Modulation of asymmetric dimethylarginine in critically ill patients receiving intensive insulin treatment: A possible explanation of reduced morbidity and mortality?*

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LEARNING OBJECTIVES

On completion of this article, the reader should be able to:

1. Describe the influence of insulin therapy in asymmetric dimethylarginine (ADMA) production.
2. Explain the relationship between blood sugar levels, ADMA, and insulin therapy.
3. Use this information in a clinical setting.

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Objective: **Asymmetric dimethylarginine, which inhibits production of nitric oxide, has been shown to be a strong and independent predictor of mortality in critically ill patients with clinical evidence of organ dysfunction. Interestingly, intensive insulin therapy in critically ill patients improved morbidity and mortality, but the exact mechanisms by which these beneficial effects are brought about remain unknown. Therefore, we aimed to investigate whether modulation of asymmetric dimethylarginine concentrations by intensive insulin therapy is involved in these effects.**

Design: **A prospective, randomized, controlled trial.**

Setting: **A 56-bed predominantly surgical intensive care unit in a tertiary teaching hospital.**

Patients: **From a study of 1,548 critically ill patients who were randomized to receive either conventional or intensive insulin therapy, we included 79 patients who were admitted to the intensive care unit after complicated pulmonary and esophageal surgery and required prolonged (≥7 days) intensive care.**

Interventions: **Determination of asymmetric dimethylarginine concentrations.**

Measurements and Main Results: **Asymmetric dimethylarginine concentrations were determined with high-performance liquid chromatography on the day of admission, on day 2, on day 7, and on the last day at the intensive care unit. Although the asymmetric dimethylarginine levels did not change between day 0 and day 2 in patients receiving intensive insulin treatment, there was a significant increase during this period in the conventionally treated patients ($p = .043$). Interestingly, the mean daily insulin dose was inversely associated with the asymmetric dimethylarginine concentration on the last day ($r = -.23$, $p = .042$), and the asymmetric dimethylarginine concentration on the last day at the intensive care unit was significantly lower in the intensive insulin treatment group ($p = .048$). Furthermore, asymmetric dimethylarginine was positively associated with duration of intensive care unit stay, duration of ventilatory support, duration of inotropic and vasopressor treatment, number of red cell transfusions, duration of antibiotic treatment, presence of critical illness polyneuropathy, mean Acute Physiology and Chronic Health Evaluation II score, and cumulative Therapeutic Intervention Scoring System-28 score. In addition, asymmetric dimethylarginine levels in patients who died were significantly higher compared with survivors, and changes in the course of asymmetric dimethylarginine plasma concentrations were predictive for adverse intensive care unit outcome.**

Conclusions: **Modulation of asymmetric dimethylarginine concentration by insulin at least partly explains the beneficial effects found in critically ill patients receiving intensive insulin therapy. (Crit Care Med 2005; 33:504–510)**

***See also p. 674.**

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Critically ill patients are in a hypercatabolic state, a condition in which endogenous sources are consumed to meet increased demand of nutrients. Accordingly, protein breakdown of skeletal muscle and visceral structures takes place. An inadequate response renders the patient more susceptible for developing infectious complications, possibly leading to sepsis, subsequent multiple organ failure (MOF), and eventually death. Hyperglycemia caused by insulin resistance is another common metabolic disorder during critical illness, and pronounced hyperglycemia has been shown to cause complications in these patients (1–3). Interestingly, a recent study provided evidence that maintaining normoglycemia with intensive insulin therapy during critical illness improves outcome and reduces morbidity (4). However, the exact mechanisms by which these beneficial effects are brought about remain unknown. One of the possible explanations may be endothelial dysfunction, which is present in the insulin resistance syndrome and generally results from diminished availability of the gaseous molecule nitric oxide (NO) (5–7).

NO is produced by the enzyme NO synthase, which is directly inhibited by asymmetric dimethylarginine (ADMA) (8). In addition, ADMA may interfere with NO synthesis by competing with arginine for cellular transport across cationic amino acid transporters (CAT) of system y^+ (9). ADMA is produced by methylation of arginine residues in proteins and is released during proteolysis. The elimination of ADMA occurs via both urinary excretion and degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) (10). This enzyme converts ADMA into dimethylamine and citrulline and is mainly present in the liver, kidney, pancreas, and endothelial cells (11, 12). Overexpression of human DDAH in transgenic mice results in a reduction of plasma ADMA levels with a concomitant increase in tissue NO synthase activity, providing strong evidence for an important role of endogenous ADMA in regulating NO synthase activity (13). Theoretically, conditions that decrease DDAH activity could lead to increased concentrations of ADMA and consequently reduced formation of NO. Ito and coworkers (14) observed a reduced activity of DDAH after incubation of endothelial cells with tumor necrosis factor-α or oxidized low-density lipoprotein. Interestingly, hyperglycemia stimulates production of tumor necrosis factor-α and increases free radical generation, whereas insulin has anti-inflammatory actions (15). High glucose levels have also been demonstrated to impair activity of DDAH, thereby causing ADMA accumulation (16). Therefore, it could be hypothesized that tight regulation of glucose levels by intensive insulin treatment preserves DDAH activity. Moreover, insulin may decrease protein breakdown and thereby reduce the production of ADMA.

Recently, we showed seriously elevated concentrations of ADMA in critically ill patients with clinical evidence of organ dysfunction (17). Moreover, in these patients, ADMA proved to be the strongest predictor of intensive care unit (ICU) mortality with a 17-fold increased risk for patients who were in the highest quartile for ADMA. In the present study, we examined whether modulation of ADMA concentrations is involved in the beneficial effects on morbidity and mortality in critically ill patients receiving intensive insulin therapy.

METHODS

Patients. The samples used for the present study were obtained from a prospective, randomized, controlled study including all patients receiving mechanical ventilation admitted to the Department of Intensive Care Medicine of the University Hospital Gasthuisberg, Leuven, Belgium, between February 2, 2000, and January 18, 2001. The complete study protocol and the results of this study have been described in detail previously (4, 18). The protocol was approved by the institutional review board of the University Hospital Gasthuisberg, and written informed consent was obtained from the closest family member before participation in the study. From the total study population of 1,548 patients, we included patients who underwent pulmonary or esophageal surgery and who postoperatively developed complications and therefore were admitted to the ICU. From this group of patients, we included the patients who remained in the ICU for ≥7 days. This patient group was chosen because it contained critically ill patients with the highest probability of developing serious complications and it has been shown that critically ill patients with MOF have highly elevated concentrations of ADMA, which predict ICU mortality (17). Therefore, a beneficial effect of insulin on ADMA levels may be expected in the "complicated pulmonary and esophageal surgery" group.

Insulin Therapy. On the day of admission to the ICU, all patients were randomly assigned to receive either conventional or intensive insulin therapy. In the conventional treatment group, insulin (Actrapid HM, Novo Nordisk, Copenhagen, Denmark) was administered as a continuous infusion of insulin (50 IU Actrapid HM in 50 mL of 0.9% sodium chloride) by using an infusion pump (Perfusor-FM, B. Braun, Melsungen, Germany) only when blood glucose concentration exceeded 11.9 mmol/L. The infusion was adjusted to maintain blood glucose levels between 10.0 and 11.1 mmol/L. In the intensive treatment group, insulin infusion was started when blood glucose concentration exceeded 6.1 mmol/L and was adjusted to maintain normoglycemia (4.4–6.1 mmol/L).

Whole blood glucose levels were measured on site in undiluted arterial blood. During the first 12–24 hrs after admission to the ICU, until the targeted range was reached, measurement of blood glucose was advised every 1–2 hrs. Thereafter, blood glucose was measured every 4 hrs, unless steep decreases or increases in blood glucose level occurred, for which hourly control after each dose adjustment was advised.

Nutritional Support. At ICU admission, all patients were started on partial nutritional support with mainly intravenous glucose (8–12 g/hr) and from the next day onward with a standardized feeding schedule, intended to deliver 20–30 nonprotein calories/kg/24 hrs with a balanced composition (0.13–0.26 g nitrogen/kg/24 hrs and 20–40% of nonprotein calories as lipids) of total parenteral, combined parenteral/enteral, or full enteral feeding. Enteral feeding was attempted as early as possible, at the discretion of the attending physician.

Data Collection. At baseline, demographic data and clinical information was obtained. Scores to evaluate the severity of illness were calculated for the Acute Physiology and Chronic Health Evaluation (APACHE II) (19) and cumulative simplified Therapeutic Intervention Scoring System (TISS)-28 (20). Higher scores indicate more severe illness and a higher number of therapeutic interventions, respectively. Blood cultures were obtained whenever the central body temperature exceeded 38.5°C, and a septic episode was defined by the first positive culture in a series.

Clinical Outcome Measures. The primary outcome measure was death during intensive care. The causes of death were defined as "MOF + sepsis" as MOF + systemic inflammatory response (SIRS; as defined by the Bone criteria) (21) together with a proven septic focus and "MOF + SIRS" as MOF + SIRS without a proven septic focus. The causes of death were determined both clinically, by the attending physician, and by postmortem examination by a pathologist unaware of treatment assignment. The Sepsis-Related Organ Failure Assessment score was used in patients who died to objectively determine MOF as a cause of death. MOF was present when two or more organs were failing. Organ failure was defined as a Sepsis-Related Organ Failure Assessment score ≥3 points for each individual organ system (22). The postmortem examina-

tion differentiated between MOF with and without a septic focus, acute cardiovascular collapse, and severe brain damage. Secondary outcome measures used in our study were number of days in the ICU, duration of ventilatory support, inotropic and vasopressor support, the presence of critical illness polyneuropathy, duration of antibiotic treatment, and transfusion requirements.

Blood Sampling and Laboratory Procedures. Blood samples were drawn after admission to the ICU and subsequently on day 2, on day 7, and on the last day at the ICU (i.e., day before discharge or day before death) for determination of ADMA. ADMA was measured by high-performance liquid chromatography with fluorescence detection, as recently described (23). Briefly, 0.1 mL of plasma was mixed with 0.1 mL of a 40 μmol/L solution of the internal standard MMA and 0.8 mL of phosphate buffered saline. This mixture was applied to Oasis MCX solid-phase extraction columns (Waters, Milford, MA) for extraction of basic amino acids. The columns were consecutively washed with 1.0 mL of 100 mM HCl and 1.0 mL of methanol. Analytes were eluted with 1.0 mL of concentrated ammonia/water/methanol (10/40/50). After evaporation of the solvent under nitrogen, the amino acids were derivatized with orthophthaldialdehyde reagent containing 3-mercaptopropionic acid. The derivatives were separated by isocratic reversed-phase chromatography on a Symmetry C18 column (3.9 × 150 mm; 5 μm particle size; Waters). Potassium phosphate buffer (50 mM, pH 6.5) containing 8.7% acetonitrile was used as mobile phase at a flow rate of 1.1 mL/min and a column temperature of 30°C. Fluorescence detection was performed at excitation and emission wavelengths of 340 and 455 nm, respectively. After elution of the last analyte, strongly retained compounds were quickly eluted by a strong solvent flush with 50% acetonitrile, resulting in a total analysis time of 30 mins. The coefficients of variation were 1.2% within assay and 2.0% between assay for ADMA. Reference values for ADMA have been obtained from plasma of healthy laboratory personnel and medical students (23). The concentration of ADMA in these individuals is normally distributed with a mean value of 0.42 μmol/L and a 95% confidence interval of 0.30–0.54. Other biochemical parameters were measured by standard laboratory methods.

Statistical Analyses. General linear model (GLM) for repeated measurements was used to investigate the effect of treatment on glucose plasma levels and ADMA plasma concentrations and to investigate whether ADMA levels differed between survivors and nonsurvivors. In addition, independent-samples Student's *t*-test was used to investigate differences among various time points between two groups. These data are presented as mean and SEM. Since ADMA levels on the day of admission differed significantly between males (0.58 ± 0.02) and females (0.45 ± 0.03, $p = .003$), the GLM was corrected for gender. For the analysis of differences in clinical outcome measures between groups, Mann-Whitney U test was used. These data are presented as medians and interquartile ranges. Relations between variables were investigated by Spearman's rho. The associations of relevant variables with ICU mortality were investigated by univariate regression analysis. Thereafter, these variables were stepwise included in a logistic regression model to study predictive values of variables on ICU mortality. Predictive values are presented as odds ratios with corresponding 95% confidence intervals. We considered $p < .05$ to be statistically significant. Statistical analyses were performed using SPSS (version 11.0 for Windows, SPSS, Chicago, IL).

RESULTS

Patients. Seventy-nine patients were admitted to the ICU after complicated

Table 1. Demographics of patient population

Characteristic	Conventional Treatment (n = 36)	Intensive Treatment (n = 43)
Gender, male/female	26/10	31/12
Age, median (range)	67 (33–84)	66 (41–83)
Body mass index	25.6 (21.8–29.7)	25.7 (22.0–29.4)
ICU stay, days[a]	26 (13–42)	17 (9–30)
Intake of nutrients, g/day		
Carbohydrate	226 (200–247)	226 (198–248)
Protein	73 (64–82)	73 (65–80)
Fat	66 (52–79)	67 (57–79)
Arginine	7.4 (6.4–8.2)	7.3 (6.5–8.0)
Patients receiving insulin, n (%)[b]	16/36 (44)	43/43 (100)
Duration of ventilatory support, days	21 (10–37)	14 (8–23)
Patients receiving inotropics/vasopressors, n (%)	29/36 (81)	34/43 (79)
Patients requiring red cell transfusion, n (%)	30/36 (83)	34/43 (79)
Duration of antibiotic treatment, days[a]	19 (10–40)	12 (8–17)
Critical illness polyneuropathy, n (%)[a]	22/36 (61)	13/43 (30)
APACHE II score, day		
0	15 (9–22)	15 (9–20)
2	14 (11–18)	12 (8–16)
7	12 (9–15)	11 (9–14)
Last	9 (8–15)	10 (5–13)
Cumulative TISS-28 score[a]	813 (220–4553)	553 (178–5241)
ICU mortality, n (%)[a]	12/36 (33)	5/43 (12)

ICU, intensive care unit; APACHE, Acute Physiology and Chronic Health Evaluation; TISS, Therapeutic Intervention Scoring System.

[a]$p < .05$; [b]$p < .001$. Data are presented as medians and interquartile ranges unless otherwise stated.

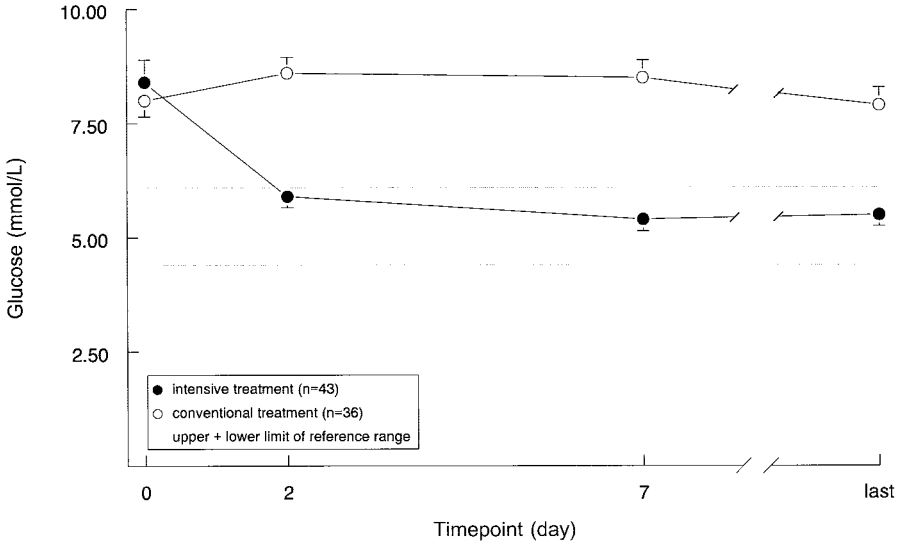

Figure 1. The course of glucose concentrations in critically ill patients receiving either conventional or intensive insulin therapy. Data represent mean ± SEM. General linear model for repeated measurements showed a statistically significant ($p < .001$) overall difference between groups.

pulmonary and esophageal surgery and required intensive care for ≥7 days. Forty-three patients received intensive insulin treatment, whereas 36 patients were treated according to the conventional approach. Patient characteristics are shown in Table 1.

Glucose and Insulin Treatment. Figure 1 shows glucose plasma levels in the two patient groups. GLM for repeated measurements showed that during the period after initiation of insulin therapy, glucose levels were significantly lower in the intensively treated patients in comparison with patients who received conventional treatment ($p < .001$).

ADMA and Insulin Treatment. On the day of admission, ADMA concentrations did not differ between patients receiving either conventional or intensive insulin treatment (Fig. 2). GLM for repeated measurements showed a significant difference in the course of ADMA levels from day 0 to day 2 between both groups ($p = .043$). Whereas the ADMA levels did not change between day 0 and day 2 in patients receiving intensive insulin treatment, there was a significant increase during this period in the conventionally treated patients. During the remaining period, ADMA levels were lower in the intensively treated patients. However, this difference did not reach statistical significance. Furthermore, on the last day at the ICU, ADMA concentrations were significantly lower in patients receiving intensive insulin treatment ($p = .048$). Moreover, the mean daily insulin dose was inversely correlated with the ADMA concentration of all patients on the last day ($r = -.23$, $p = .042$).

ADMA and Morbidity. ADMA levels were significantly related to duration of ICU stay, duration of ventilatory support, duration of inotropic and vasopressor treatment, number of red cell transfusions, duration of antibiotic treatment, presence of critical illness polyneuropathy, mean APACHE II score, and cumulative TISS-28 score (Table 2).

ADMA and Mortality. GLM for repeated measurements revealed that during the whole period, ADMA levels were significantly ($p < .001$) higher in patients who died during their ICU stay (Fig. 3). Main causes of death were MOF due to sepsis (n = 7) and SIRS (n = 8), whereas two patients died due to cardiac shock. Mortality rates were significantly lower in the intensively treated group (12%) compared with the group that received conventional insulin treatment (33%, $p = .02$). In addition, the ADMA concentration on the last day was associated with mortality (Table 2). Furthermore, univariate regression analyses revealed significant relationships between mortality and the following: type of treatment, APACHE II score on the last day, duration of inotropic and vasopressor treatment, duration of antibiotic treatment, number of red cell transfusions, ADMA increase between day 0 and day 2, and ADMA increase between day 7 and the last day at the ICU (Table 3). After these univariate regression analyses, all potential predictors of ICU mortality were stepwise included in a logistic regression model (Table 4). Results showed that a 10%

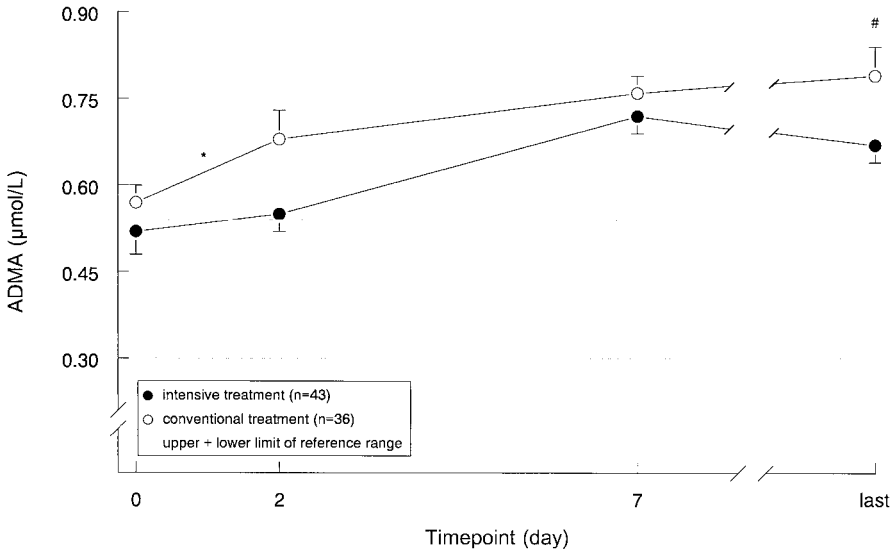

Figure 2. The course of asymmetric dimethylarginine (*ADMA*) concentrations in critically ill patients receiving either conventional or intensive insulin therapy. Data represent mean ± SEM. General linear model for repeated measurements showed a statistically significant (*$p = .043$) different course from day 0 to day 2 between groups. #$p = .048$ between groups.

Table 2. Relations between asymmetric dimethylarginine (ADMA) concentration and outcome variables

	ADMA Day 0		ADMA Day 2		ADMA Day 7		ADMA Last Day	
Outcome Variable	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Duration of ICU stay	.28	.014	.29	.009	.29	.012	.35	.001
Duration of ventilatory support	.27	.018	.31	.006	.25	.035	.43	<.001
Duration of inotropic treatment		NS		NS		NS	.27	.018
Duration of vasopressor treatment		NS		NS		NS	.37	.001
Number of red cell transfusions	.24	.032	.31	.005		NS	.47	<.001
Duration of antibiotic treatment		NS	.26	.020		NS	.40	<.001
Presence of critical illness polyneuropathy	.33	.003	.29	.011	.27	.023		NS
Mean APACHE II score		NS	.31	.006		NS	.36	.001
Cumulative TISS-28 score	.27	.014	.32	.004	.28	.016	.40	<.001
ICU mortality		NS	.22	.050		NS	.44	<.001

ICU, intensive care unit; NS, not significant; APACHE, Acute Physiology and Chronic Health Evaluation; TISS, Therapeutic Intervention Scoring System.

Relations between variables were tested by Spearman's rho.

increase in ADMA concentration from day 0 to day 2 was associated with a 29% higher risk of adverse outcome and that a 10% increase of ADMA between day 7 and the last day at the ICU was associated with a 27% higher risk of adverse outcome. In addition, the APACHE II score on the last day at the ICU was associated with a higher mortality risk, 26% increased risk per point added.

DISCUSSION

The results of this study elucidate a potential mechanism of the reported reduced morbidity and mortality in critically ill patients receiving intensive insulin treatment (4). The most important finding of the present study is the different course in ADMA levels from day 0 to day 2 due to type of insulin treatment: The ADMA plasma concentration significantly increased during the first 2 days after assignment of conventional insulin treatment, whereas the ADMA levels did not change between day 0 and day 2 in patients receiving intensive insulin treatment. Moreover, at the end of the ICU period, ADMA levels were still significantly lower in the intensive treatment group compared with the conventional treatment group. These results, together with the positive association between mean daily insulin dose and ADMA concentration of all patients on the last day, strongly suggest that ADMA is influenced by insulin therapy. Our study is in accordance with the study of Stühlinger and coworkers (24), who showed a significant relationship between ADMA plasma concentrations and insulin resistance in patients with impaired glucose tolerance. In addition, it was shown that glucose regulation by pharmacologic intervention with rosiglitazone enhanced insulin sensitivity and reduced ADMA levels.

Another interesting finding of our study is that ADMA levels were significantly higher in nonsurvivors compared with survivors, regardless of the type of treatment received. Moreover, ADMA changes at the beginning and at the end of the ICU period were independent predictors of mortality. These results confirm our previous findings in a population of critically ill patients where ADMA proved to be a stronger predictor of ICU mortality than traditional risk factors such as age, failure of individual organ systems, and total organ failure score (17). However, the design of this study was cross-sectional, and therefore the novel finding that a change in the course of ADMA levels in ICU patients acts as a predictor of outcome is even more important.

The most likely mechanism by which ADMA increases the risk of adverse outcome in critically ill patients is inhibition of the constitutively expressed endothelial NO synthase (25). NO produced by endothelial NO synthase is important for preservation of organ blood flow by regulating vascular tone and influencing the interaction of white blood cells and platelets with the endothelium. In addition, NO is involved in host defense by acting as a cytotoxic agent. During inflammation, the inducible iso-

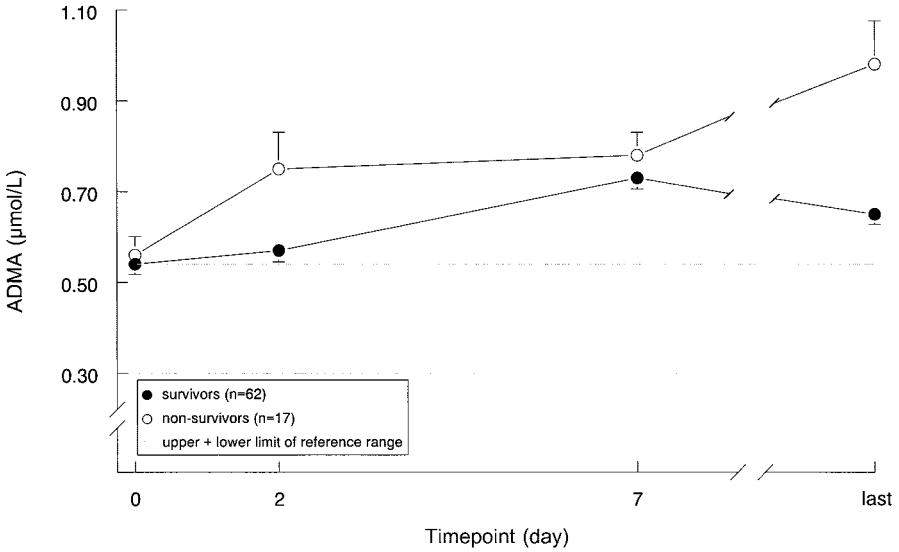

Figure 3. The course of asymmetric dimethylarginine (*ADMA*) concentrations in critically ill patients who survived or died. Data represent mean ± SEM. General linear model for repeated measurements showed a statistically significant ($p < .001$) overall difference between groups.

Table 3. Univariate relationships between relevant predictors and intensive care unit mortality

Risk Factor	OR	95% CI	*p*
Gender	1.10	0.34–3.60	.871
Age (per decade)	1.11	0.70–1.77	.662
Type of treatment (conventional vs. intensive)	3.80	1.19–12.14	.024
APACHE II $score_{day\ 0}$ (per point increase)	1.05	0.97–1.13	.263
APACHE II $score_{day\ 2}$ (per point increase)	1.05	0.96–1.15	.262
APACHE II $score_{day\ 7}$ (per point increase)	1.11	0.99–1.24	.070
APACHE II $score_{last\ day}$ (per point increase)	1.20	1.10–1.30	.001
Duration of ventilatory support (per day)	1.01	0.99–1.03	.177
Duration of inotropic treatment (per day)	1.11	1.04–1.19	.003
Duration of vasopressor treatment (per day)	1.08	1.03–1.14	.004
Duration of antibiotic treatment (per day)	1.03	1.00–1.07	.049
Number of red cell transfusions	1.17	1.03–1.34	.021
Presence of critical illness polyneuropathy	1.56	0.53–4.58	.420
$\Delta ADMA_{day\ 0–day\ 2}$ (per 10% increase)	1.21	1.03–1.42	.020
$\Delta ADMA_{day\ 2–day\ 7}$ (per 10% increase)	0.86	0.71–1.05	.130
$\Delta ADMA_{day\ 7–last\ day}$ (per 10% increase)	1.39	1.12–1.72	.003

OR, odds ratio; CI, confidence interval; APACHE, Acute Physiology and Chronic Health Evaluation; ADMA, asymmetric dimethylarginine.

Table 4. Independent predictors of intensive care unit mortality in critically ill patients

Predictor	OR	95% CI	*p*
$\Delta ADMA_{day\ 0–day\ 2}$ (per 10% increase)	1.29	1.01–1.66	.041
$\Delta ADMA_{day\ 7–last\ day}$ (per 10% increase)	1.27	1.02–1.58	.034
APACHE II $score_{last\ day}$ (per point increase)	1.26	1.08–1.47	.004

OR, odds ratio; CI, confidence interval; ADMA, asymmetric dimethylarginine; APACHE, Acute Physiology and Chronic Health Evaluation.

Stepwise logistic regression analysis of effect of all relevant variables on intensive care unit mortality.

Modulation of asymmetric dimethylarginine concentration by insulin at least partly explains the beneficial effects found in critically ill patients receiving intensive insulin therapy.

form of NO synthase is able to produce large amounts of NO. Overproduction of NO may aggravate tissue damage and may cause systemic vasodilation with therapeutically refractory hypotension and coagulation disorders as seen in septic shock. Assuming that inhibition of these adverse effects has therapeutic potential, the pharmacologic NO synthase inhibitor monomethylarginine has been given to septic patients. However, the results of this study were very recently published and revealed increased mortality rates in patients receiving monomethylarginine (26). In several human and animal studies reporting adverse effects of NO synthase inhibition, the inhibitors were nonselective, inhibiting both endothelial NO synthase and the inducible isoform of NO synthase. ADMA is also a nonselective inhibitor of NO synthase and is endogenously produced. Therefore, when plasma levels of ADMA increase, interference with physiologic functions of NO may be expected.

An interesting study from the group of Avontuur and coworkers (27) showed that even a small amount of endotoxin significantly increased blood flow through the heart. If NO production was inhibited by administration of N^G-nitro-L-arginine, local areas of cardiac ischemia could be detected, which was not seen when N^G-nitro-L-arginine was given without endotoxin. These data strongly suggest that inhibition of NO production may become important when increased demands for the heart are present. A recent study of Achan and coworkers (28) demonstrated that low-dose ADMA infusion in healthy volunteers reduced heart rate and cardiac output and increased mean blood pressure and systemic vascular resistance. Moreover, handgrip exercise increased cardiac output in control patients by 96.8%, but in subjects given ADMA cardiac output increased by only 35.3% ($p < .05$). In addition, our finding that ADMA was positively associated with duration of treatment with inotropic and vasopressor medication, combined with the recent finding that myocardial blood flow is regulated by local activity of DDAH (29), also points to a potential ability of ADMA to adversely influence perfusion, and thus function, of the heart.

Plasma ADMA concentrations probably represent cellular spillover and therefore weakly mirror intracellular concentrations. Both cellular export and import of ADMA occur via CAT proteins of the system y^+, which transport arginine but also methylated arginines across cell membranes (9). Thus, ADMA is able to impair NO production both by direct inhibition of NO synthase and by reducing substrate availability of NO synthase through competition with arginine for the y^+ pump. Interestingly, activity and expression of CAT are affected by glucose and insulin, probably making these substances important in regulating NO synthesis in critically ill patients. CAT-2B is expressed in various kinds of cells and is usually induced under inflammatory conditions (30). Experiments in human umbilical vein endothelial cells revealed that CAT-2B messenger RNA levels increased two-fold after 4 hrs of exposure to 25 mM glucose (31). Thus, when ADMA plasma levels are increased in diseased states accompanied by hyperglycemia and inflammation, increased uptake of ADMA by cells could be expected. In turn, increased intracellular ADMA concentrations may diminish NO release. On the other hand, in ADMA-eliminating organs such as the liver (32, 33), CAT may be of relevance in regulating ADMA concentrations by taking up large amounts of ADMA from the circulation in order to break it down. Hepatic expression of the CAT-1 transporter is negligible under physiologic conditions. However, under circumstances that require hepatic transport of cationic amino acids, such as after feeding or during illness, insulin has been shown to induce expression of CAT-1 (34). Therefore, it could be hypothesized that insulin treatment in critically ill patients stimulates ADMA uptake in the liver by increasing CAT-1 gene expression. Subsequently, ADMA could be degraded by DDAH, which is present in high amounts in the liver. Considering that DDAH is inhibited by hyperglycemia (16), tight regulation of glucose plasma concentrations by intensive insulin therapy may indirectly lead to lower systemic ADMA levels. This hypothesis is supported by the results of the present study, which show an early effect of insulin on ADMA during the first 2 days after initiation of intensive insulin therapy and a significantly lower ADMA level on the last day at the ICU in patients receiving intensive insulin treatment.

Besides the association between ADMA and insulin, ADMA was also related to several outcome variables. Moreover, ADMA levels in patients who died were significantly higher compared with survivors, and a change in the early and late course of the ADMA concentration proved to be a strong and independent predictor of ICU mortality. These relationships strongly suggest that ADMA influences recovery of the critically ill patient, but they do not prove causality between ADMA and insulin. Therefore, our study must be regarded as a first step in the field of clinical research on ADMA and glucose/insulin. Future studies lowering ADMA levels with, for example, selective hemodialysis or up-regulation of the ADMA-degrading enzyme DDAH must confirm the finding that ADMA is associated with outcome variables. Two other studies have also reported potential explanations of improved morbidity and mortality due to intensive insulin therapy in critically ill patients (35, 36). C-reactive protein and mannose-binding lectin are both inflammatory markers that decrease due to intensive insulin treatment (36). In addition, intensive insulin therapy increases low-density lipoprotein and high-density lipoprotein, which may play a significant role in the binding and processing of endotoxins (35). Thus, decreasing the inflammatory response via insulin results in beneficial effects on C-reactive protein, mannose-binding lectin, low-density lipoprotein, and high-density lipoprotein, but it also affects ADMA levels.

CONCLUSIONS

We show that insulin therapy modulates plasma concentration of ADMA. It seems most likely that this modulation is caused by a combination of factors, including preservation of DDAH, reduced protein breakdown and thus less ADMA release, and increased uptake of ADMA via transport systems in organs that eliminate ADMA. Moreover, ADMA is related to several outcome variables, and changes in the course

of ADMA during the ICU period is independently associated with ICU mortality. Therefore, we suggest that modulation of ADMA concentration by insulin at least partly explains the reduced morbidity and mortality in critically ill patients receiving intensive insulin therapy.

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