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Interstitial cortisol obtained by microdialysis in mechanically ventilated septic patients: Correlations with total and free serum cortisol

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Abstract

Purpose: The aim of this study was to measure subcutaneous tissue cortisol obtained by microdialysis (MD) in 35 mechanically ventilated septic patients.

Materials and Methods: Upon intensive care unit admission, an MD catheter was inserted into the subcutaneous tissue of the thigh. Cortisol (CORT) was determined in a 5:00 to 9:00 AM microdialysate sample collected within 72 hours. Concurrently, serum total (T-CORT) and free CORT (F-CORT) were measured. The Acute Physiology and Chronic Health Evaluation (APACHE II) and Sequential Organ Failure Assessment scores were calculated. Both T-CORT less than 10 μg/dL and F-CORT less than 0.8 μg/dL were considered as indicating critical illness–related corticosteroid insufficiency. *Adrenal adequacy* was defined as T-CORT greater than 20 μg/dL or F-CORT greater than 2.0 μg/dL.

Results: Total CORT correlated significantly with F-CORT ($r_s = +0.8$, $P < .0001$). Microdialysis CORT had a lower correlation with T-CORT ($r_s = +0.6$, $P < .0001$) and F-CORT ($r_s = +0.7$, $P < .0001$) and a weak correlation with APACHE II score ($r_s = +0.4$, $P < .01$). On the basis of MD-CORT, the patients were divided in quartiles. Although the median F-CORT and T-CORT levels were significantly different ($P < .001$) among the MD-CORT quartiles, there was a considerable overlap between the subgroups. All patients with T-CORT less than 10 μg/dL and all but 3 patients with F-CORT less than 0.8 μg/dL had tissue CORT in the lower

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quartile. However, only 50% and 58% of patients with adequate T-CORT and F-CORT levels, respectively, had concordant MD-CORT in the highest quartile.
Conclusions: Microdialysis CORT levels correlate moderately with circulating T-CORT and F-CORT. Of note, several patients presented with discrepant measurements between interstitial and circulating CORT concentrations. Thus, interstitial CORT measurements represent an additional tool to investigate the tissue CORT availability in critically ill patients.

1. Introduction

The spectrum of responses of the hypothalamic-pituitary-adrenal axis in critical illness and, in particular, in sepsis is varied and multifaceted [1]. The notion of absolute or relative adrenal insufficiency in the setting of critical illness has been introduced about 2 decades ago and has raised much debate about its existence and diagnostic approach [1]. Serum baseline and/or stimulated total cortisol has been used in most studies of patients with sepsis to assess hypothalamic-pituitary-adrenal functional integrity. However, because of the dramatic fall in cortisol binding globulin (CBG) and albumin levels that occurs in sepsis, measurements of total cortisol do not necessarily reflect the free cortisol levels, which is considered the biologically active moiety [1,2]. Free cortisol can be measured directly in serum; alternatively, measurement of cortisol in fluids that do not contain binding proteins, such as saliva, has been proposed as a surrogate marker for free cortisol levels [3]. The free cortisol fraction is readily available to tissues to exert its action by passing through the cell membrane and binding to the glucocorticoid receptor. However, besides the amount of circulating free cortisol, other factors may also determine how much cortisol is available at the cellular level. In this line, a substantial increase in the serum cortisol/cortisone ratio, consistent with a global increase in the conversion of cortisone to cortisol, has been shown in acute illness [4–6]. This presumably results from an increased 11β-hydroxysteroid-dehydrogenase type 1 (11β-HSD1) activity [7]. Such a prereceptor, tissue-specific, intracellular cortisol metabolism by the 11 β-HSD system may alter tissue levels, irrespective of circulating cortisol levels [7]. Cortisol in the interstitium may better reflect the ultimately available glucocorticoid pool for intracellular handling, and thus, measurements of interstitial cortisol may provide additional pathophysiologically relevant information in the setting of critical illness [8]. Bedside in vivo microdialysis (MD) is a diffusion-based sampling technique during which a catheter is implanted in organs or tissues of interest—mimicking a blood capillary—that permits continuous analysis of patient's interstitial fluid chemistry. As the interstitial fluid bathes the cells, its composition reflects the local metabolic activity and the hormonal milieu of the cells [9]. Recently, MD has been used to measure interstitial fluid cortisol in obesity, severe burns, or traumatic brain injury [10–12]. Interestingly, the correlation between serum total cortisol and interstitial fluid cortisol that is obtained with MD remains controversial [11,12]. So far, MD-derived cortisol measurements have been performed in a limited number of critically ill patients, and there is no study measuring cortisol in tissue microdialysates from septic patients.

Thus, the aims of this prospective pilot study were to measure cortisol in the interstitial fluid by MD in a group of mechanically ventilated septic patients and to examine the correlation between interstitial cortisol levels and total along with free serum cortisol.

2. Subjects and methods

This prospective 16-month study included consecutively hospitalized, mechanically ventilated, critically ill, septic patients (diagnosed according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Committee criteria) [13]. The hospital's ethics committee approved the study, and informed consent was obtained from the patients' relatives. Critically ill patients analyzed in the current study are partly shared with other publications by our research group [14–16]. Inclusion criteria were as follows: mechanically ventilated patients with diagnosis of sepsis, severe sepsis, or septic shock. Patients were not enrolled in the study if one of the following exclusion criteria was present: age less than 18 years, mechanical ventilation for more than 48 hours before intensive care unit (ICU) admission, no need for intubation and mechanical ventilation during ICU stay, do-not-resuscitate clinical conditions, brain death upon ICU entry, HIV infection, chronic intake of corticosteroids, or administration of glucocorticoids at any time point during ICU stay.

Sepsis was defined as the presence of a microbiologically documented or clinically diagnosed infection with at least 2 of the following: (*a*) core temperature above 38°C or below 36°C, (b) heart rate of more than 90 beats/min, (c) respiratory rate of more than 20 breaths/min or partial pressure of carbon dioxide below 32 mm Hg, and (d) leukocytosis (white blood cell count >12 000/mm^3) or leukopenia (white blood cell count <4000/mm^3) or more than 10% bands in peripheral blood. *Severe sepsis* was defined as sepsis aggravated by the acute dysfunction of at least 1 organ due to tissue hypoperfusion. *Septic shock* was defined as severe sepsis aggravated by systolic arterial pressure of less than 90 mm Hg requiring administration of vasopressors [13].

A total of 113 patients were admitted with sepsis during the study period; 78 received low-dose hydrocortisone therapy and were thus excluded, whereas 35 did not receive glucocorticoids at any time in the course of their illness and were eligible for this study protocol.

In all patients, the following variables were recorded: age, sex, and sepsis stage (sepsis, severe sepsis, or septic shock). The Acute Physiology and Chronic Health Evaluation (APACHE II) on day 1 and the Sequential Organ Failure Assessment (SOFA) scores were calculated [17,18]. Administration of vasopressors was noted as well as death in the ICU and 28-day mortality. Body mass index was calculated as weight (in kilograms)/[height (in meters)]^2.

Upon ICU admission, an MD catheter (CMA 60; CMA Microdialysis AB, Stockholm, Sweden) was inserted under sterile conditions into the subcutaneous adipose tissue of the upper thigh, as previously described [14–16,19]. The recovery of cortisol in MD has already been previously assessed with in vitro studies and is in the order of 56% [10]. The first dialysate samples were collected in microvials at least 2 hours after the insertion of the catheter to avoid the effect of placement trauma on metabolite measurements. Within 72 hours from ICU admission, cortisol concentrations were determined in a 4-hour microdialysate collection (from 5:00 to 9:00 AM; approximately 72 μL each) to ensure enough amount of sample for the analysis. Microdialysates were also analyzed for glucose, pyruvate, lactate, and glycerol (the latter 4 are used as markers of altered or disrupted local energy production [19]) with an automated analyzer (CMA 600 Microdialysis Analyzer; CMA Microdialysis AB). On the same day when the microdialysate was obtained, a blood sample was taken between 05:00 and 06:00 AM for measurement of total cortisol and free cortisol and for routine hematologic and biochemical testing (including glucose, albumin, and lactate).

Cortisol in microdialysates was determined using an enzyme immunoassay kit (Salimetrics Inc, State College, Penn; sensitivity was 0.007 μg/dL and interassay coefficients of variation [CVs] over the range of low to high values were 5.7%-6.8%, whereas the respective intra-assay CVs were 3.2% and 6.3%; cross-reactivity for cortisone in the cortisol assay was 0.13%). Serum total cortisol was assayed using an automated immunochemiluminescence method with the ADVIA Centaur CP Immunoassay System (ACS: 180 cortisol assay; Bayer Tarrytown, NY; lower detection limit of the assay, 0.19 μg/dL; intra-assay CVs, 8.0% for 5.43 μg/dL, 6.4% for 14.86 μg/dL, and 9.2% for 31.78 μg/dL). For serum free cortisol, the unbound cortisol fraction was separated by temperature-controlled ultrafiltration (0.5 mL of serum was incubated at 37°C for 30 minutes) and then centrifuged in preconditioned ultrafiltration devices (Amicon Ultra-4, 10 kd; Millipore, Bedford, Mass) for 30 minutes at 37°C. The concentration of free cortisol in the ultrafiltrate was then measured with a liquid chromatography–tandem mass spectrometry, assay as previously described [20,21]. The cumulative intra-assay and interassay between-device CVs for the ultrafiltration–liquid chromatography–tandem mass spectrometry method are less than 9.5% and 8%, respectively.

A total cortisol value of less than 10μg/dL was used as suggestive of critical illness–related corticosteroid insufficiency (CIRCI) [1] and a value above 20μg/dL as indicating corticosteroid adequacy. Currently, there are no widely accepted normal serum free cortisol concentrations during critical illness. For analysis purposes, however, we applied cutoff values proposed in previous studies [2,22]; for CIRCI, we applied a free cortisol cutoff of less than 0.8μg/dL, and for corticosteroid adequacy, we applied a free cortisol cutoff of more than 2 μg/dL. Because there is no available reference range for microdialysis cortisol levels in the literature, we arbitrarily considered relatively low the values in the lower quartile and extremely high those values in the higher quartile.

3. Statistical analysis

Normality of the parameters' distribution was assessed using the Kolmogorov-Smirnov test. Results are expressed as means ± SD or medians/ranges. Correlations between variables were assessed by Spearman rank correlation coefficient, denoted as r_s. Comparisons of continuous variables between 2 groups were analyzed with the Mann-Whitney nonparametric test, whereas the Kruskal-Wallis nonparametric test was applied to compare continuous variables between 3 or more groups. IBM SPSS statistical package, version 20 (IBM Software Group, Chicago, IL, USA), and GraphPad Prism, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA), were used for analyses. Statistical significance was set at $P < .05$.

4. Results

All patients were admitted with sepsis; none developed sepsis in the ICU. The characteristics of the 35 subjects that were included in the study are given in Table 1. The most common sites of infection were the lungs (n = 23) and the abdomen (n = 5). Other infections included encephalitis (n = 2), soft-tissue infection and pneumonia (n = 2), catheter-related infection (n = 1), central nervous system abscess and pneumonia (n = 1) and unspecified site (n = 1). The 28-day mortality rate was 17%. Twenty-one patients having septic shock required norepinephrine. In the whole cohort, the median values for total serum cortisol, free serum cortisol, and MD cortisol were 17.3 μg/dL (range, 1.40-49.20 μg/dL), 1.7 μg/dL (range, 0.07-12.6 μg/dL), and 0.6 μg/dL (range, 0.09-5.00 μg/dL), respectively. The individual values for serum total cortisol, serum free cortisol, and MD cortisol are given as an electronic supplementary material. Of note, a substantial number of patients had markedly low serum total and/or free cortisol values. There was considerable overlap in total (Fig. 1A), free serum cortisol (Fig. 1B), and MD (Fig. 1C)

Table 1 Characteristics of the study's patients

Men/women (n)	20/15
Age (y), mean ± SD	64 ± 18
BMI (kg/m^2), mean + SD	27.6 ± 3.1
Sepsis grade (n)	Severe sepsis: 14 Septic shock: 21
ICU mortality (n)	12
28-d mortality (n)	6
ICU stay (d): nonsurvivors, median	23
ICU stay (d): survivors, median	21
APACHE II score (day 1), mean ± SD	15.9 ± 5.4
SOFA score (day 1), mean ± SD	6.4 ± 2.8
Heart rate (day 1) (beats/min), mean ± SD	98 ± 23
Mean arterial blood pressure (day 1) (mm Hg), mean ± SD	98 ± 12
White blood cell count (day 1) (×10^3/μL), mean ± SD	11.7 ± 5.3
Hemoglobin (day 1) (g/L), mean ± SD	10.2 ± 2.0

cortisol levels in patients having severe sepsis and septic shock. There were no significant differences in serum and MD corisol measurements between survivors and nonsurvivors (median total serum cortisol, 17.3 μg/dL vs 20.0 μg/dL; medial free cortisol, 1.7 μg/dL vs 2.7μg/dL; median MD cortisol, 0.6 μg/dL vs 1.5 μg/dL, for survivors and nonsurvivors, respectively). Similarly, there were no differences in serum and MD cortisol levels between the patients requiring vasopressors and those who did not (median total serum cortisol, 16.6 μg/dL vs 18.8 μg/dL; medial free cortisol 1.7 μg/dL vs 1.8 μg/dL; median MD cortisol, 0.5 μg/dL vs 0.7 μg/dL, for patients requiring vasopressors and those who did not, respectively). No correlation between MD cortisol and patients' body mass index was seen.

4.1. Correlations between serum and tissue cortisol levels

Total cortisol levels correlated significantly with serum free cortisol levels (r_s = +0.8, P < .0001) (Fig. 2A). Cortisol obtained by MD had a significant, albeit lower, correlation with serum total cortisol (r_s = +0.6, P < .0001) (Fig. 2B) and with serum free cortisol (r_s = +0.7, P < .0001) (Fig. 2C).

4.2. Assessment of MD cortisol quartiles

On the basis of tissue cortisol levels, obtained by subcutaneous adipose tissue MD, the patients were divided in quartiles. Although, as shown in Fig. 3, the median serum free and total cortisol levels were significantly different (P < .001) among the interstitial tissue cortisol quartiles, there was a considerable overlap between the subgroups. Among patients with tissue cortisol levels in the lowest quartile, 6 of the 10 patients (60%) had serum total cortisol levels compatible with CIRCI (<10 μg/dL), and none had more than 20 μg/dL (Fig. 3A); 7 (70%) of 10 of these patients had

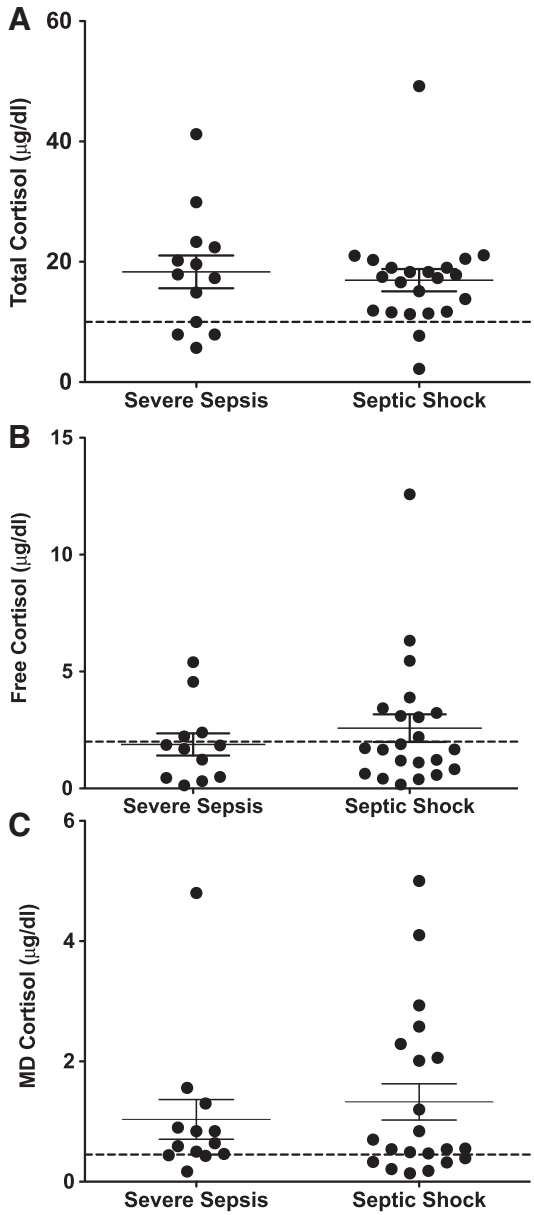

Fig. 1 Serum total cortisol (A), serum free cortisol (B), and MD cortisol (C) levels according to sepsis stage. Dashed lines in panels A and B indicate the cutoff values of total serum cortisol and free serum cortisol, respectively, applied for the diagnosis of CIRCI. Dashed line in panel C indicates the 25th MD cortisol percentile.

serum free cortisol levels below 0.8 μg/dL (Fig. 3B). In addition, none of the patients with interstitial cortisol levels in the highest quartile had total or free serum cortisol levels consistent with corticosteroid insufficiency (ie, of 10 <10 and <0.8 μg/dL, respectively).

4.3. Assessment based on total and serum free cortisol levels

Patients with total cortisol levels less than 10 μg/dL had significantly lower interstitial tissue cortisol levels compared

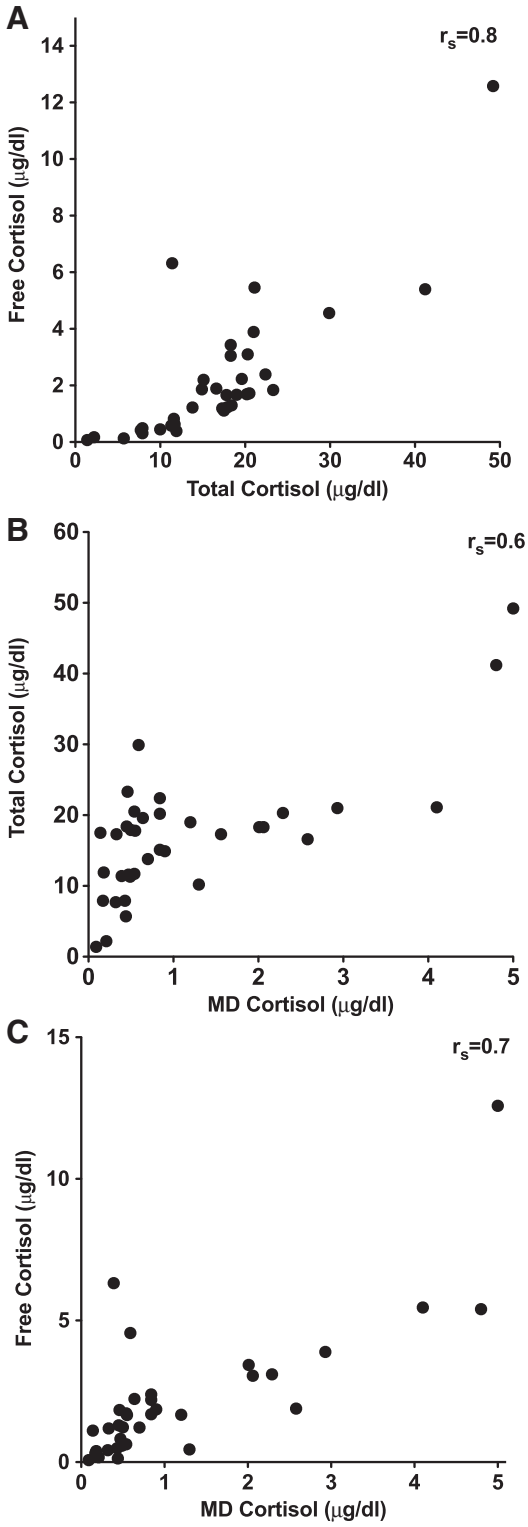

Fig. 2 Correlations between total serum cortisol and free serum cortisol (A), total serum cortisol and MD cortisol (B), and free serum cortisol and MD cortisol. r_s indicates Spearman rank correlation coefficient.

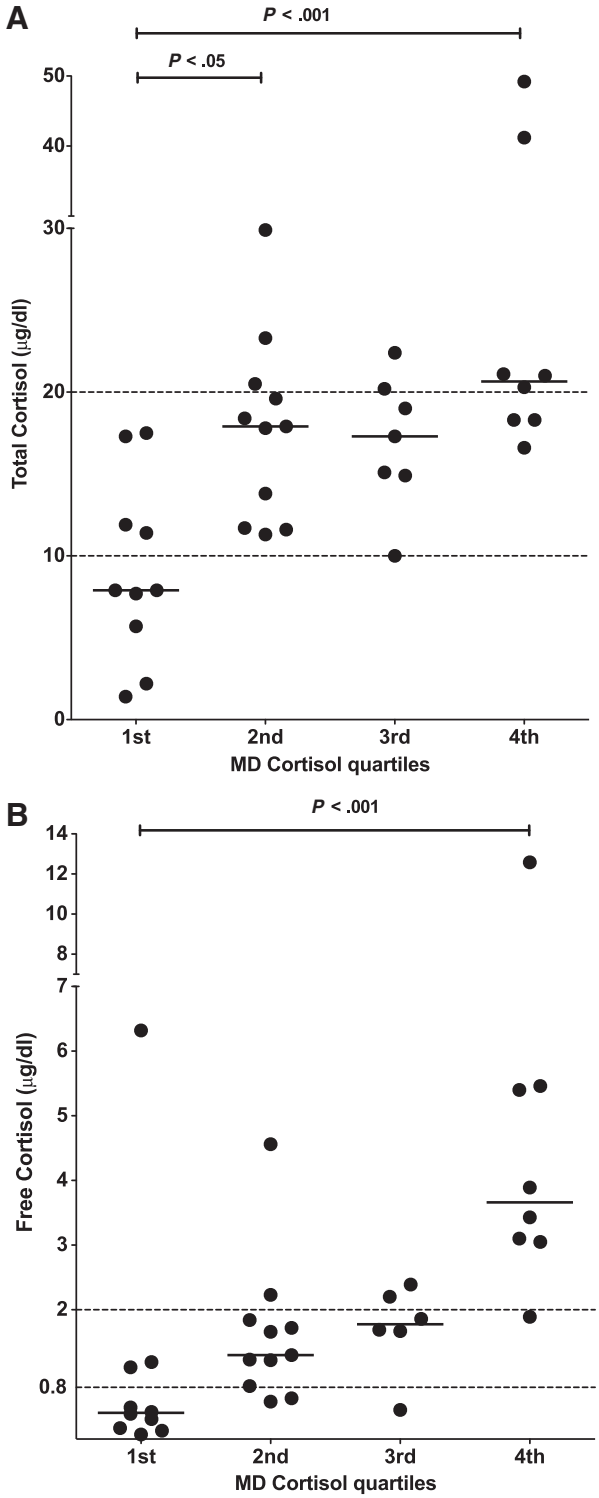

Fig. 3 Serum total cortisol (A) and serum free cortisol (B) according to MD cortisol quartiles. Dashed lines in panel A indicate the total cortisol values suggesting CIRCI (<10 μg/dL) or indicating corticosteroid adequacy (>20 μg/dL). Dashed lines in panel B indicate the free serum cortisol values suggesting CIRCI (<0.8μg/dL) or indicating corticosteroid adequacy (>2μg/dL).

with patients with serum total cortisol levels between 10 and 20 μg/dL and compared with patients with serum total cortisol levels greater than 20 μg/dL (Fig. 4A). Also, patients

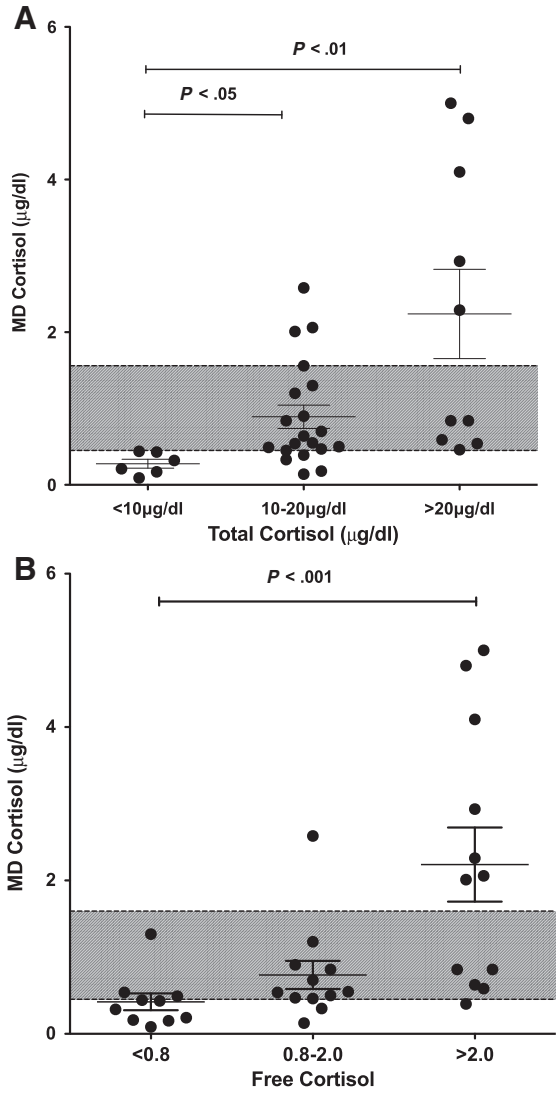

Fig. 4 Microdialysis cortisol levels in groups according to serum total cortisol levels (A) and to serum free cortisol levels (B). The shaded area indicates the values between the 25th and the 75th percentiles.

with serum free cortisol levels less than 0.8 μg/dL had significantly lower tissue cortisol levels compared with patients with serum free cortisol levels greater than 2 μg/dL ($P < .01$) (Fig. 4B). Of note, all patients with serum total cortisol less than 10 μg/dL (Fig. 4A) and all but 3 patients with serum free cortisol levels less than 0.8 μg/dL (Fig. 4B) had tissue cortisol levels in the lower quartile. However, only 5 (50%) of the 10 patients with high total serum cortisol and 7 (58%) of the 12 patients with high free serum cortisol had interstitial cortisol measurements in the highest quartile.

4.4. Correlations between tissue cortisol levels, clinical severity scores, and metabolic parameters

Microdialysis cortisol correlated weakly with the APACHE II score ($r_s = +0.4$, $P < .01$) and the SOFA score ($r_s = +0.5$, $P < .01$). Analogous correlations were observed between serum free cortisol and APACHE II score ($r_s = +0.5$, $P < .01$) and SOFA score ($r_s = +0.6$, $P < .001$), whereas serum total cortisol correlated with the SOFA score ($r_s = +0.5$, $P < .001$) but not with the APACHE II score. Free serum cortisol but not serum total cortisol or MD cortisol correlated with the cardiovascular SOFA subscore ($r_s = +0.5$, $P < .01$). There were no significant correlations between MD cortisol and MD lactate, glucose, glycerol, pyruvate, and the lactate-to-pyruvate ratio.

5. Discussion

This is the largest study in critically ill patients and the first study in the setting of sepsis that measured interstitial cortisol levels by subcutaneous adipose tissue MD. A key finding of the present investigation is that cortisol concentrations in the interstitium of subcutaneous adipose tissue correlate moderately with the concentrations of total and free cortisol in serum. This finding suggests that tissue cortisol availability in critically ill septic patients is only, to some extent, reflected by total and free serum cortisol concentrations and provides some ground that other factors are likely to be involved.

So far, interstitial cortisol by MD has been measured only in 2 previous small-scale investigations on critically ill patients with burns and head trauma [11,12]. In patients with brain injury, the mean ± SD cerebral interstitial cortisol at 8.00 AM was 1.1 ± 1.0 μg/dL, and in patients with burns, the median MD cortisol in the nonburn tissue was 0.80 μg/dL. In our study, the median and mean ± SD subcutaneous interstitial cortisol were 0.7 and 1.2 ± 1.3 μg/dL, respectively. However, because of different flow rates [12] and time intervals of sample collection [11,12], we cannot compare the absolute values of MD cortisol in our study with the previously reported [11,12]. In these studies, either a moderate correlation [12], in accordance with our results, or a lack of correlation, were observed between serum total and/or free and MD cortisol [11]. In our study, although there was some correlation of tissue cortisol with both total and free serum cortisol, analysis of individual patients showed that a substantial number of patients presented with discrepant results between cortisol measurements obtained in circulating and interstitial fluid compartments. In particular, some patients with total or free serum cortisol values in the upper range had no concordant tissue cortisol concentrations. Nevertheless, a low serum cortisol below the level which is regarded as indicating corticosteroid insufficiency was almost always associated with tissue levels of cortisol in the lowest quartile.

The underlying pathophysiology of the observed correlations and discrepancies of cortisol measurements in different compartments is currently not well understood. Tissue availability of cortisol may be affected in several ways: in critically ill patients, even when systemic

hemodynamics are within satisfactory goals, alterations in the microcirculation can be observed [23]. Sepsis, in particular, is associated with interstitial edema related to fluid overload and increased capillary permeability. In addition, there is impaired regional blood flow distribution due either to sepsis itself or to the administration of vasopressors. Such pathophysiologic changes are expected to account, at least in part, for an incomplete equilibrium of substances from plasma to tissue and may thus explain the moderate correlations of cortisol between the circulating and tissue compartments observed in our study. Indeed, studies investigating the levels of anti-infective agents in septic patients support this concept [24]. Extracellular cortisol availability is also determined by the presence of plasma binding proteins such as CBG and albumin. During sepsis, neutrophil elastase, an enzyme originating from polymorphonuclear leukocytes, cleaves cortisol from CBG, thereby increasing the amount of interstitial cortisol levels at the site of inflammation [25]. Moreover, temperature changes affect CBG resulting in cortisol release in response to fever or in tissue-specific delivery in areas with higher temperatures [26]. Finally, cortisol generation or inactivation by the intracellular enzymes 11β-HSD1 and 11β-HSD2, respectively, may affect interstitial tissue levels [11,27]. Differential activation or inactivation of these processes may underlie the low interstitial tissue levels observed in some patients, despite the elevated circulating levels of total and serum free cortisol. Moreover, saturable CBG binding is compatible with a nonlinear association between total cortisol and both free and MD cortisol. In fact, curve-fitting analysis in healthy subjects has shown that the relationship between total and salivary cortisol fits better to an exponential curve [21]. It is unknown whether this applies to critically ill patients, where there are dramatic quantitative and qualitative changes in CBG [28]. However, in our cohort, curve-fitting analysis was not possible, given the relatively small sample size and lack of normally distributed values.

In our study, tissue cortisol correlated with the widely applied APACHE II score in assessing severity of disease. Similar associations have already been reported between serum cortisol and clinical severity scores in critically ill patients [29]. It has been suggested that a sign indicating the presence of relative adrenal insufficiency in critically ill patients is vasopressor dependency itself [30]. We did not observe, however, any differences in serum or tissue cortisol levels between patients who required vasopressors and those who did not. Also, in our selected population of patients who did not receive glucocorticoids through the course of their illness, interstitial cortisol was not linked to clinical outcome.

Our study has certain limitations that need to be pointed out. First, a control group is lacking; thus, we cannot provide reference normal values for interstitial cortisol. Although the inclusion of a control group is not essential for addressing the issue of whether there are differences in the relationship between free and interstitial cortisol in various sepsis states, it would address the question of whether the relationship between the circulation and the interstitium is generally disturbed in critical illness. Second, it should be noted that cortisol concentrations measured in the dialysate represent a percentage of the actual concentration of cortisol in the interstitial fluid, referred to as *recovery*. Recovery depends on the perfusion flow, the size of the dialysis membrane, and the diffusivity of substances in the tissue. As previously assessed in vitro, the recovery of cortisol by MD with flow rates of 0.3 μL/min is in the order of 56% [10]. As a result, this means that the amount of cortisol in the collected samples is systematically underestimated compared with the actual levels of tissue cortisol. Third, the sample size is relatively small; however, it is much larger compared with other studies reporting tissue cortisol data in critically ill patients. So far, only 10 burned patients were studied by Cohen et al [11] and 10 patients with trauma by Llompart-Pou et al [12]. However, it should be taken into account that MD represents a recently introduced laborious and expensive method, and this is a major limitation for recruiting a large number of patients. Finally, micodialysates were only obtained from subcutaneous tissue, and thus, our findings cannot be extrapolated to all extracellular compartments.

In conclusion, in patients with critical sepsis, we found that interstitial subcutaneous tissue cortisol is only moderately correlated with total and serum free circulating cortisol, suggesting that serum cortisol may not always reflect tissue cortisol availability. In particular, our findings indicate that although low total or free cortisol concentrations are associated with low tissue cortisol levels, high circulating cortisol concentrations do not always guarantee similar changes at the tissue level. Thus, MD measurements represent an additional tool that, with the accumulation of further data and particularly of serial (and/or time integrated) measurements, will provide further insight into the cortisol conundrum in critical illness.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcrc.2012.07.008.

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