADMA was related to the presence of hepatic dysfunction and to lactic acid and bilirubin as biochemical markers of hepatic function. Moreover, ADMA proved to be the strongest predictor of ICU mortality with a 17-fold increased risk for patients who were in the highest quartile of ADMA.

Since, apart from other effects, arginine and ADMA both influence NO metabolism, we hypothesize that the combination of low arginine levels and high ADMA levels, as found in critically ill patients after thoracoabdominal aortic surgery, might influence NO-induced vasodilatation and organ blood flow.

Therefore, we have evaluated the effects of low arginine plasma concentrations in combination with high plasma concentrations of ADMA on hemodynamics and organ blood flow in a rat model.

Materials and Methods

Animal model

The study conforms to the Guide for the Care and Use of Laboratory Animals (NIH Publication N0. 85-23, revised 1996), and the local ethics committee for animal experiments approved the procedures. Twenty-one male Wistar rats (Harlan, Horst, The Netherlands) weighing 250 – 350 g, were housed under standard conditions and were randomly assigned to three groups, a control group (n = 8), an ADMA group (n = 7) and an Arginase/ADMA (ASE/ADMA) group (n = 6). Rats were anesthetized with pentobarbital sodium (60 mg/kg i.p.) and ketamine HCL (70 mg/kg i.m.) and received a pentobarbital sodium maintenance dose of 15 mg/kg via an intra arterial catheter of the left femoral artery. The animals were placed in a supine position on a heating pad maintaining body temperature at 37 ºC. The trachea was intubated with polyethylene tubing to facilitate breathing. The animals received 75 IU/kg heparin intravenously (Leo Pharmaceutical products, Weesp, The Netherlands) to prevent catheter clotting.

The control group received at the beginning of the experiment (t=0) an iv infusion of 1.5 mL NaCl 0.9% over a 20-min period and after 60 minutes (t=60) an iv bolus of 1.0 mL NaCl 0.9%. The ADMA group received at the beginning an iv infusion of 1.5 mL NaCl 0.9% over a 20-min period and after 60 minutes an iv bolus of 1.0 mL ADMA (20 mg/kg). ADMA was obtained from Sigma Aldrich, Zwijndrecht, The Netherlands. The ASE/ADMA group received at the beginning an iv infusion of 1.5 mL arginase (3200 IU) over a 20-min period as described previously (22). Arginase was obtained from Sigma Aldrich, Zwijndrecht, The Netherlands (bovine liver enzyme, activity 131 IU/mg). After 60 minutes the rats received an iv bolus of 1.0 mL ADMA (20 mg/kg).

Hemodynamic measurements

The right carotid artery, and left femoral artery were cannulated with polyethylene tubing. The left femoral artery catheter was connected to a pressure transducer and the mean arterial pressure (MAP) and heart rate (HR) were continuously monitored during the experiment. A thermistor derived from an acetone stripped Swan-Ganz catheter, was placed in the thoracic aorta via the right femoral artery. Cardiac Output (CO; Cardiac Output Computer 9520A, Edwards Lifesciences, Irvine, CA) was obtained using the thermodilution method; 200 µl of saline was injected via the right jugular vein catheter as described previously (23).

Systemic vascular resistance (SVR) was calculated by dividing MAP by CO. Stroke volume (SV) was calculated by dividing CO by HR.

Blood flow measurement

In each animal, organ blood flow was measured after 90 minutes and at the end of the experiment (t=180 min.), using FluoSpheres polystyrene microspheres (15 µm scarlet fluorescent (645/680) and green fluorescent (450/480), Molecular Probes Europe, Leiden, the Netherlands). Blood flow was calculated according to the reference sample method as previously described in detail (24). Briefly, an intraventricular injection of microspheres was performed. A reference blood sample was obtained from the left femoral artery at a rate of 0.4 ml/min over 120 seconds. In order to determine the distribution of the microspheres, the left and right triceps muscle blood flow was determined. Portal venous flow was computed as the sum of the arterial flows through the splanchnic organs i.e. the stomach, pancreas, spleen, small intestine and colon.
Organ vascular resistance was calculated by dividing MAP by organ blood flow.

Plasma amino acids and chemical analyses

In order not to affect hemodynamics during the experiment, blood samples were taken at the end of the experiment and immediately placed on ice and centrifuged at 3000 rpm for 10 min at 4°C. Plasma was immediately put in liquid nitrogen, and stored at -80°C before analysis. The concentrations of arginine, ADMA and symmetric dimethylarginine (SDMA) were determined by high-performance liquid chromatography (HPLC) as described previously (25). In brief, solid-phase extraction on polymeric cation-exchange columns was performed after addition of monomethylarginine as the internal standard. After derivatization with ortho-phthaldialdehyde reagent containing 3-mercaptopropionic acid, analytes were separated by isocratic reversed-phase HPLC with fluorescence detection. Intra- and inter-assay coefficients of variation were better than 1.2 and 3.0%, respectively. In addition, the arginine/ADMA ratio was calculated.
Laboratory parameters indicating kidney function (creatinine) and liver function (urea, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactic acid) were measured by standard methods in the clinical laboratory using the Modular P800 (Roche Diagnostics BV, Almere, The Netherlands).
A Sievers 280 NOA Nitric Oxide Analyzer with Radical Purger (Sievers Instruments, Boulder, Colo) was used to detect NO (µM) and its reaction products in biologic samples (26).

Liver histology

Directly after sacrificing the animals, the liver was taken, immediately snap frozen in liquid nitrogen and stored at -80°C. For histological examination, liver specimens were sectioned (6 µm) and routinely stained with hematoxylin and eosin (H&E). All liver specimens were assessed blindly for apoptosis, necrosis and inflammation by an experienced liver pathologist (E.B.).

Statistical Analysis

Data are expressed as means ± standard deviation (SD). Generalized estimation equations (GEEs) were used to investigate differences between groups GEE adjusts for the correlation between repeated observations taken in the same subjects and has the advantage of handling longitudinal data on subjects with varying numbers of unequally spaced observations.

A two-tailed P-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 15.0 (SPSS Inc, Chicago, IL).

Results

Plasma concentrations of arginine, ADMA and SDMA and blood chemistry

Plasma levels of arginine, ADMA, SDMA and the arginine/ADMA ratio at t=180 minutes are shown in table 1. In a pilot study in ASE/ADMA treated rats, plasma concentrations of arginine and ADMA at t=90 minutes were 2.1 μmol/L and 7.2 μmol/L respectively. Infusion of arginase resulted in significantly lower arginine plasma levels in the ASE/ADMA treated rats compared to the control and ADMA groups ($p<0.001$). Infusion of ADMA resulted in significantly higher ADMA plasma levels in the ADMA and ASE/ADMA groups, compared to the controls ($p<0.001$). In the ASE/ADMA group SDMA plasma levels were significantly higher ($p<0.001$) than the SDMA levels in both the control and ADMA groups. Compared to the controls, the arginine/ADMA ratio was significantly lower in the ADMA and ASE/ADMA groups ($p<0.001$).

Creatinine, urea, bilirubin, ALT, lactic acid and nitric oxide did not differ among the three groups (Table 2). AST was significantly higher in the ASE/ADMA group ($p<0.001$).

Table 1. Plasma concentration (μmol/L) of arginine, ADMA, SDMA and the arginine/ADMA ratio in control, ADMA and ASE/ADMA rats at t=180 min.

	Control (N=8)	*ADMA infusion (N=7)*	*ASE/ADMA infusion (N=6)*
Arginine (μmol/L)	82 ± 18	87 ± 17	2 ± 0.4*
ADMA (μmol/L)	0.52 ± 0.09	3.3 ± 0.63*	3.7 ± 1.1*
SDMA (μmol/L)	0.29 ± 0.04	0.31 ± 0.04	0.45 ± 0.03*
Arginine/ADMA ratio	158 ± 32	27.4 ± 7*	0.4 ± 0.17*

Data are presented as means ± SD. *p<0.001 ADMA and/or ASE/ADMA vs controls.

Table 2. Renal and hepatic function and NO concentration in control, ADMA and ASE/ADMA rats at t=180 min.

	Control (N=8)	*ADMA infusion (N=7)*	*ASE/ADMA infusion (N=6)*	*p-value*
Creatinine (µmol/L)	44 ± 5	49 ± 9	45 ± 3.8	0.44
Urea (mmol/L)	5.8 ± 0.9	6.1 ± 0.9	5.2 ± 1.4	0.35
Bilirubin (µmol/L)	2.1 ± 0.3	2.1 ± 0.7	2.0 ± 0.6	0.89
AST (U/L)	249 ± 86	192 ± 51	8631 ± 2300	<0.001
ALT (U/L)	72 ± 46	52 ± 8	87 ± 28	0.19
Lactic acid (mmol/L)	3.4 ± 1.3	3.6 ± 0.6	3.4 ± 0.7	0.92
Nitric Oxide (µmol/L)	19 ± 8	14 ± 5	21 ± 5	0.144

Data are presented as means ± SD.

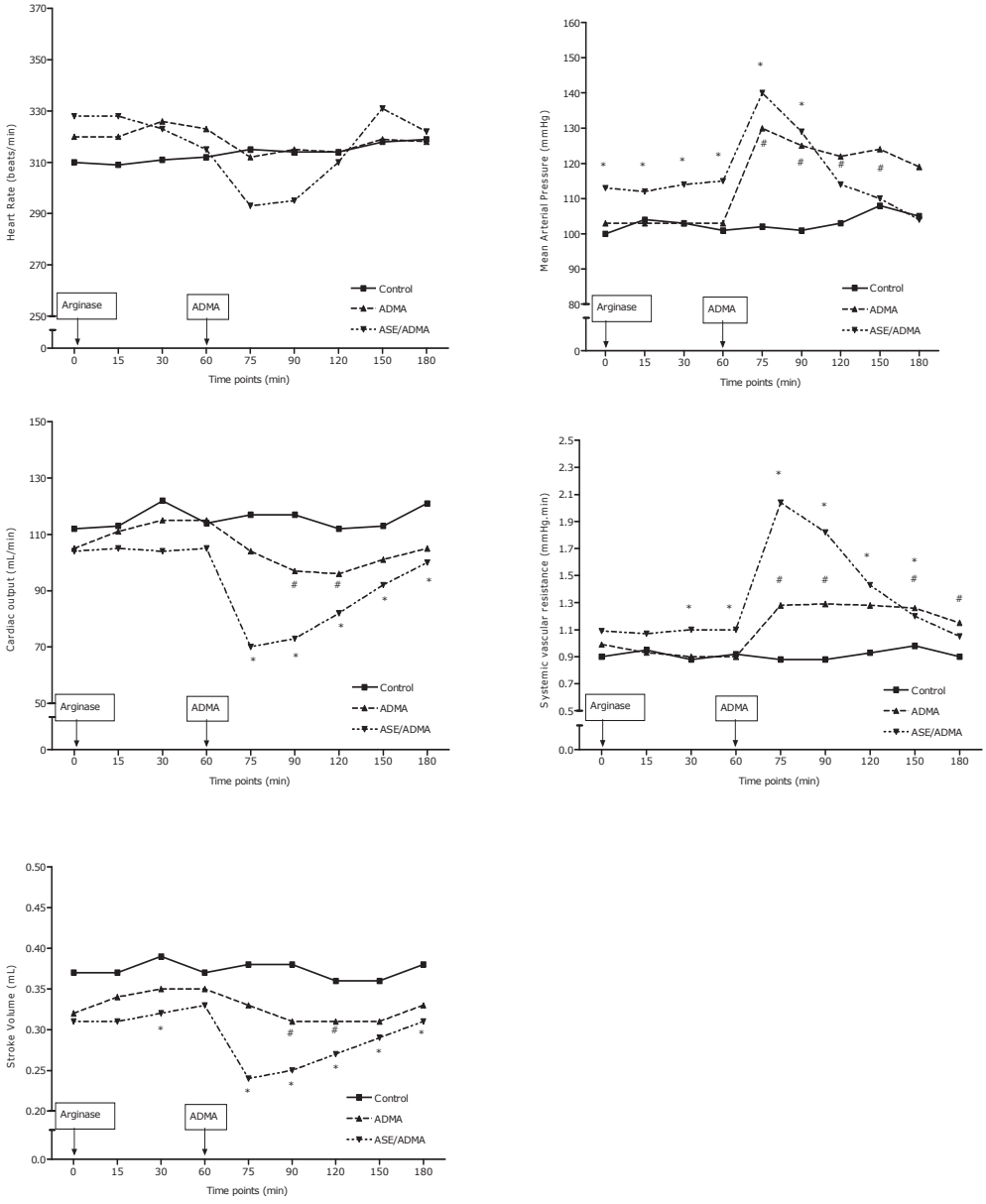
Hemodynamic data

Systemic hemodynamic variables are depicted in figure 1. In the control group, there were no significant changes in hemodynamics during the experiment.

In the ADMA group, MAP increased significantly with 27% ($p<0.001$) at t=75 minutes to 15% ($p=0.034$) at t=150 minutes with respect to the control group. CO decreased significantly with 17% ($p=0.043$) at t=90 minutes and 14% at t=120 minutes ($p=0.002$). SVR increased significantly with 45% ($p<0.001$) at t=75 minutes to 27% ($p=0.016$) at t=180 minutes. SV decreased significantly with 18% ($p=0.024$) at t=90 minutes and 14% ($p=0.044$) at t=120 minutes.

In the ASE/ADMA group, MAP increased significantly with 37% ($p<0.001$) at t=75 minutes and 28% ($p<0.001$) at t=90 minutes compared to the control group. CO decreased significantly with 40% ($p<0.001$) at t=75 minutes to 17% ($p< 0.038$) at t= 180 minutes. SVR increased significantly with 132% ($p<0.001$) at t=75 minutes to 22% ($p=0.035$) at t= 150 minutes. SV decreased significantly with 37% ($p<0.001$) at t=75 minutes to 18% ($p=0.001$) at t=180 minutes.

Figure 1. Changes in systemic hemodynamics in control, ADMA and ASE/ADMA rats adjusted for dependence.



At t=0 arginase (3200 IU) was infused in 20-minutes. At t=60, a bolus of ADMA (20mg/kg) was administered. Results are expressed as means. * $p<0.05$ ASE/ADMA vs controls, # $p<0.05$ ADMA vs controls.

Organ blood flow

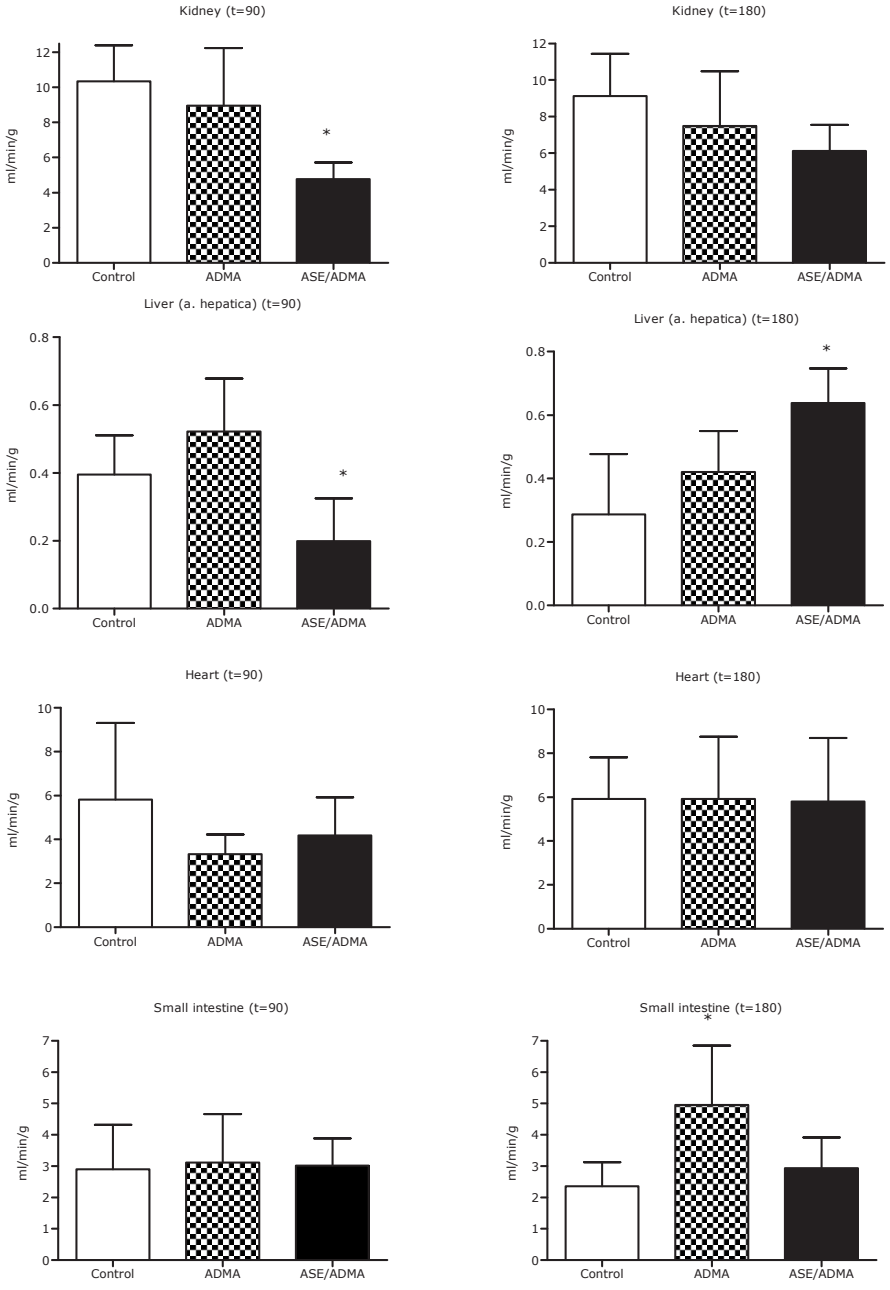
Organ blood flow and the organ blood flow as percentage of the cardiac output are shown in figure 2 and table 3, respectively. ADMA infusion reduced splenic blood flow significantly (p=0.036) after 90 minutes compared to the control group. After 180 minutes, splenic blood flow was still significantly lower (p=0.033). In the ASE/ADMA group, blood flow through the kidney (p=0.001), liver (hepatic artery) (p=0.042) and spleen (p=0.003) was significantly reduced after 90 minutes. After 180 minutes, only splenic blood flow was still decreased (p=0.033) whereas the hepatic arterial blood flow increased significantly (p=0.012).

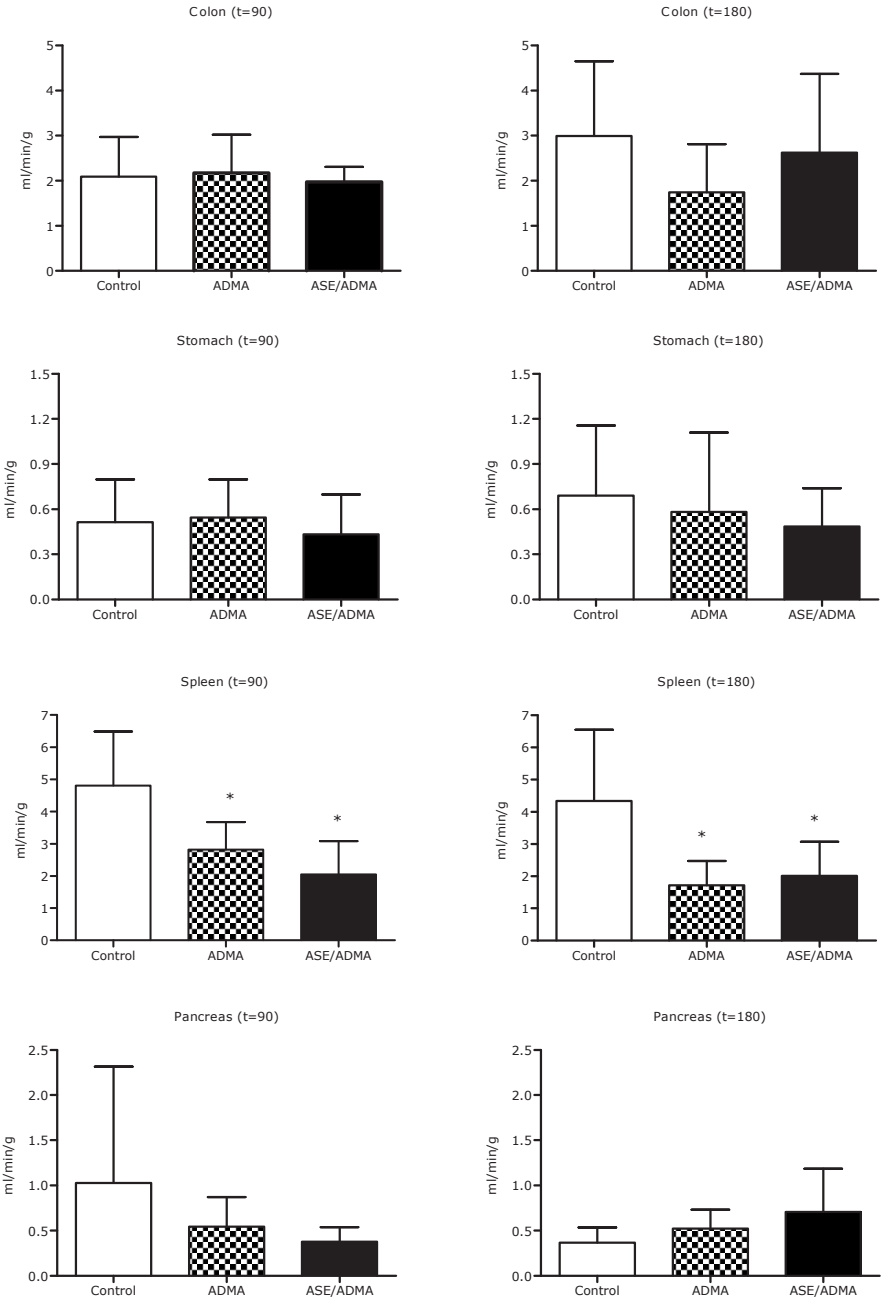
Compared to the control group, a significantly higher percentage of the cardiac output was distributed to the liver in the ASE/ADMA group after 180 minutes (p=0.011). Furthermore, a significantly increased percentage of the cardiac output was distributed to the small intestine in the ADMA group after 180 minutes (p=0.002) and in the ASE/ADMA group after 90 minutes (p=0.016). In the ASE/ADMA group, a significantly increased percentage of the cardiac output was distributed to the colon after 90 minutes (p=0.04). Compared to the ADMA group, a significantly higher percentage of the cardiac output was distributed to the heart in the ASE/ADMA group after 90 minutes (p=0.038).

Organ vascular resistance

Organ vascular resistance is shown in table 4. Compared to the control group, in the ASE/ADMA group significantly higher vascular resistance was found in the kidney (p=0.001) and spleen (p=0.031). Furthermore, the vascular resistance of the liver was also increased in the ASE/ADMA group, but this did not reach statistical significance.

Figure 2. Organ blood flow (ml/min/g) after 90 min. (left column) and 180 min. (right column).




Colon (t=90)
ml/min/g
0
1
2
3
4
5
Control
ADMA
ASE/ADMA
Colon (t=180)
ml/min/g
0
1
2
3
4
5
Control
ADMA
ASE/ADMA
Stomach (t=90)
ml/min/g
0.0
0.3
0.6
0.9
1.2
1.5
Control
ADMA
ASE/ADMA
Stomach (t=180)
ml/min/g
0.0
0.3
0.6
0.9
1.2
1.5
Control
ADMA
ASE/ADMA
Spleen (t=90)
ml/min/g
0
1
2
3
4
5
6
7
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*
Control
ADMA
ASE/ADMA
Spleen (t=180)
ml/min/g
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7
*
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Control
ADMA
ASE/ADMA
Pancreas (t=90)
ml/min/g
0.0
0.5
1.0
1.5
2.0
2.5
Control
ADMA
ASE/ADMA
Pancreas (t=180)
ml/min/g
0.0
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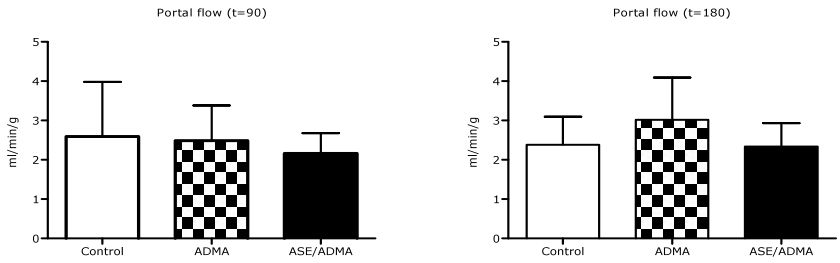
2.5
Control
ADMA
ASE/ADMA



Results are expressed as means ± SD. * p<0.05 compared to controls.

Table 3. Organ blood flow per gram as percentage of the cardiac output.

	Control (N=8)		ADMA infusion (N=7)		ASE/ADMA infusion (N=6)	
	90 min	180 min	90 min	180 min	90 min	180 min
Kidney	7.9 ± 1.8	7.5 ± 2.2	9.0 ± 2.7	6.8 ± 3.2	7.3 ± 1.4	6.1 ± 1.5
Liver (a. hep.)	0.38 ± 0.1	0.23 ± 0.2	0.56 ± 0.2	0.38 ± 0.16	0.28 ± 0.2	0.66 ± 0.3†
Heart	4.7 ± 1.6	5.2 ± 1.3	3.4 ± 0.5	5.3 ± 2.6	5.9 ± 1.9*	10.3 ± 10
Small intestine	2.3 ± 0.7	1.8 ± 0.6	3.3 ± 0.9	4.3 ± 1.4†	4.1 ± 1.2†	3.0 ± 1.1
Colon	1.8 ± 0.3	2.3 ± 1.6	2.3 ± 0.7	1.6 ± 1.1	2.7 ± 0.7†	2.7 ± 2.1
Stomach	1.9 ± 3.1	0.52 ± 0.4	0.5 ± 0.2	0.5 ± 0.4	0.6 ± 0.3	0.5 ± 0.2
Spleen	3.6 ± 1.2	3.2 ± 2	2.8 ± 0.5	1.5 ± 0.5	2.7 ± 1.3	2.0 ± 1.0
Pancreas	0.79 ± 0.7	0.73 ± 0.9	0.53 ± 0.2	0.71 ± 0.4	0.53 ± 0.2	0.71 ± 0.4
Portal	10.4 ± 4.3	9.4 ± 2.9	9.5± 1.7	8.4 ± 2.9	10.7± 2.5	8.8 ± 3.4

Data are presented as means ± SD. †p<0.05 versus controls, *p<0.05 versus ADMA.

Liver histology

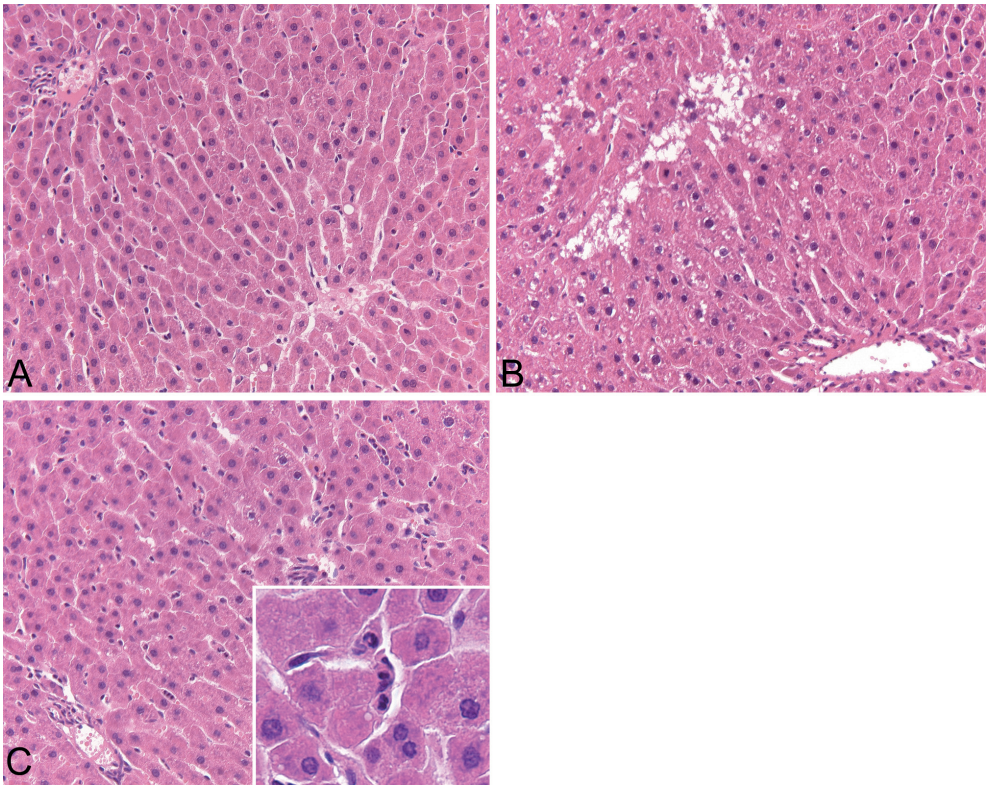
Compared to the control group, different patterns of apoptosis and necrosis were observed in both ADMA and the ASE/ADMA group (Figure 3). In the ADMA group, particularly pericentral apoptosis and necrosis was observed, whereas the liver of the animals in the ASE/ADMA group was characterized by panlobular apoptosis and necrosis of the hepatocytes.

Table 4. Organ vascular resistance (mmHg.g.min.mL^{-1}).

	Control (N=8)		ADMA infusion (N=7)		ASE/ADMA infusion (N=6)	
	90 min	180 min	90 min	180 min	90 min	180 min
Kidney	9.9 ± 2	12 ± 3	14 ± 6	17 ± 8	28 ± 8*	18 ± 6
Liver (a. hep.)	275 ± 85	500 ± 528	242 ± 110	286 ± 96	1229 ± 1207	192 ± 91
Heart	22 ± 11	19 ± 6	36 ± 9	22 ± 9	36 ± 19	22 ± 10
Small intestine	52 ± 35	48 ± 15	38 ± 10	25 ± 8	45 ± 15	39 ± 10
Colon	71 ± 63	49 ± 32	56 ± 24	106 ± 101	65 ± 14	70 ± 61
Stomach	199 ± 151	254 ± 220	233 ± 71	304 ± 195	368 ± 189	287 ± 170
Spleen	23.0 ± 8	33 ± 23	43 ± 13	71 ± 21	88 ± 66†	67 ± 37
Pancreas	292 ± 291	336 ± 184	271 ± 145	312 ± 272	388 ± 169	230 ± 187

Data are presented as means ± SD. †p<0.05 versus controls, *p<0.05 versus controls and ADMA.

Figure 3. Histology of the liver in control (A), ADMA (B) and ASE/ADMA (C) rats.



In the ADMA group (B): pericentral apoptosis and necrosis. In the ASE/ADMA group (C): panlobular apoptosis and necrosis.

Discussion

This study shows that an increase of ADMA levels influences systemic hemodynamics by increasing the MAP and SVR and reduces splenic blood flow. Addition of arginase deteriorates the systemic hemodynamics, i.e. MAP, CO, SVR and SV, reduces blood flow through the kidney and spleen and leads to an initial reduction in hepatic arterial flow, followed by a later major increase.

This is the first study that reports on the effects of arginase and ADMA infusion on systemic hemodynamics in combination with organ blood flow. In contrast to the effect of high plasma ADMA levels, which has been investigated extensively, little is known about the combination of high ADMA levels and low arginine levels on hemodynamics and organ blood flow. Since, apart from other effects, arginine and ADMA play also a crucial role in the synthesis of NO, which is important for the regulation of the vascular tone and for preservation of organ blood flow, it is essential to obtain more insight into the consequences of alterations of ADMA and arginine (19).

In adult mammals, the kidney has a key role in maintaining a constant supply of arginine, an amino acid which is converted into NO by the enzymes NOS (27). Arginine degradation and/or utilization is increased during growth, wound healing, sepsis, shock, injury and in surgical vascular procedures (4;28;29). However, despite the increased need of arginine, low arginine plasma levels are found in patients with major trauma, severe sepsis and after thoracoabdominal aortic surgery (4;7).

ADMA is a naturally occurring methylarginine which inhibits all isoforms of NOS and thereby decreases NO synthesis (14). ADMA is produced as a result of proteolysis of methylated proteins. Methylation of arginine incorporated in proteins is a process of post-translational modification of protein function that is carried out by a group of enzymes known as the protein arginine methyltransferases (PRMT) (30). ADMA is metabolized into citrulline and dimethylamine by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes (31;32). DDAH is mainly present in the liver and the kidney but also in the pancreas, spleen, lung, brain and the endothelium (33-35).

Increased plasma ADMA concentrations occur in a wide range of diseases in which cardiovascular events are increased (32). Particularly in critically ill patients, high ADMA concentrations (highest quartile) proved to be a strong and independent risk factor for ICU death (21).

In the current rat model, low arginine plasma levels and elevated ADMA levels are measured. In order to realize low arginine levels, arginase was infused in a dose (3200 IU per rat in a 20 min period) as previously described (22). Arginase is an enzyme that catalyzes the hydrolysis of arginine in the final step of the urea cycle to ornithine and urea (36). Two isoforms (I and II) of arginase have been identified. Arginase I is the hepatic isoform, whereas arginase II has a mainly extrahepatic, mitochondrial localization. Arginase has recently emerged

as a critical regulator of NO synthesis that may contribute to the development of numerous pathologies, including vascular disease.

Apart from arginase, 20 mg/kg ADMA was infused which led to a 7-fold increase in circulating ADMA. A difference between our model and patients that has to be addressed is the acute effect of administered ADMA. Normally, ADMA is formed continuously and is actively metabolized by DDAH. Although we have only studied the acute effects of ADMA, there is evidence to suggest that chronic exposure to ADMA is likely to produce even greater effects (37-40).

The separate effects of arginase and ADMA on hemodynamics and blood flow were in line with other studies. As previously shown by Prins et al., infusion of only arginase neither affected systemic hemodynamic variables nor organ blood flow (22).

This result was confirmed by the present study, since, in the course of the first 60 minutes, infusion of arginase did not influence systemic hemodynamics. The hemodynamic changes after infusion of ADMA were also in line with the results of other studies (41). In1992, Vallance and co-workers demonstrated that infusion of ADMA into guinea pigs dose dependently increased the MAP (20). Furthermore, local infusion of ADMA into the brachial artery of healthy volunteers caused a dose-dependent fall in forearm blood flow. In humans, Achan and co-workers showed that intravenous administration of ADMA increased the MAP and SVR (32).

However, as shown in the current study, the combination of low arginine levels and high ADMA levels deteriorated systemic hemodynamics e.g. MAP, CO, SVR and SV and reduced the blood flow through important organs such as the kidney and liver. Since the cardiac output is of significance importance for maintaining blood flow through the organs, particularly the decreased cardiac output is probably of significance importance concerning the alteration of regional blood flows as indicated in this study. Infusion of both arginase and ADMA, may lead to a reduced NO synthesis which increases the systemic vascular resistance and mean arterial pressure and consequently a reduced cardiac output. However, NO plasma levels did not differ significantly between the three groups. A possible explanation could be that due to the highly reactive properties of NO, its short half-life (few seconds) and because plasma levels are affected by many factors such as intake (food and water) and excretion (faeces, urine, expired air), no differences could be found (42;43).

Next to the importance of NO as vasodilator, NO is also of great importance in regulating organ perfusion (10). As shown in this study, the reduction of kidney, liver and splenic blood flow could be a result of the decreased NO synthesis and concomitantly a decreased cardiac output. This result is supported by the demonstrated increase of the vascular resistance of the kidney, liver and spleen. However, concerning the liver blood flow it is noteworthy that the proportional hepatic arterial flow increased markedly, whereas the portal blood flow remained stable despite the major reduction of the cardiac output. This could signify that

the liver is capable to preserve blood flow. However, since liver histology revealed increased liver injury, this could conceivably be explained by alteration of the microcirculation. Another potential cause of the manifested liver damage could be by hepatic ischemia-reperfusion injury given that the hepatic arterial flow firstly decreased (90 min.) followed by a later major increase (180 min.).

In addition to the altered plasma concentrations of arginine and ADMA, SDMA levels were significantly increased after infusion of both arginase and ADMA. In contrast to ADMA, SDMA has an indirect effect on NO synthesis. SDMA inhibits the y^+ transporters that mediates the intracellular uptake of arginine and inhibits renal tubular arginine absorption (44-46). These two mechanisms could indirectly inhibit NO synthesis by interfering with arginine uptake. SDMA is almost completely eliminated by the kidney by which SDMA may also be an excellent marker of renal function. Notwithstanding that creatinine did not differ between the three groups, increased SDMA levels could be a result of a reduced kidney function due to the decreased renal blood flow.

Finally, AST levels were significantly increased after infusion of arginase and ADMA. Increased release of AST into serum could indicate increased damage of different organs such as the liver and kidney. Indeed, this hypothesis is further supported by the fact that increased detriment of the hepatocytes (panlobular apoptosis and necrosis) was observed in liver histology of the ASE/ADMA group.

The limitations of our study need to be addressed. Although the aim of this study was to study the effects of altered ADMA and arginine levels, extrapolating from our animal model to the complex entity of human critical illness should be done with great caution. Furthermore, due to ethical reasons, in the present study we only investigated three groups (control, ADMA and arginase/ADMA) since in a previous study our research group already investigated the effect of arginase on systemic hemodynamics and organ perfusion. However, since histology of the liver was not performed in that study, we cannot exclude a direct effect of arginase on the liver (22).

In conclusion, infusion of ADMA increased MAP and SVR and decreased splenic blood flow. Combined with infusion of arginase, causing low arginine plasma levels, administration of ADMA led to deterioration of systemic hemodynamics, decreased kidney and splenic blood flow and an initial reduction of the arterial flow through the liver, followed by a later major increase. In addition, increased detriment of the hepatocytes was found. These results are in concordance with the hypothesis paper of Nijveldt et al. suggesting that the arginine-NO pathway could play a major role in the onset of organ failure (47). Maintaining the arginine and ADMA concentration at physiological levels seems to be a prerequisite for maintaining systemic hemodynamics optimally and in sustaining organ blood flow through important organs such as the kidney and liver.

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