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ORIGINAL

Tight glycemic control does not affect asymmetric-dimethylarginine in septic patients

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Abstract *Objective:* We investigated whether preventing hyperglycemia in septic patients affected the plasma concentration of asymmetric-dimethylarginine and if this was associated with clinical benefit. *Design:* A prospective, multicenter, randomized, controlled, clinical study. *Setting:* Intensive care units (ICU) in three university hospitals. *Patients:* A total of 72 patients admitted for severe sepsis or septic shock, who stayed at least 3 days in the ICU. At admission the patients were assigned to receive either tight or conventional glycemic control. *Interventions:* Determination of circulating levels of asymmetric-dimethylarginine, arginine, interleukin-6, C-reactive-protein and tumor-necrosis-factor-α. *Measurements and results:* Blood was sampled at admission (no differences between groups), and on the 3rd, 6th, 9th, and 12th (T12) days. Sequential organ failure assessment was scored at each sampling time. All the data were analyzed on an intention-to-treat basis. The control and treatment groups received the same energy intake, glycemia (110.4 ± 17.3 vs. 163.0 ± 28.9 mg/dL, $P < 0.001$) and insulin ($P = 0.02$) supply differed. No differences were found in high plasma levels of asymmetric-dimethylarginine ($P = 0.812$) at any time during the ICU stay. The clinical course, as indicated by markers of inflammation, average and maximum organ failure score, ICU stay and ICU and 90-day mortality, was the same. *Conclusions:* Intensive insulin treatment, while achieving glucose control, did not reduce asymmetric-dimethylarginine in high-risk septic patients fed with no more than 25 kcal/kg per day to limit ventilatory demand and to simplify glucose control. *Descriptor:* 45 (SIRS/sepsis: clinical studies).

Keywords ADMA · Sepsis · Glycemic control · Insulin therapy · Endocrine

Introduction

Nuclear proteins release asymmetric-dimethylarginine (ADMA) upon proteolytic degradation during physiological protein turnover. The main metabolic route for ADMA is decomposition to citrulline and dimethylamine in a reaction catalyzed by dimethylarginine-dimethylaminohydrolase, highly expressed under normal conditions in the liver, kidney, pancreas and endothelial cells. ADMA is also eliminated by renal excretion [1–4].

Asymmetric-dimethylarginine competes with arginine for cationic amino acid transporters of the y+ system [5, 6] and is also an endogenous inhibitor of all the isoforms of nitric oxide synthase [7].

Oxidative stress and cytokines enhance the activity of protein arginine methyltransferase, increase methylarginine release due to protein catabolism and variably influence [8, 9] dimethylarginine-dimethylaminohydrolase activity in liver, kidney and pancreas, further contributing to ADMA plasma levels [8]. In the presence of high ADMA levels active dimeric isoforms of nitric oxide synthase may become uncoupled, with the generation of superoxide anion. ADMA, therefore, is not just a marker but might also be a mediator of oxidative stress [10].

High plasma levels of ADMA were recently described in patho-physiologic states associated with endothelial dysfunction, oxidative injury, and inflammation, such as cardiovascular diseases [6, 11, 12], renal diseases [3, 4], idiopathic pulmonary arterial hypertension [13], and critical illness [14–16]. ADMA was also a strong predictor of mortality in critically ill patients [14, 15], in surgical patients [17], in patients with end-stage renal disease [18], coronary heart diseases [19, 20], and idiopathic pulmonary arterial hypertension [13]. Siroen and coworkers [15] reported a modulation of ADMA concentrations in critically ill surgical patients receiving insulin for glucose control, and an association between a low concentration of ADMA and improved survival.

In a multicenter, prospective, randomized, controlled clinical trial we investigated whether prevention of hyperglycemia could affect some metabolic pathways involved in the patho-physiology of sepsis. Here we report its effects on plasma ADMA concentration.

Materials and methods

Study population

Patients were studied from December 2004 to March 2007 at three university hospitals. The study was approved by the institutional review boards of each hospital and patients or next-of-kin gave informed written consent. Patients were enrolled if they met the criteria for severe sepsis [21]. Exclusion criteria were: (1) age less than 16 years; (2) hematological malignancies or bone marrow transplantation; (3) diabetes; (4) dialysis; (5) likelihood of early death because of the underlying disease; (6) enrolled patients who were discharged or died before the third day (second sample for ADMA assay) were excluded.

Study design

On enrolment, patients were assigned by block randomization to receive during the ICU stay either tight glycemic control (treatment group) or conventional glycemic control (control group). Glucose control and insulin treatment in the two groups were as described by Van den Berghe et al. [22]. Briefly, in the treatment group, prompt insulin was administered whenever blood glucose was higher than 110 mg/dL (6.1 mmol/L) and titrated to maintain glycemia between 80 and 110 mg/dL (4.4 and 6.1 mmol/L). In the control group, insulin was administered whenever glycemia exceeded 215 mg/dL (12 mmol/L) and titrated to keep the levels between 180 and 200 mg/dL (10 and 11 mmol/L). Glycemia was measured every 4 h, or more frequently when indicated. Parenteral/enteral nutrition was given, as soon as tolerated, aiming at 25 kcal/kg ideal body weight during the acute phase of ICU stay.

Data collection

At baseline, the patients' demographic and clinical characteristics were recorded, including severity of illness, using the Simplified Acute Physiologic Score II (SAPS II) [23], and morbidity, using the Sequential Organ Failure Assessment (SOFA) score [24]. SOFA was also scored at each sampling time.

Blood sampling for ADMA, arginine, and, as markers of inflammatory activation, interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) was done immediately after enrolment (T0), and on the 3rd (T3), 6th (T6), 9th (T9) and 12th (T12) days. Circulating levels of ADMA were determined in plasma by extraction and high-performance liquid chromatography (HPLC), as previously described [25]. Briefly plasma, with L-homoarginine added as internal standard, was applied to a cation-exchange solid phase extraction (SPE) cartridge (Phenomenex STRATA SCX 100 mg/mL, Chemtek Analitica, Bologna, Italy), activated beforehand with methanol and trichloroacetic acid (TCA), 2 mL. After column washings (TCA 2%, 150 mmol/L phosphate buffer pH 8.0, methanol), the arginines were eluted with 2% triethylamine in methanol:water (70:30 v/v). The eluate was dried under nitrogen, redissolved in 0.1 mL bi-distilled water and derivatized with *ortho*-phthaldialdehyde reagent before analysis. The HPLC was equipped

with a fluorescence detector (λ 340 nm, λ_{em} 455 nm) and an Ultrasphere ODS column (250 × 4.6 mm, 5 μm). The baseline separation of the methylated arginines was achieved with a gradient between mobile phase A (sodium citrate buffer, 50 mmol/L, pH 6.2) and B (distilled water:acetonitrile:methanol; 1:2:2 v/v/v). The levels found in 77 healthy volunteers (38 males, age 41 ± 16 years, creatinine 0.76 ± 0.13 mg/100 mL) were ADMA 0.46 ± 0.13, arginine 77.2 ± 31.0 μmol/L.

Statistical analysis

All the data were analyzed on an intention-to-treat basis. Results are given as mean ± standard deviation (SD) when normally distributed, or median (interquartile range) if not. To assess the differences of each variable over time and between the treatment and control groups, we built up a general linear mixed model for repeated measures (GLM) based on every single patient. Non-normally distributed variables were log-transformed for the analysis. The model took into account the effect of treatment (conventional or tight blood glucose control) as a between-subject factor, and the effect of time as a within-subject factor. In addition, we used Student's *t* test or Mann–Whitney *U* test, as needed, to assess differences between groups, chi-square tests to compare categorical data, and linear or Spearman's rank correlation analyses for the associations of variables. We considered $P < 0.05$ statistically significant. Statistical analysis was done using Stata Statistical Software, release 9.2 (Stata Corporation, College Station, TX, USA).

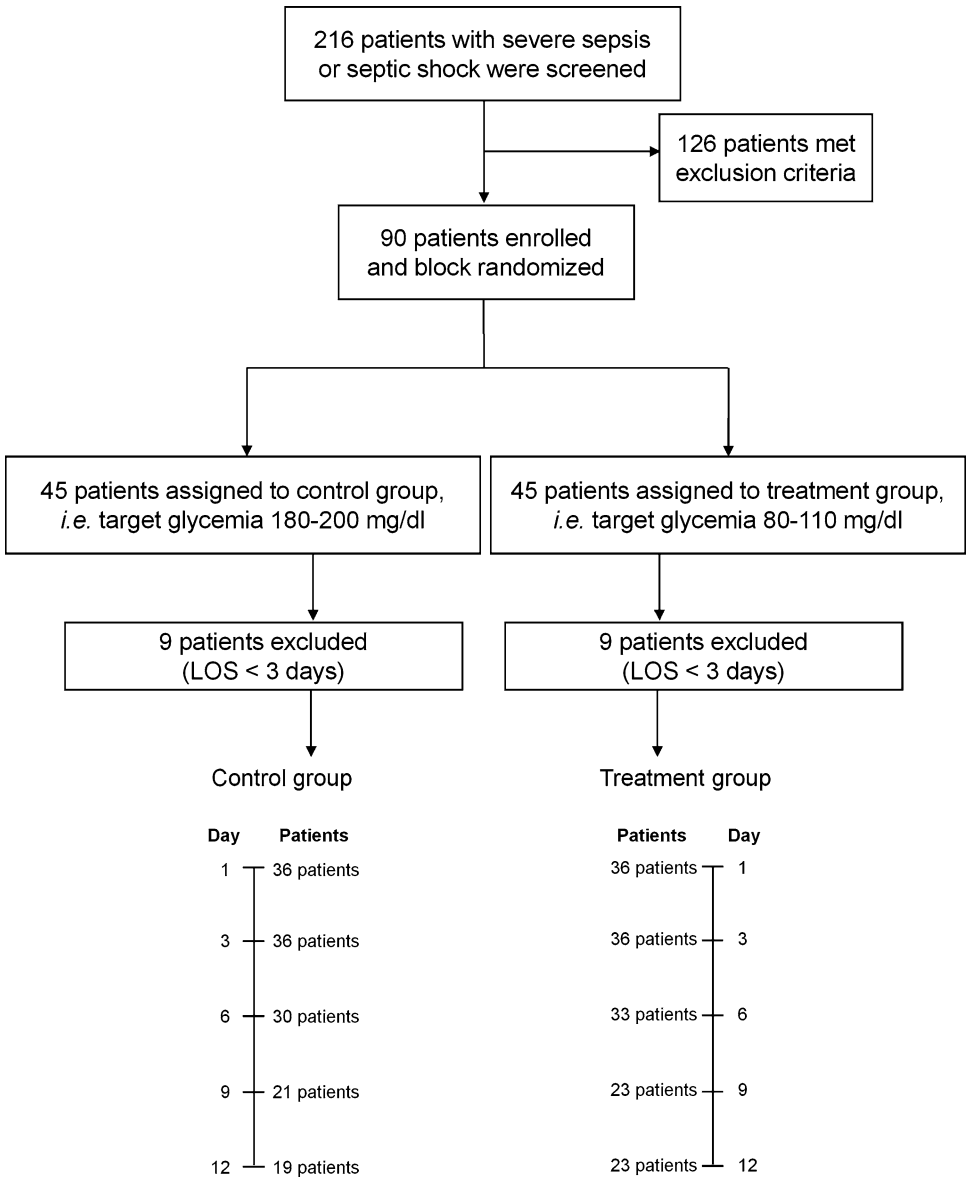

Fig. 1 Study flow-chart

Results

During the study, 216 patients were screened; 14 were excluded for age less than 16 years, 31 for hematological malignancies/bone marrow transplantation, 21 for diabetes, 15 for any type of artificial renal support and 45 with likelihood of early death because of the underlying disease. Finally, 90 were eligible for enrolment (Fig. 1). A total of 45 patients were randomized to the treatment group, and 45 to the control group. A total of 36 patients in both arms had T0 and T3 samples. Table 1 illustrates the clinical characteristics of the study population and the baseline profile of biochemical markers. Basal values of glycemia, CRP, IL-6, TNF-α, ADMA (as such or normalized for creatinine, data not presented), and creatinine were higher than normal, but did not differ between groups. Arginine was normal.

Treatment

Half of the patients completed the ICU stay within T12. With the same overall energy intake, the treatment group received higher daily dosages of insulin than the control group. The clear separation over time of daily average glycemia of patients assigned to tight treatment and controls is clear from Fig. 2. The percentage of glycemia samples reaching the target was 43.3% in the treatment group. The rates of severe hypoglycemia (<40 mg/dL) was 22.2% (8 patients) in the treatment and 8.3% (3

Table 1 Baseline characteristics and profile of biochemical markers of the study population

	Treatment group ($n = 36$)	Control group ($n = 36$)	P value
Male sex, no. (%)	22 (61.1)	25 (69.4)	0.45
Age (year)	60.3 ± 14.2	64.3 ± 14.3	0.23
IBW (kg)	62.2 ± 8.1	65.1 ± 6.8	0.12
ICU admission type, no. (%)			
Medical	24 (72.7)	22 (61.1)	0.56
Surgical unscheduled	8 (24.2)	13 (36.1)	0.56
Surgical scheduled	1 (3.0)	1 (2.7)	0.56
Infection site, no. (%)			
Pulmonary	19 (52.7)	16 (44.4)	0.47
Abdominal	8 (22.2)	10 (27.7)	0.58
Urinary tract	5 (13.8)	5 (13.8)	1.00
Others	10 (27.7)	7 (19.4)	0.40
Comorbidities, no. (%)			
Hypertension	19 (52.7)	14 (38.8)	0.23
Ischemic heart disease	2 (5.5)	2 (5.5)	1.00
Heart failure	2 (5.7)	1 (2.7)	0.53
Liver failure	3 (8.5)	7 (19.4)	0.18
Diabetes mellitus	7 (19.4)	5 (13.8)	0.52
Chronic obstructive pulmonary disease	8 (22.2)	9 (25.0)	0.78
Chronic renal failure	4 (11.7)	2 (5.5)	0.35
Severe sepsis, no. (%)	6 (16.6)	8 (22.2)	0.55
Septic shock, no. (%)	30 (83.3)	28 (77.7)	0.55
SAPS II score (points)	41.3 ± 14.4	42.2 ± 12.2	0.77
SOFA score (points)	9.3 ± 2.8	8.7 ± 3.3	0.30
SOFA score (organ system >3 points)			
Respiratory failure, no (%)	20 (55.6)	23 (63.9)	0.47
Coagulation failure, no (%)	7 (19.4)	4 (11.1)	0.33
Hepatic failure, no (%)	4 (11.1)	2 (5.6)	0.39
Cardiovascular failure, no (%)	26 (72.2)	27 (75.0)	0.79
Neurological failure, no (%)	1 (2.8)	2 (5.6)	0.56
Renal failure, no (%)	7 (19.4)	9 (25.0)	0.57
Creatinine (mg/dL)	2.4 ± 1.8	1.9 ± 1.6	0.24
Blood glucose (mg/dL)	136.7 ± 45.0	151.7 ± 36.6	0.18
CRP (μg/mL) (normal 0.15–6.55)	165.6 (130.3–231.1)*	148.6 (88.9–223.6)*	0.34
IL-6 (pg/mL) (normal 0.19–12.00)	254.5 (62.3–465.8)*	197.6 (61.2–427.0)*	0.88
TNF-α (pg/mL) (normal 3.75–18.25)	33.9 (17.2–74.3)*	42.3 (15.5–99.2)*	0.62
ADMA (μmol/L) (normal 0.33–0.59)	0.86 ± 0.25*	1.05 ± 0.52*	0.06
Arginine (μmol/L) (normal 55.0–95.0)	66.6 ± 34.2	67.3 ± 25.4	0.92

Values are presented as mean ± SD or median (interquartile range) as needed

IBW Ideal body weight; *ICU* intensive care unit; *SAPS II* simplified acute physiological score II; *SOFA* sepsis-related organ failure assessment; *CRP* C-reactive protein; *IL-6* interleukin-6; *TNF-α* tumor necrosis factor-α; *ADMA* asymmetric dimethylarginine

*$P < 0.001$ vs. normal value

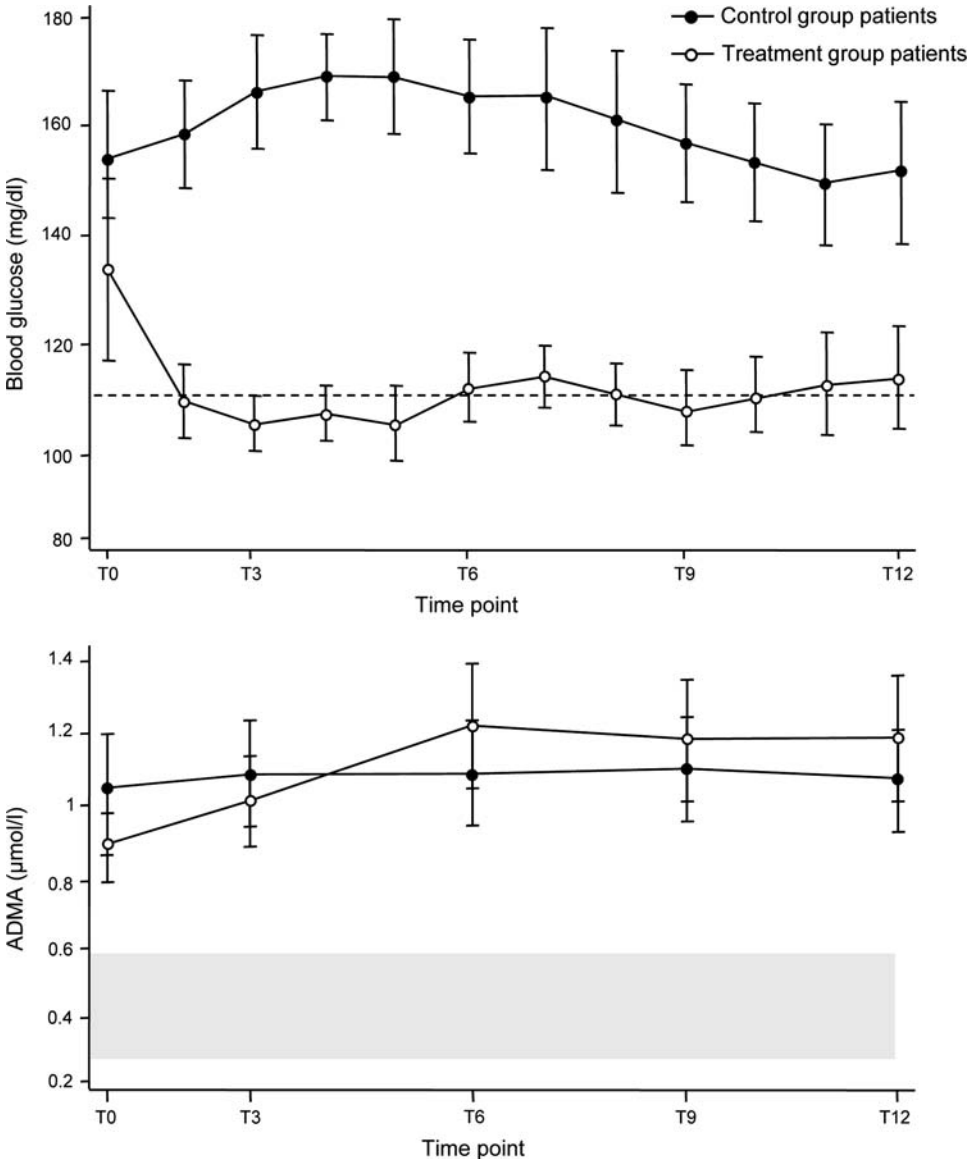

Fig. 2 Time course of recorded average daily blood glucose (general linear mixed model between groups: $P < 0.001$). Asymmetric-dimethylarginine levels increase with time (general linear mixed model within group: $P = 0.012$) in treatment group, but do not differ between groups ($P = 0.812$). Data are expressed as mean ± SD. The *dotted line* represents the upper limit of blood glucose level for the treatment group, *shaded area* represent the normal range (up to the 95th percentile) for asymmetric-dimethylarginine

patients) in the control group ($P = 0.189$). No neurological consequences were observed (Table 2).

Asymmetric-dimethylarginine level increased over time in treatment group (GLM within group: $P = 0.012$), but, during the sample period, ADMA levels were not different between groups (GLM between groups: $P = 0.812$) (Fig. 2). The same was true for values corrected for creatinine level ($P = 0.439$) and for arginine ($P = 0.692$). No difference was found between groups for CRP, IL-6, TNF-α, transfusions, incidence of septic shock, daily and maximum SOFA, ICU stay, and ICU and 90-day mortality.

Daily ADMA concentration at the 6th day was associated with ICU stay (Spearman's rank correlation coefficient $r = 0.52$, $P = 0.036$) in control group, while in treatment group an association was found with SOFA score at the 9th day ($r = 0.53$, $P = 0.015$).

Discussion

During the last few years, the relation between ADMA plasma levels and adverse effects has been frequently described [3, 4, 6, 9, 11–20]. Elevation of ADMA, like other methylated aminoacids [26], quite likely results from increased protein breakdown, possibly coupled with impairment of the degrading enzyme in liver, kidney or endothelium [8], and/or renal failure.

The possibility that ADMA not only indicates risk, but actually contributes to adverse events interfering with the

Table 2 Biochemical markers and clinical characteristics during care

Variable	Treatment group ($n = 36$)	Control group ($n = 36$)	P value
T3–T12 days			
Creatinine (mg/dL)	1.8 ± 2.4	1.5 ± 1.2	0.09
Blood glucose (mg/dL)	110.4 ± 17.3	163.0 ± 28.9	<0.0001
CRP (μg/mL)	74.3 (34.6–136.8)*	77.0 (29.6–121.4)*	0.48
IL-6 (pg/mL)	56.0 (28.4–167.7)*	47.6 (21.9–146.7)*	0.18
TNF-α (pg/mL)	21.6 (14.3–34.7)	19.0 (15.0–32.7)	0.84
Arginine (μmol/L)	78.8 ± 33.2	81.3 ± 30.8	0.56
Insulin (UI/day)	74.5 ± 141.1	38.8 ± 44.8	0.02
kcal/kg/day	20.3 ± 16.3	18.9 ± 2.7	0.74
Whole ICU stay			
Red cell transfusion, no (%)	21 (58.3)	24 (66.6)	0.53
Daily SOFA score (points)	7.4 ± 3.6	6.7 ± 3.5	0.08
Maximum SOFA score (points)	10.4 ± 2.6	10.0 ± 3.1	0.22
Septic shock, no (%)	31 (86.1)	32 (88.9)	0.55
Length of stay (days)	16 [8.1–28.5]	13 [6.5–23.5]	0.74
ICU mortality, no (%)	8 (22.2)	6 (16.7)	0.55
90-day mortality, no (%)	13 (36.1)	11 (30.6)	0.62

Values are presented as mean ± SD or median (interquartile range) as needed
CRP C-reactive protein; *IL-6* interleukin-6; *TNF-α* tumor necrosis factor-α; *ICU* intensive care unit; *SOFA* sepsis-related organ failure assessment
*$P < 0.001$ vs. normal values

nitric oxide fine tuning of the microcirculation involving the endothelium, platelets and white blood cells [5–7, 9] could open new therapeutic perspectives. Attempts to lower the plasma ADMA concentration have not been encouraging [17]; however, in surgical intensive care patients, Siroen et al. [15] have suggested that ADMA levels could be modulated by insulin-mediated normoglycemia and this partly explained clinical beneficial effects, reduced morbidity, and mortality associated with insulin treatment.

The assumptions of the normoglycemia/insulin hypothesis in inflammatory conditions are as follows [9]: a catabolic status results in cellular ADMA spillover and high plasma levels, while hyperglycemia increases expression of the cationic amino acid transport system y+ [9, 27, 28] and inhibits the activity of dimethylarginine-hydrolase [29]. In this scenario, insulin reduces protein catabolism [26], affects the cationic amino acid y+ transport system and may thereby increase the uptake of ADMA in organs able to eliminate it [27, 28, 30, 31]. Insulin may also preserve dimethylarginine-hydrolase inhibition due to hyperglycemia [29]. All these data work in favor of a compensatory ADMA metabolism with ADMA disappearing from plasma with insulin treatment able to achieve glycemic control.

We set out to test the interesting hypothesis of metabolic control of ADMA in the first 12 days of ICU stay in the most stressed critically ill patients, i.e. those admitted with severe sepsis and septic shock [20]. Patients were really at high-risk: about 50% had pneumonia, 25% peritonitis, and about 80% were admitted in septic shock. Their severity at admission, the elevated levels of the inflammatory markers CRP, IL-6, TNF-α, and the number and degree of organ failures during the ICU stay (our patients had a mean daily SOFA score of 7 and a worst score of 10 out of a maximum of 14 points), resulted in a substantial mortality rate and quite long ICU stays (Table 2). As expected, mean basal ADMA plasma level was high, higher than levels reported in less critically ill patients [14, 15, 17] and fitted with those reported in the only study on septic patients [16].

Despite the significant differences in glycemia and insulin supply between the treatment and control groups, we were unable to find differences in plasma levels of ADMA during the first 12 days ICU stay, as well as differences in the overall clinical course of sepsis as indicated by markers of inflammation, mean and maximum SOFA score, ICU stay, and (but the study was not powered enough) for ICU/90-day mortality.

Searching for a possible explanation, we analyzed the differences between our and Siroen's study [15]: the number of patients was similar (79 for Siroen and 72 in our study), all Siroen's patients had undergone thoracic surgery whereas in our case-mix only 33% were surgical patients (abdominal and thoracic); Siroen et al. did not report the incidence of severe sepsis, all our patients had severe sepsis and 88% of them had septic shock during ICU stay. Noteworthy, according to a more severe stress reaction, our basal ADMA levels were consistently higher than Siroen et al. Moreover, to limit ventilatory demand [32] and simplify glycemic control during the acute phase of illness, we supplied less energy than Siroen et al. (he reports about 1,700 kcal/day). Despite this, glucose control was less optimal than that reported by Siroen et al. Both these factors could have reduced the anabolic effect of the treatment. However, even in this scenario, our data

confirm ADMA as a marker of stress/severity; its levels at the peak of stress were significantly related with organ failure score, and ICU stay.

Asymmetric-dimethylarginine plasma level in severe sepsis/septic shock is massively increased due to systemic inflammatory response and organ failure. At least during the first 12 days of ICU stay, intensive insulin treatment was unable to reduce ADMA levels as reported (15). Differences in composition (sepsis incidence), severity of the case-mix, very high ADMA levels, hypo-caloric energy supply, and not easy glucose control, might partially explain the finding. However, the cost/benefit ratio of a more aggressive energy supply (and of insulin to ensure normoglycemia) in the early care of such critically ill patients needs to be carefully evaluated in the light of the risks in critically ill patients, and particularly in the septic ones, as we ourselves observed and as reported in a wider case-mix by Glucocontrol and VISEP studies [33, 34].

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