Chapter 7

The effect of rosiglitazone on asymmetric dimethylarginine (ADMA) in critically ill patients

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Abstract

Introduction
Asymmetric dimethylarginine (ADMA) plays a crucial role in the arginine-nitric oxide pathway. Critically ill patients have elevated levels of ADMA which proved to be a strong and independent risk factor for ICU mortality. The aim of this study was to investigate the effect of the peroxisome proliferator-activated receptor (PPAR)-gamma agonist rosiglitazone on ADMA plasma levels in critically ill patients.

Methods
In a randomized controlled pilot study, ADMA, arginine and symmetric dimethylarginine (SDMA) were measured in 21 critically ill patients on the intensive care unit (ICU). Twelve patients received 4 mg rosiglitazone once a day for a maximum of 6 weeks or until discharge or death. Nine patients served as control patients. In addition, total sequential organ failure assessment (SOFA score), kidney function and liver function were determined.

Results
Compared to the ADMA levels of healthy individuals as specified in earlier studies, ADMA plasma levels of critically ill patients were significantly higher (0.42 ± 0.06 versus 0.73 ± 0.2 μmol/L, respectively; $p<0.001$). Both ADMA (B=3.5; 95% CI: 0.5 to 6.5; p=0.023) and SDMA (B= 1.7; 95% CI: 0.7 to 2.7; p=0.001) were independently related to SOFA scores. Overall, rosiglitazone treatment had no effect on ADMA levels, which only significantly differed between the rosiglitazone and control groups at day 7 (p=0.028). The SOFA score in the rosiglitazone group was lower compared to the control group but the difference was only statistically significant at day 10 (p=0.01).

Conclusions
In critically ill patients plasma ADMA levels were elevated and associated with the extent of multiple organ failure, but no significant ADMA-lowering effect of the PPAR-gamma agonist rosiglitazone was observed.

Introduction

One of the major endothelium-derived vasoactive mediators in the human body is nitric oxide (NO). NO is a gaseous signalling molecule which is involved in a wide variety of regulatory mechanisms of the cardiovascular system, including regulation of the vasomotor tone, cell adhesion to the endothelium, inhibition of platelet aggregation, and vascular smooth muscle cell proliferation (1). Furthermore, NO plays also an important role in several pathophysiological aspects of critical illness, such as infection, inflammation and organ injury (2).
NO is synthesized from the amino acid arginine by the action of NO-synthases (NOS), a family of enzymes with endothelial, neuronal, and inducible isoforms (3). Dysfunction of the arginine-NO pathway is a common mechanism by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall, including diabetes mellitus, hypercholesterolemia, hypertension, smoking, and hyperhomocysteinemia (4).
Recent insights into NO metabolism have shown an important role of endogenously produced inhibitors of the enzyme NOS; in particular asymmetric dimethylarginine (ADMA) (5). Several studies have shown elevated concentrations of ADMA in patients with conditions characterized by endothelial dysfunction, including peripheral arterial disease (6), diabetes mellitus (7), hypercholesterolemia (8), and hyperhomocysteinemia (9).
Apart from patients suffering endothelial dysfunction, our group found elevated levels of ADMA in critically ill patients, and moreover, ADMA proved to be a strong and independent risk factor for ICU mortality (10).
Stühlinger and co-workers (11), studied the effect of pharmacological intervention with rosiglitazone, a peroxisome proliferator-activated receptor (PPAR) -gamma agonist on plasma ADMA levels. They found that rosiglitazone improved insulin sensitivity and reduced plasma ADMA levels by 30% in seven insulin-resistant patients. Consistent with this study, Wang and co-workers (12) observed reduced plasma ADMA levels in patients with metabolic syndrome after treatment with rosiglitazone.
We hypothesize that rosiglitazone reduces plasma ADMA levels, and concomitantly has a beneficial effect on organ function and morbidity in critically ill patients. However, since rosiglitazone has never been given to critically ill patients, the primary objectives of this (pilot) study were to investigate the pharmacokinetics of rosiglitazone and its effect on plasma ADMA levels in critically ill patients.

Patients and methods

Patients and study design
In this randomized controlled pilot study, blood samples were drawn from 21 critically ill patients on the intensive care unit (ICU) of the VU University medical

center, Amsterdam, The Netherlands, the Erasmus Medical Center, Rotterdam, The Netherlands and the University Hospital Leuven, Leuven, Belgium. The Institutional Review Boards and the Hospital Ethics Committees of all participating hospitals approved the study. Written informed consent was obtained from the closest family member before participation in the study. In addition, a Case Report Form (CRF) of each included patient was completed.
From January 2006 until December 2007, the senior intensivist of the ICU judged on the suitability for inclusion. Patients were included if they met both criteria: (1) clinical evidence of dysfunction of ≥ 2 organs, irrespective of the cause of organ dysfunction and (2) calculated total sequential organ failure assessment (SOFA) score (13) ≥ 7. Exclusion criteria were an age under 18 or above 75 years, impaired hepatic function (prothrombin time > 1.5 times the upper limit of normal or alanine aminotransferase (ALT) > 2.5 times upper limit of normal), previous (known) history of diabetes type I or II, hypercholesterolemia and/or hyperhomocysteinemia.
After inclusion, patients were randomised into either the rosiglitazone group receiving 4 mg rosiglitazone once a day or the control group. Rosiglitazone tablets were grinded to a powder, mixed with water, and administered via the gastric tube.

Procedures
After inclusion, a heparinised blood sample was drawn from an indwelling arterial line for determination of ADMA, arginine and SDMA at baseline. Subsequently, on day 2, 5, 7, 10, 14, 21, 28, 35 and 42 a blood sample was drawn. Simultaneously, laboratory parameters indicating renal function (creatinine, urea) and hepatic function (aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphatase (AP), prothrombin time (PT), lactic acid) were determined. In patients receiving rosiglitazone, the rosiglitazone plasma concentration was determined on day one at baseline and 1, 2, 4, 8, 12 and 24 hours after administration of rosiglitazone.
The patients were followed for a maximum of 42 days or until they deceased (non-survivors) or were discharged (survivors) from the ICU.

Laboratory procedures
The heparinised arterial blood samples for the determination of ADMA, arginine and SDMA, were 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 concentration of ADMA, arginine and SDMA were determined by high-performance liquid chromatography (HPLC) as described previously (14), with modified chromatographic conditions (15). In brief, solid-phase extraction on polymeric cation-exchange columns was performed after addition of monomethylarginine as the internal standard. After derivatization with ortho-phtaldialdehyde reagent containing 3-mercaptopropionic acid,

analytes were separated by isocratic reversed-phase HPLC with fluorescence detection. Inter-assay coefficients of variation were <3.0% for arginine and ADMA and <4% for SDMA. The arginine/ADMA ratio was calculated.
Laboratory parameters indicating liver function (AST, ALT, AP, PT, lactic acid) and renal function (creatinine and urea) were measured by standard methods in the clinical laboratory.
Rosiglitazone plasma concentrations were quantitatively determined by a validated validated Liquid Chromatography Tandem Mass Spectrometry (LCMS/MS) as described previously(16).

Pharmacokinetic analysis
Pharmacokinetic parameters were derived by use of noncompartmental methods in WinNonlin (version 1.5; Scientific Consulting, Inc., Cary, NC). The highest observed serum concentration was defined as the C_{max} with the corresponding sampling time as T_{max}. The area under the plasma rosiglitazone concentration versus time curve from 0 to 24 hours ($AUC_{[0-24h]}$) was obtained by use of the linear trapezoidal rule. The concentration at 24 hours after administration of rosiglitazone was defined as the trough concentration (C_{min}). The terminal log-linear period (log C versus T) was defined by the last data points ($n \geq 3$) by visual inspection. The absolute value of this slope ([lambda]z) was calculated by least squares regression analysis. The elimination half-life ($T_{1/2}$) was calculated using $T_{1/2}$ = ln2/[lambda]z. The clearance was calculated by dividing the dose by the area under the concentration curve extrapolated to infinity (AUC_{inf}). The volume of distribution in steady state (V_{ss}) was estimated from the mean residence time extrapolated to infinity times clearance.

Statistical analysis
Data are expressed as means ± standard deviation (SD). Differences between the baseline characteristics of the rosiglitazone and control groups were analysed by using Chi-square, Mann-Whitney U or a Students T-test. 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. In addition, multivariate linear regression analyses were performed to determine the relation between the SOFA score and the plasma concentrations of arginine, ADMA and SDMA and between the plasma ADMA concentration and liver function.
A two-tailed P-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 15.0 (SPSS Inc, Chicago, IL).

Results

In total 22 critically ill patients were included in the study. One patient received shortly after inclusion plasmapheresis and was therefore excluded. Twelve patients received 4 mg rosiglitazone once a day and nine patients served as controls. Baseline patient characteristics and biochemical variables including amino acid concentrations are shown in Tables 1 and 2, respectively.

Table 1. Demographics of the patient population.

Characteristic	*Rosiglitazone (n=12)*	*Control (n=9)*
Male : Female, no.	8 : 4	5 : 4
Age (year)	64 (6)	64 ± 10
Body mass index	22.6 (14.2-39.7)	25.4 (20.1-31.1)
ICU admission type, no. (%)		
Medical	5 (42)	4 (45)
Surgical unscheduled	2 (16)	2 (22)
Surgical scheduled	5 (42)	3 (33)
Comorbidities, no. (%)		
Hypertension	2 (17)	1 (11)
Ischemic heart disease	6 (50)	2 (22)
Cardiac failure	-	2 (22)
Chronic obstructive pulmonary disease	1 (8)	-
Renal failure	3 (25)	-
SOFA score (total)	11 (7-16)	12 (7-16)
SOFA score, no. (%)		
Respiratory failure	12 (100)	8 (89)
Coagulation failure	8 (67)	4 (45)
Hepatic failure	6 (50)	6 (67)
Cardiovascular failure	11 (92)	7 (78)
Neurological failure	4 (33)	3 (33)
Renal failure	10 (83)	8 (89)
ICU stay (days)	45 (11-175)	31 (14-62)
Duration of ventilatory support (days)	20 (6-118)	25 (9-43)
ICU mortality, no. (%)	3/12 (25)	5/9 (56)

Data are presented as means ± SD or medians and range unless otherwise is stated.

Table 2. Biochemical variables and amino acid concentration at baseline (t=0).

	Rosiglitazone (n=12)	*Control (n=9)*
Blood glucose (mmol/L)	8.5 ± 2.2	7.5 ± 1.9
CRP (mg/L)	198 ± 131	155 ± 94
Creatinine (µmol/L)	175 ± 104	273 ± 173
Lactic acid (mmol/L)	2.57 ± 2.3	1.56 ± 0.5
Urea (mmol/L)†	19.2 ± 7.8	36.2 ± 21.6
Alanine aminotransferase (ALT)	29.6 ± 20.9	37.7 ± 24.8
Aspartate aminotransferase (AST)	54.6 ± 28.1	69.3 ± 50.8
Bilirubin	19.7 ± 13	138 ± 246
Alkaline phosphatase (AP)	329 ± 286	174 ± 126
Prothrombin time (PT)	1.39 ± 0.35	1.46 ± 0.24
ADMA (µmol/L)	0.77 ± 0.21	0.67 ± 0.17
Arginine (µmol/L)	61 ± 26	78 ± 31
SDMA (µmol/L)	1.52 ± 0.78	1.43 ± 0.53
Arginine/ADMA ratio†	81 ± 32	115 ± 33

Data are presented as means ± SD. †$p<0.05$.

Rosiglitazone was well tolerated, and no adverse drug reactions occurred. The median plasma concentrations of rosiglitazone versus time curve are shown in Figure 1. Pharmacokinetic parameters of rosiglitazone are shown in Table 3.

Table 3. Pharmacokinetic parameters of rosiglitazone in critically ill patients.

Parameter	*Median (range)*
$AUC_{(0\text{-}24)}$ (h*ng/L)	679 (201-2812)
C_{max} (ng/L)	93 (43-246)
T_{max} (h)	1 (1-2)
$T_{1/2}$ (h)	4.4 (2.3-7.5)
Clearance (L/h)	0.0056 (0.001-0.02)
V_{ss} (L)	33.9 (12.9-118.5)

Data are presented as median and range.

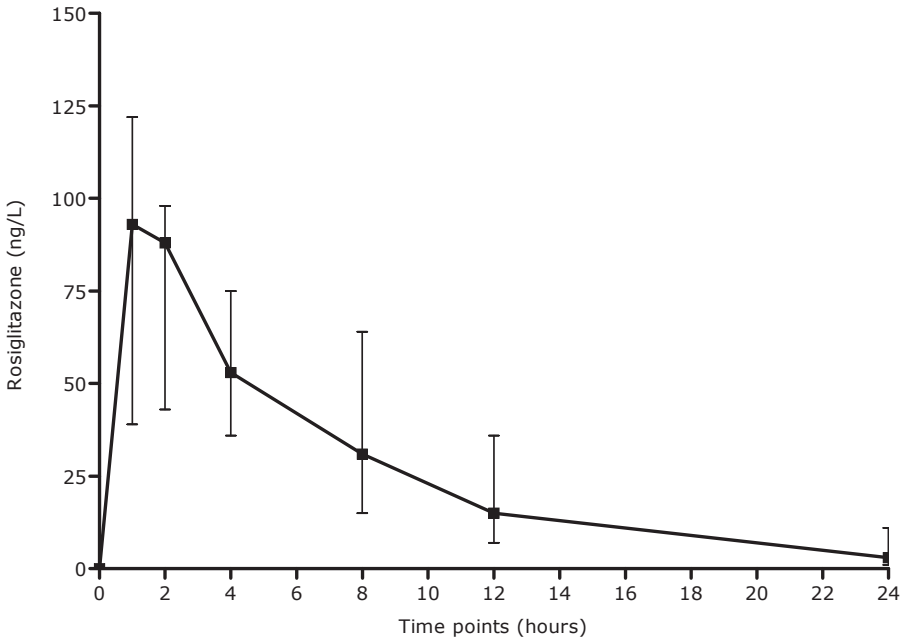

Figure 1. Median rosiglitazone plasma concentration versus time curve. The error bars represent the 25% and 75% percentiles.

At baseline, ADMA and SDMA plasma levels were significantly higher and arginine levels lower in critically ill patients (ADMA: 0.73 ± 0.2 μmol/L, SDMA: 1.48 ± 0.68 μmol/L, arginine: 68 ± 29 μmol/L) compared to healthy volunteers(14) (ADMA: 0.42 ± 0.06 μmol/L; $p<0.001$, SDMA: 0.47 ± 0.08 μmol/L; $p<0.001$ and arginine: 94 ± 29 μmol/L; $p<0.001$ respectively).
The course of the amino acids ADMA, arginine, SDMA, the arginine/ADMA ratio, the SOFA score and glucose levels are shown in Figure 2. The arginine/ADMA ratio reflects the amount of substrate relative to inhibitor of NOS and is often considered as an indicator of potential NO production. ADMA plasma levels did only significantly differ between the rosiglitazone and control groups at day 7 ($p=0.028$). Arginine concentration was only significantly higher in the control group at day 2 ($p=0.045$). In the control group, SDMA concentration was significantly higher at day 21 ($p=0.003$) and day 35 ($p=0.05$). The arginine/ADMA ratio was significantly lower in the rosiglitazone group compared to the control group at baseline ($p=0.013$), day 2 ($p=0.003$) and day 7 ($p=0.029$). The SOFA score in the rosiglitazone group was lower compared to the control group but the difference was only statistically significant at day 10 ($p=0.01$). Glucose concentration was significantly lower ($p=0.002$) in the rosiglitazone group at day 35 compared to the control group. Insulin levels did not show significant differences.

Additionally, the average percentage change of the amino acids between both groups was analysed. However, no significant effects were found in this analysis (data not shown).

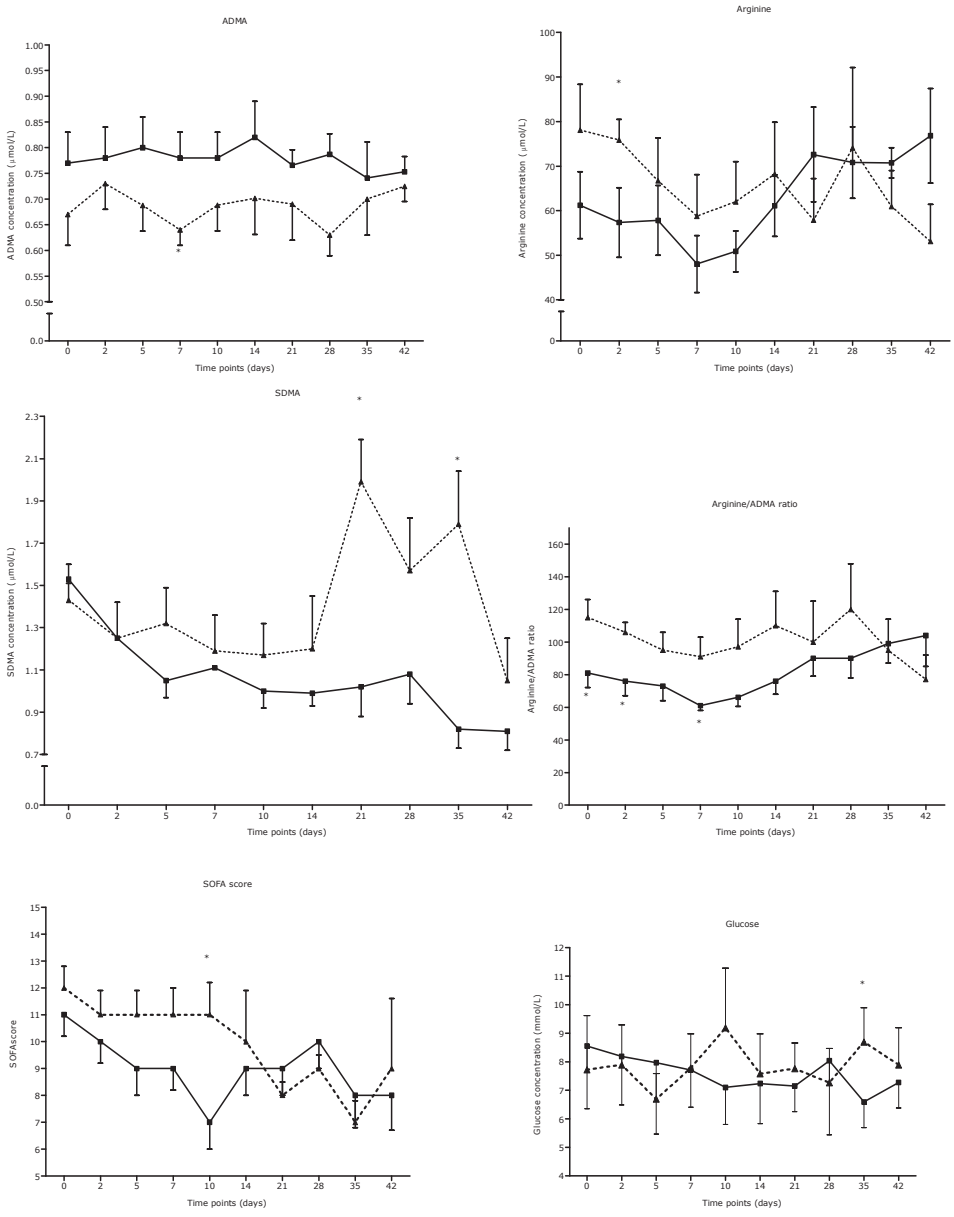

Figure 2. Changes of ADMA, arginine, SDMA, arginine/ADMA ratio, SOFA score, and glucose levels of critically ill patients adjusted for dependence. Results are expressed as means ± SD. The broken line represents the control group (n=9) and the uninterrupted line the rosiglitazone group (n=12).* $p<0.05$ rosiglitazone groups vs. controls.

Table 4. Multivariate linear regression model of the relation between plasma concentrations of ADMA, arginine, SDMA and the SOFA score at baseline in critically ill patients (n=21).

Independent variables	*Regression coefficient (B)*[¶]	*95% CI*	*p-value*
ADMA (µmol/L)	3.5	0.5 to 6.5	0.023
Arginine (µmol/L)	-0.002	-0.023 to 0.018	0.82
SDMA (µmol/L)	1.7	0.7 to 2.7	0.001

[¶] Regression coefficients are expressed as increase/decrease of the SOFA score per 1 µmol/L increase of the independent variable.

Multivariate analysis of the relationship between the SOFA score and the amino acids ADMA, arginine and SDMA revealed that both ADMA (regression coefficient 3.5, p=0.023) and SDMA (regression coefficient 1.7, p=0.001) were independently related to SOFA scores at baseline (Table 4), whereas arginine did not significantly contribute to the model. This indicates that every 1 µmol/L increase of ADMA or SDMA was associated with an increase of 3.5 or 1.7 points of the SOFA score, respectively.
Since the liver is a major ADMA metabolizing organ, we also investigated the relation between variables related to liver function and plasma ADMA concentration. Upon multivariable linear regression analysis, only lactic acid was independently associated with ADMA (regression coefficient 0.04; 95% CI: 0.013 to 0.067; p=0.004), whereas ALT and bilirubin did not significantly contribute to the model.

Discussion

The main findings of the present study are that in critically ill patients plasma levels of ADMA are elevated and positively associated with the severity of illness, but are not significantly lowered upon treatment with the PPAR-gamma agonist rosiglitazone.
During the last decennium, ADMA has been identified as an important risk factor, not only for cardiovascular diseases, but also as an important determinant in the development of critical illness, (multiple) organ failure and ICU death (10;17;18). Indeed, the present study confirms the association between increased ADMA plasma levels and severity of organ failure.
Regrettably, there are currently no specific ADMA-lowering therapies available. Yet, both Stühlinger and co-workers (11) and Wang and co-workers (12), suggested that plasma ADMA levels could be reduced by administration of the PPAR-gamma agonist rosiglitazone. Rosiglitazone belongs to a class of medications called thiazolidinediones which reduce blood glucose levels by improving insulin sensitivity in organs such as liver and muscle. In addition, thiazolidinediones have been shown to reduce free fatty acid plasma levels (19), enhance endothelial function (20), increases NO production (21) and may be

vasoprotective by their anti-inflammatory (22) and anti-oxidative properties (23). However, recent analyses showed rosiglitazone to increase the risk of myocardial infarction, indicating that the molecular mechanisms by which rosiglitazone affects the cardiovascular system are complex (24).

In this study, rosiglitazone was well tolerated, and no adverse drug reactions occurred. Furthermore, pharmacokinetics of rosiglitazone in critically ill patients was consistent with the pharmacokinetics in type 2 diabetes patients (25) and healthy volunteers (26). The present study shows, in accordance with studies performed in experimental animals and in healthy and diabetic human subjects (27-29), no reduction of ADMA concentrations in critically ill patients after administration of rosiglitazone. Strikingly, in our study, ADMA levels were even higher in the rosiglitazone group but this difference reached only statistical significance at day 7. In addition, the arginine/ADMA ratio was significantly lower during the first 7 days in the rosiglitazone group which could indicate a reduced NO production in this group of critically ill patients. However, we must take into account that in the rosiglitazone group the arginine/ADMA ratio was already significantly lower at baseline.

The adverse consequences of a decreased NO production were shown by López and co-workers (30). They demonstrated in a randomized placebo controlled study, increased mortality in septic critically ill patients who were treated with the nonselective NO synthase inhibitor N^G-methyl-L-arginine hydrochloride. A possible mechanism underlying this adverse effect could be myocardial ischemia. As shown by Avontuur and co-workers (31), inhibition of the NO pathway in septic rats reduced the coronary flow and caused areas of myocardial ischemia. Conceivably, although the underlying mechanism for the apparent increase in myocardial infarction and death from cardiovascular causes associated with rosiglitazone remains uncertain, it could be hypothesized that a reduction of the arginine/ADMA ratio may contribute, at least partly, to the negative effects of rosiglitazone.

In accordance with other studies (10;17;32), compared to healthy individuals, ADMA levels in critically ill patients were significantly higher. Although we cannot designate a single mediator for the increased ADMA concentration in critically ill patients, theoretically an increased metabolic turnover and/or a reduced function of the liver and kidneys in critically ill patients could be a possible reason. ADMA and its stereo-isomer SDMA are formed by the methylation of arginine residues in proteins. In this process, methyl groups from S-adenosylmethionine are transferred to the terminal guanidine group of arginine residues by the enzyme family of protein arginine methyltransferases (PRMT) (33). ADMA is partially excreted by the kidney, but the most important way of eliminating ADMA is by means of metabolization by dimethylarginine dimethylaminohydrolases (DDAH) enzymes which convert ADMA into citrulline and dimethylamine (34). From the approximately 300 µmol of ADMA which is daily generated in humans, approximately 250 µmol (>80%) is metabolised by

DDAH (35). Since DDAH has a high activity in the liver (36), it is not surprising that in critically ill patients, lactic acid was significantly associated with ADMA plasma levels as shown also previously by O`Dwyer and co-workers (17).

The limitations of our study need to be addressed. As far as we know, this is the first study to investigate the effect of rosiglitazone on ADMA plasma levels in critically ill patients. A priori, a power analysis (α = 0.05, β = 0.8) was performed based on a mean ADMA concentration of 0.74 µmol/L and standard deviation of 0.24 µmol/L as demonstrated by Nijveldt and co-workers (10). Based on these assumptions, we calculated a total of 30 critically ill patients to detect a 30% reduction in ADMA levels as shown in the study of Stühlinger and co-workers (11).

Since we did not know the (side)effects of rosiglitazone on critically ill patients, we formulated strict in- and exclusion criteria resulting in a decrease in the size of the study population. Consequently, only 22 critically ill patients could be randomized. Given the a priori assumptions and actual number of included patients, the power of this study decreased to 60%. Furthermore, we did not measure NO or its oxidation products nitrite and nitrate. Due to the highly reactive properties of NO, its short half-life (< 0.1 second in human circulation) and because plasma levels of nitrite/nitrate are affected by many factors such as intake (food and water), excretion (faeces, urine, expired air) and clinical and therapeutic interventions, NO production may not be reliably assessed in critically ill patients (37).

In conclusion, this study confirmed that critically ill patients have increased ADMA plasma levels compared to healthy individuals and that these levels are associated with the extent of multiple organ failure. However, no ADMA-lowering effect of the PPAR-gamma agonist rosiglitazone was found in this study.

References

(1) Andrew PJ, Mayer B. Enzymatic function of nitric oxide synthases. Cardiovasc Res 1999; 43(3):521-531.

(2) Cauwels A. Nitric oxide in shock. Kidney Int 2007; 72(5):557-565.

(3) Forstermann U, Schmidt HH, Pollock JS, Sheng H, Mitchell JA, Warner TD et al. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. Biochem Pharmacol 1991; 42(10):1849-1857.

(4) Cooke JP. Asymmetrical dimethylarginine: the Uber marker? Circulation 2004; 109(15):1813-1818.

(5) Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004; 24(6):1023-1030.

(6) Mittermayer F, Krzyzanowska K, Exner M, Mlekusch W, Amighi J, Sabeti S et al. Asymmetric dimethylarginine predicts major adverse cardiovascular events in patients with advanced peripheral artery disease. Arterioscler Thromb Vasc Biol 2006; 26(11):2536-2540.

(7) Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. Am J Cardiol 2001; 88(10):1201-1203.

(8) Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998; 98(18):1842-1847.

(9) Dayal S, Lentz SR. ADMA and hyperhomocysteinemia. Vasc Med 2005; 10 Suppl 1:S27-S33.

(10) Nijveldt RJ, Teerlink T, van der Hoven B, Siroen MPC, Kuik DJ, Rauwerda JA et al. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. Clin Nutr 2003; 22:23-30.

(11) Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP et al. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. JAMA 2002; 287(11):1420-1426.

(12) Wang TD, Chen WJ, Cheng WC, Lin JW, Chen MF, Lee YT. Relation of improvement in endothelium-dependent flow-mediated vasodilation after rosiglitazone to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome. Am J Cardiol 2006; 98(8):1057-1062.

(13) Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996; 22(7):707-710.

(14) Teerlink T, Nijveldt RJ, De Jong S, van Leeuwen PA. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. Analytical Biochemistry 2002; 303(2):131-137.

(15) De Jong S, Teerlink T. Analysis of asymmetric dimethylarginine in plasma by HPLC using a monolithic column. Anal Biochem 2006; 353(2):287-289.

(16) Chapelsky MC, Thompson-Culkin K, Miller AK, Sack M, Blum R, Freed MI. Pharmacokinetics of rosiglitazone in patients with varying degrees of renal insufficiency. J Clin Pharmacol 2003; 43(3):252-259.

(17) O'Dwyer MJ, Dempsey F, Crowley V, Kelleher DP, McManus R, Ryan T. Septic shock is correlated with asymmetrical dimethyl arginine levels, which may be influenced by a polymorphism in the dimethylarginine dimethylaminohydrolase II gene: a prospective observational study. Crit Care 2006; 10(5):R139.

(18) Siroen MP, Teerlink T, Nijveldt RJ, Prins HA, Richir MC, van Leeuwen PA. The clinical significance of asymmetric dimethylarginine. Annu Rev Nutr 2006; 26:203-228.
(19) Miyazaki Y, Mahankali A, Matsuda M, Glass L, Mahankali S, Ferrannini E et al. Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. Diabetes Care 2001; 24(4):710-719.
(20) Pistrosch F, Passauer J, Fischer S, Fuecker K, Hanefeld M, Gross P. In type 2 diabetes, rosiglitazone therapy for insulin resistance ameliorates endothelial dysfunction independent of glucose control. Diabetes Care 2004; 27(2):484-490.
(21) Vinik AI, Stansberry KB, Barlow PM. Rosiglitazone treatment increases nitric oxide production in human peripheral skin: a controlled clinical trial in patients with type 2 diabetes mellitus. J Diabetes Complications 2003; 17(5):279-285.
(22) Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T et al. Evidence for a potent antiinflammatory effect of rosiglitazone. J Clin Endocrinol Metab 2004; 89(6):2728-2735.
(23) Tao L, Liu HR, Gao E, Teng ZP, Lopez BL, Christopher TA et al. Antioxidative, antinitrative, and vasculoprotective effects of a peroxisome proliferator-activated receptor-gamma agonist in hypercholesterolemia. Circulation 2003; 108(22):2805-2811.
(24) Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007; 356(24):2457-2471.
(25) Werner AL, Travaglini MT. A review of rosiglitazone in type 2 diabetes mellitus. Pharmacotherapy 2001; 21(9):1082-1099.
(26) Cox PJ, Ryan DA, Hollis FJ, Harris AM, Miller AK, Vousden M et al. Absorption, disposition, and metabolism of rosiglitazone, a potent thiazolidinedione insulin sensitizer, in humans. Drug Metab Dispos 2000; 28(7):772-780.
(27) Kelly AS, Thelen AM, Kaiser DR, Gonzalez-Campoy JM, Bank AJ. Rosiglitazone improves endothelial function and inflammation but not asymmetric dimethylarginine or oxidative stress in patients with type 2 diabetes mellitus. Vasc Med 2007; 12(4):311-318.
(28) Wang S, Jiang JL, Hu CP, Zhang XJ, Yang DL, Li YJ. Relationship between protective effects of rosiglitazone on endothelium and endogenous nitric oxide synthase inhibitor in streptozotocin-induced diabetic rats and cultured endothelial cells. Diabetes Metab Res Rev 2006; 23:157-164.
(29) Mittermayer F, Schaller G, Pleiner J, Krzyzanowska K, Kapiotis S, Roden M et al. Rosiglitazone prevents free fatty acid-induced vascular endothelial dysfunction. J Clin Endocrinol Metab 2007; 92(7):2574-2580.
(30) Lopez A, Lorente JA, Steingrub J, Bakker J, McLuckie A, Willatts S et al. Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. Crit Care Med 2004; 32(1):21-30.
(31) Avontuur JA, Bruining HA, Ince C. Inhibition of nitric oxide synthesis causes myocardial ischemia in endotoxemic rats. Circ Res 1995; 76(3):418-425.
(32) Siroen MP, van Leeuwen PA, Nijveldt RJ, Teerlink T, Wouters PJ, Van den BG. Modulation of asymmetric dimethylarginine in critically ill patients receiving intensive insulin treatment: a possible explanation of reduced morbidity and mortality? Crit Care Med 2005; 33(3):504-510.
(33) Paik WK, Kim S. Protein methylase I. Purification and properties of the enzyme. J Biol Chem 1968; 243(9):2108-2114.
(34) Ogawa T, Kimoto M, Sasaoka K. Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. J Biol Chem 1989; 264(17):10205-10209.
(35) Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 2003; 23(8):1455-1459.
(36) Teerlink T. ADMA metabolism and clearance. Vasc Med 2005; 10 Suppl 1:S73-S81.

(37) Baylis C, Vallance P. Measurement of nitrite and nitrate levels in plasma and urine--what does this measure tell us about the activity of the endogenous nitric oxide system? Curr Opin Nephrol Hypertens 1998; 7(1):59-62.