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Assessing adrenal insufficiency of corticosteroid secretion using free versus total cortisol levels in critical illness

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Abstract *Purpose:* To study the value of free versus total cortisol levels in assessing relative adrenal insufficiency during critical illness-related corticosteroid insufficiency. *Methods:* A prospective study in a mixed intensive care unit from 2004 to 2007. We consecutively included 49 septic and 63 non-septic patients with treatment-insensitive hypotension in whom an adrenocorticotropic hormone (ACTH) test (250 μg) was performed. Serum total and free cortisol (equilibrium dialysis), corticosteroid-binding globulin (CBG) and albumin were assessed. *Results:* Although a low CBG resulted in a high free cortisol level relative to total cortisol, free and total cortisol and their increases were well correlated ($r = 0.77$–0.79, $P < 0.001$). In sepsis, hypoalbuminemia did not affect total and free cortisol, and increases in total cortisol upon ACTH predicted increases in free cortisol regardless of low binding proteins. In non-sepsis, total cortisol was lower with than without hypoalbuminemia; free cortisol did not differ, since hypoalbuminemia concurred with a low CBG. Increases in total cortisol depended less on binding proteins than on raw levels. The areas under the receiver operating characteristic curve for predicting increases in free from total cortisol were 0.93–0.97 in sepsis and 0.79–0.85 in non-sepsis ($P = 0.044$ or lower for sepsis vs. non-sepsis). *Conclusions:* Although the biologically active free cortisol fraction depends on binding proteins, total cortisol correlates to free cortisol in treatment-insensitive hypotension during critical illness. In sepsis, albumin is not an important binding molecule. Subnormal increments in total cortisol upon ACTH suffice in assessing relative adrenal insufficiency, particularly in sepsis.

Keywords Adrenocorticotropic hormone · Corticosteroid-binding globulin · Equilibrium dialysis · Relative adrenal insufficiency · Sepsis

Introduction

Severe illness activates the hypothalamic-pituitary-adrenal (HPA) axis [1]. Relatively insufficient adrenal secretion of cortisol for the severity of illness, in the course of critical illness-related corticosteroid insufficiency (CIRCI), commonly occurs in the critically ill. This may warrant treatment by corticosteroids to survive septic shock, although this is hotly debated [2–7]. Adrenal function is mostly assessed by total cortisol measurements prior to and after injection of adrenocorticotropic hormone (ACTH). Insufficiency is usually defined at cutoffs of peak levels, increases of circulating cortisol upon ACTH (<250 nmol/l) or combinations of these [1–3, 5].

We found a lower cutoff (<100 nmol/l) for ACTH-induced increases to be associated with mortality and predicted a survival benefit of corticosteroid treatment in septic shock [6].

Foregoing studies can be criticized because of changes in binding proteins such as cortisol-binding globulin (CBG) and albumin, confounding estimation of biologically active free cortisol from total cortisol measurements and thereby assessment of adrenal function [1, 4, 5, 7–20]. Indeed, low binding proteins may overestimate relative adrenal insufficiency assessed from (increases in) total cortisol. This issue was refuted by others, however [5, 15, 21]. Differences in case mix may partly underlie the discrepancies between studies, because of healthy volunteers or non-septic patients predominating in some studies [4, 11, 14, 17] and of septic patients in others [5, 15, 21]. Moreover, CIRCI may be more frequent in sepsis than in non-sepsis patients, and sometimes only calculated free cortisol was evaluated, thereby assuming normal albumin levels, while both CBG and albumin may be lowered [1–3, 5, 6, 8, 18, 22, 23]. We previously used (increases in) cortisol/albumin ratios to adjust for protein binding in critical illness, but these ratios were not validated, even though CBG and albumin levels commonly change in the same direction, and hypoalbuminemia has been suggested to increase the free cortisol fraction [4, 6, 18]. Other authors, however, suggest that CBG and albumin and their cortisol binding are differently regulated in sepsis as compared to non-sepsis [8, 18, 23, 24]. Free cortisol can be directly measured by equilibrium dialysis, which is not routinely used because of its non-automated and laborious nature [4, 13, 15–17, 25, 26].

The aim of the current study was to investigate the value of total cortisol versus free cortisol (by equilibrium dialysis) measurements for the assessment of relative adrenal insufficiency in the critically ill undergoing a 250-μg ACTH test for suspected CIRCI. Furthermore, we analyzed the effects of CBG and albumin levels, and separately studied septic and non-septic patients. The hypothesis was that binding molecules affect free cortisol for a given total cortisol level, and thereby confound diagnosis of adrenal insufficiency from total cortisol levels, depending on the underlying condition.

Patients and methods

Patient population and ACTH test

This prospective study was carried out in the intensive care unit (ICU) of a university hospital from December 2004 to March 2007. Dutch legislation waived the need for informed consent as the ACTH test is routinely performed in our department, no extra blood was drawn for this study, and the results were treated anonymously. One hundred twelve critically ill patients over 18 years old with a clinical suspicion of CIRCI on the basis of >6 h hypotension (<100 mmHg systolic) requiring repeated fluid challenges and/or vasopressor/inotropic treatment were consecutively included. Patients were excluded if they had a history of hypothalamic-pituitary or adrenal disease, or if they received glucocorticoid treatment within 3 months of testing.

All patients underwent a short 250 μg ACTH (tetracosactide-hexa-acetate, Synacthen®, Novartis Pharma, Basel Switzerland) test. Blood samples were taken at baseline and 30 and 60 min after intravenous injection. Serum total cortisol was measured by competitive immunoassay (Advia Centaur, Siemens Diagnostics, Deerfield, IL). The intra- and interassay coefficients of variation (CV) are 3 and 6%, respectively, and the detection limit was 30 nmol/l (500 nmol/l = 18 μg/dl). The peak (at 30 or 60 min) minus baseline cortisol level was taken to calculate increases upon ACTH. A subnormal response to ACTH in critical illness was defined by a total cortisol increase <250 nmol/l or increase <100 nmol/l, as reported by others and ourselves [5, 6]. Serum free cortisol levels were measured by equilibrium dialysis of undiluted serum samples followed by radioimmunoassay (Orion Diagnostica, Espoo, Finland; <6% cross reactivity with cortisone) [4, 13, 16, 17, 25, 26]. The intra- and interassay coefficients of variation were less than 7 and 8%, respectively. See the electronic supplement for more information about how total cortisol, free cortisol, ACTH, CBG and albumin were measured.

Data collection

At study entry, the day of the ACTH test, the following parameters were recorded: time from ICU admission, age and sex, ICD-10 definitions of common clinical conditions at admission and the severity of illness, as assessed by the Acute Physiology, Age and Chronic Health Evaluation (APACHE) II scores. Sepsis was defined as the presence of systemic inflammatory response syndrome (SIRS) with a positive microbiological local (urine, trachea or other) and/or blood culture. SIRS was defined as two or more of the following criteria: a temperature >38° or <35.5°C, a leukocyte count >12 or <4 × 10^9/l, a heart rate >90/min, and a respiratory rate >20/min or the presence of mechanical ventilation. Suspected or microbiologically proven sources of sepsis were recorded. Laboratory measurements included total white blood cells and serum levels of CBG, albumin and creatinine. Interventions including type and doses of vasopressor/inotropes, intubation, use of etomidate in the preceding 48 h to facilitate endotracheal intubation, need for mechanical ventilation and renal replacement therapy were recorded. During follow-up, 28-day and ICU mortality and length of ICU stay were recorded.

Statistical analysis

Data were distributed normally (Kolmogorov-Smirnov test $P > 0.05$), except for increases in cortisol. The CBG and albumin levels obtained at baseline were also used for 30 and 60 min, since they were assumed not to change in this time interval [5, 13]. Data were arbitrarily dichotomized around median CBG and albumin levels. Categorical data were compared with the use of the chi-square test and Fisher's exact tests. When appropriate the Pearson or partial correlation coefficients were used to express relations between variables. Furthermore, we performed generalized estimating equations (GEE), multivariable analyses, Mann-Whitney *U* test and receiver-operating characteristic curves (see the electronic supplement for the detailed information). A two-sided $P < 0.05$ was considered to indicate statistical significance, and exact P values <0.10 are given, unless <0.001. Data are expressed as median (interquartile range).

Results

Patient characteristics (Table 1)

Forty-four percent of patients had sepsis on the test day, and 43% of the latter had an increase of total cortisol <250 nmol/l upon exogenous ACTH. Septic patients frequently received vasopressor/inotropic support and therefore mostly suffered from septic shock. Sources and associated microorganisms were tabulated. Fifty-six percent of patients had non-sepsis and 43% of the latter a total cortisol response <250 nmol/l. A cortisol response <100 nmol/l occurred in 14% of septic and in 13% of non-septic patients (ns). Body temperature was 37.3 ± 1.5°C and 36.4 ± 1.5°C in sepsis and non-sepsis patients ($P = 0.010$), respectively. Non-septic patients had slightly higher APACHE II scores than septic patients, with 17 ± 8 and 16 ± 5, respectively ($P = 0.039$), and scores were higher in non-survivors ($P = 0.013$). Twenty-eight-day survivors had received hydrocortisone replacement doses in 92% and non-survivors in 82% (ns).

Table 1 Patient demographics and characteristics ($n = 112$)

Age (years)	66 (22)
Sex (m/f)	65 (58)/47 (42)
Admission syndromes[a]	
Trauma and postoperative	41 (37)
Cardiac surgery	19 (17)
Vascular surgery	6 (5)
Respiratory failure	38 (34)
Post-CPR	12 (11)
Shock	11 (10)
Sepsis	15 (13)
Renal insufficiency	4 (4)
Coma	1 (1)
Other	34 (30)
Length of stay (days)	15 (22)
Mortality day	28 (25)
Mortality ICU	35 (31)
At day of ACTH test	
Time admission to test (days)	2 (4)
APACHE II	17 (6)
SIRS	93 (83)
Non-sepsis	63 (56)
Sepsis	49 (44)
Source[a]	
Respiratory	32 (65)
Abdominal	9 (18)
Urogenital	2 (4)
Neurological	1 (2)
Catheter	1 (2)
Miscellaneous	7 (14)
Microorganism[a]	
Gram-negative	34 (69)
Gram-positive	17 (35)
Fungal	7 (14)
Bacteremia[a]	
Gram-negative	6 (12)
Gram-positive	8 (16)
Fungal	2 (4)
Mechanical ventilation	82 (73)
Renal replacement therapy	13 (12)
MAP (mmHg)	76 (21)
Vasopressors/inotropes	101 (90)
Norepinephrine, number (dose in mg/h)	93 (83), 0.16 (0.61)
Dopamine (number)	16 (14)
Enoximone (number)	28 (25)
Etomidate <48 h prior to test	32 (28)
Start hydrocortisone after test	100 (93)
Duration (days)	8 (10)
Cumulative dose (mg)	1213 (1250)

Data are expressed as median (interquartile range) or number (%), where appropriate

CPR Cardiopulmonary resuscitation, *ICU* intensive care unit, *APACHE* Acute Physiology, Age and Chronic Health Evaluation score, *ACTH* adrenocorticotropic hormone, *SIRS* systemic inflammatory response syndrome, *MAP* mean arterial pressure

[a] Patients may have more than one

Free, total cortisol and increases in sepsis and non-sepsis

Baseline free and total cortisol levels were somewhat directly related to the APACHE II score (minimum $r = 0.19$, $P = 0.043$ or lower). Both increases in free and total cortisol inversely related to the respective baselines (minimum $r = -0.23$, $P = 0.013$). Patients with sepsis had higher free (and not total) cortisol levels at baseline, 30 and 60 min than non-septic patients, whereas binding proteins and cortisol increases upon ACTH did not differ (Table 1 in electronic supplement). Patients with an increase of total cortisol <250 or <100 nmol/l upon ACTH had lower free cortisol increases, regardless of the underlying condition, than those with greater increases in total cortisol (Table 2). In sepsis, free related to total cortisol for raw values (partial $r = 0.79$, $P < 0.001$, Figure in electronic supplementary material). Increases in

Table 2 Results according to cortisol response to ACTH

	Cortisol increase		*P*	Cortisol increase		*P*
	≥250 nmol/l ($n = 64$)	<250 nmol/l ($n = 48$)		≥100 nmol/l ($n = 97$)	<100 nmol/l ($n = 15$)	
CBG (mg/l)	25 (11)	24 (10)		25 (11)	27 (16)	
Albumin (g/l)	18 (9)	17 (10)		18 (9)	14 (4)	0.079
ACTH (pmol/l)	1.8 (1.3)	3.1 (6.6)	<0.001	1.9 (1.8)	6.4 (17.1)	0.002
Total cortisol (nmol/l)						
Baseline	420 (253)	520 (326)		445 (312)	500 (540)	
30 min	725 (381)	635 (625)		675 (339)	625 (523)	
60 min	875 (395)	715 (349)		790 (374)	545 (570)	
Increase (nmol/l)	405 (215)	140 (125)	na	305 (240)	60 (45)	na
Increase (%)	102 (86)	24 (26)	na	70 (92)	10 (10)	na
Free cortisol (nmol/l)						
Baseline	82 (108)	134 (132)		92 (111)	139 (219)	
30 min	202 (123)	158 (141)		190 (126)	137 (175)	
60 min	234 (125)	187 (131)		210 (142)	175 (207)	
Free cortisol (% of total cortisol)						
	24 (11)	24 (9)		24 (11)	25 (7)	
Increase (nmol/l)	132 (68)	40 (46)	<0.001	103 (93)	22 (33)	<0.001
Increase (%)	176 (201)	32 (64)	<0.001	112 (194)	8 (26)	<0.001

Data are expressed as median (interquartile range); exact $P < 0.10$
CBG Cortisol-binding globulin; *ACTH* adrenocorticotropic hormone, *na* not applicable

free cortisol correlated with total cortisol at $r = 0.79$ ($P < 0.001$; Fig. 1), whereas percentage increases of free were greater than those of total cortisol ($P < 0.001$). For non-septic patients, free cortisol correlated at partial $r = 0.77$ to total cortisol for raw values ($P < 0.001$, Figure in electronic supplementary material). Increases in free cortisol correlated at $r = 0.77$ to total cortisol increases ($P < 0.001$; Fig. 1). Percentual increases of the former were greater ($P < 0.001$). In both conditions, greater percentual increases in free than total cortisol can be attributed to ACTH-induced increases in the free cortisol fraction. However, differences in percentual increases in free and total cortisol were less when responses to ACTH were less. In Table 3 statistically significant clinical correlates of free cortisol (76 nmol/l cutoff, see below) and total cortisol (250 nmol/l cutoff) increases are compared. It is shown that trauma and surgery as reasons for admission were less, and the use of etomidate was more common when free and total cortisol responses were lower. There were no hydrocortisone treatment differences between the groups.

Endogenous ACTH

Endogenous ACTH was directly related to the APACHE II score ($r = 0.44$, $P < 0.001$) and to baseline free and total free cortisol ($r = 0.33$ or higher, $P = 0.001$ or lower). It was inversely correlated to the increase in free and total cortisol upon exogenous ACTH ($r = -0.30$ and -0.19, $P = 0.003$ and 0.058, respectively), so that endogenous ACTH levels were relatively high at low cortisol responses to exogenous ACTH (Table 2).

Binding proteins; sepsis and non-sepsis

Albumin related poorly to CBG ($r = 0.23$, $P = 0.007$) in sepsis, but better ($r = 0.43$, $P < 0.001$) in non-sepsis, whereas both binding protein levels were subnormal. Total cortisol and CBG predicted free cortisol in sepsis (GEE, $P < 0.001$), while albumin did not contribute. In non-sepsis, both CBG and albumin (and first order interactions) contributed to the relation between free and total cortisol (GEE, $P = 0.036$ or lower). Increases in free cortisol were related to increases in total cortisol (GEE, $P < 0.001$), without a significant contribution of binding proteins in sepsis, but this was dependent on both proteins (and first order interactions) in non-sepsis (GEE, $P = 0.020$ or lower; Fig. 1). Indeed, free cortisol at baseline, 30 and 60 min was higher with lower CBG, particularly in septic patients, while total cortisol was similar regardless of underlying condition (Table 2 in electronic supplement). Hypoalbuminemia did not affect free and total cortisol levels at baseline, 30 and 60 min in sepsis (Table 3 in electronic supplement). However, being paralleled by a low CBG, hypoalbuminemia was associated with relatively low total (but not free) cortisol levels in non-septic patients. The increases in free and total cortisol were less affected by CBG and albumin than raw cortisol levels in both underlying conditions and less so in sepsis than in non-sepsis patients (Fig. 1).

Diagnostic values

For both underlying conditions, the optimal cutoff of free cortisol increases with the highest sensitivity and

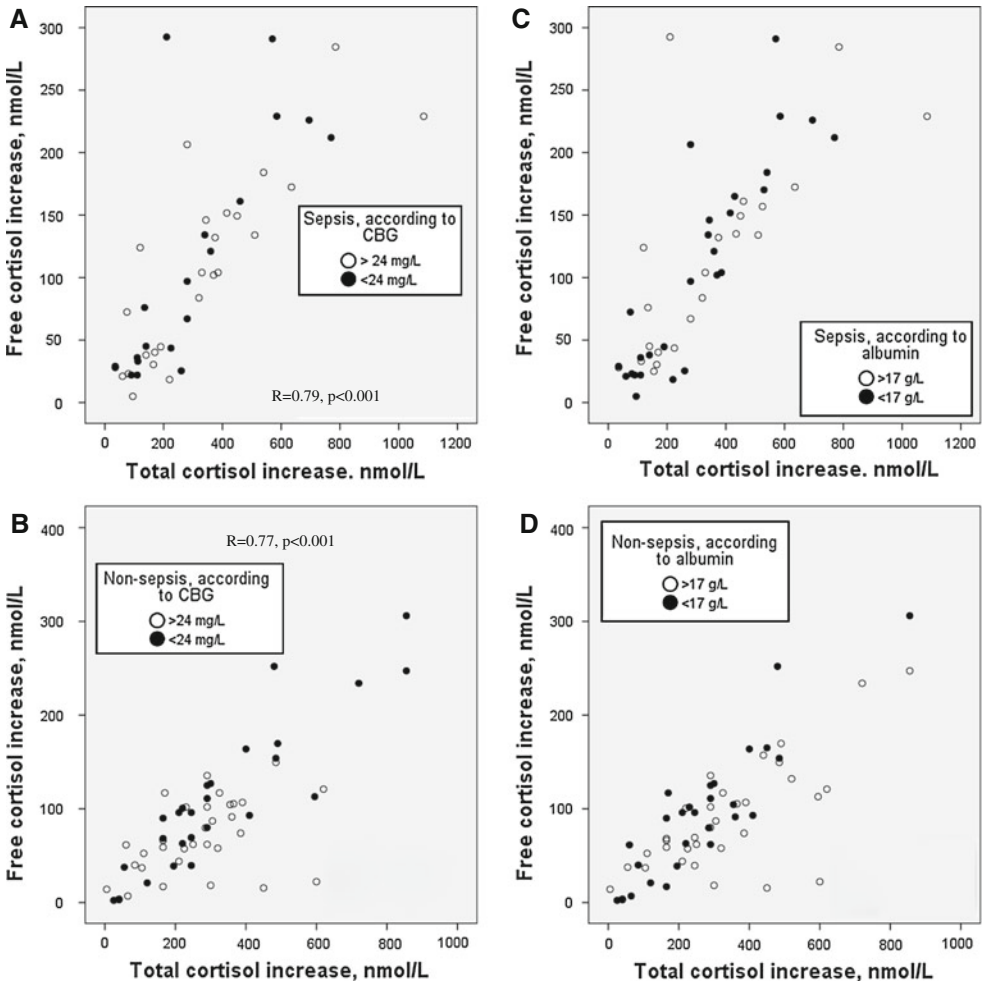

Fig. 1 Increases upon ACTH of free (measured by equilibrium dialysis) versus total cortisol in the critically ill patients with sepsis (**a**, **c**) or non-sepsis (**b**, **d**), according to dichotomized levels of cortisol binding globulin (*CBG*; **a**, **b**) and albumin (**c**, **d**). *R* Partial correlation coefficient

Table 3 Statistically significant clinical correlates of free and total cortisol responses to ACTH

	Free cortisol increase		*P*	Total cortisol increase		*P*
	≥76 nmol/l $n = 62$	<76 nmol/l $n = 49$		≥250 nmol/l $n = 64$	<250 nmol/l $n = 48$	
Admission						
APACHE II	16 (7)	17 (6)	0.156	15 (6)	17 (6)	0.037
Trauma and postoperative	29 (47)	12 (24)	0.018	29 (45)	12 (25)	0.031
Respiratory failure	17 (27)	20 (41)	0.159	16 (25)	22 (46)	0.027
Shock	1 (2)	10 (20)	0.002	6 (9)	5 (10)	0.525
Test day						
Etomidate <48 h	10 (16)	23 (47)	<0.001	12 (19)	21(44)	0.006
Norepinephrine (mg/h)	0.04 (0.08)	0.03 (0.25)	0.594	0.03 (0.05)	0.04(0.04)	0.382
Day 28						
Mortality	13 (21)	15 (31)	0.276	10 (16)	18 (38)	0.014

Data are expressed as median (interquartile range) or number (percentage), where appropriate; exact $P < 0.10$
ACTH Adrenocorticotropic hormone

specificity in the ROC curve for predicting total cortisol increases <250 nmol/l was 76 nmol/l. In sepsis, the AUC of the ROC curve of total cortisol increases predicting an increase in free cortisol <76 nmol/l was 0.97 ($P < 0.001$). In non-sepsis, the AUC was 0.85 ($P < 0.001$, $P = 0.024$ sepsis vs. non-sepsis). For both underlying conditions, the optimal cutoff of the free cortisol increase with the highest sensitivity and specificity in the ROC curve for predicting total cortisol increases <100 nmol/l was 40 nmol/l. In sepsis, the AUC of the ROC curve of total

cortisol increases predicting an increase in free cortisol <40 nmol/l was 0.93 ($P < 0.001$) and in non-sepsis 0.79 ($P = 0.001$, $P = 0.044$ sepsis vs. non-sepsis).

Discussion

Our results suggest that total cortisol closely correlates to free cortisol in critically ill, septic and non-septic patients with suspected CIRCI, even though the biologically active free cortisol fraction depends on binding proteins and free cortisol better parallels severity of disease than total cortisol. In sepsis, albumin is a less important binding molecule than in non-sepsis, and levels cannot be used to predict free from total cortisol. Finally, increases in free cortisol upon ACTH depend less on low binding proteins than raw levels, so that increases in total cortisol suffice in assessing adrenal insufficiency, particularly in sepsis.

CBG is the major cortisol binding-protein in blood, so that, as our data confirm, the free cortisol fraction is only about 25% of total cortisol even in the critically ill, and direct measurements are needed when trying to assess free from total cortisol levels [1, 4, 5, 9, 11, 14–18]. Cortisol binding to CBG is saturable (690 nmol/l) and characterized by a molar binding ratio at high affinity [18]. By contrast, cortisol binding to albumin has low affinity, is non-saturable and is characterized by complex stoichiometry and interactions [18]. Dorin et al. [18] described that albumin-bound and free cortisol fractions increase, whereas the CBG-bound cortisol fraction declines at increasing levels of total cortisol. This leads to a predominance of albumin-bound cortisol at high cortisol levels and in case of combined CBG and albumin deficiency. The latter may thus help to explain the lower total (rather than free) cortisol levels in non-septic, hypoalbuminemic patients with a relatively low CBG vs. those with higher protein levels, in line with the results in predominantly non-septic patients studied by Hamrahian et al. [4]. In contrast, hypoalbuminemia in septic patients was not associated with a low and potentially saturated CBG, and thus low total and high free cortisol, in line with others [5, 15]. CBG and albumin apparently often change in dissimilar directions in sepsis as compared to non-sepsis, as suggested before [18]. Hence, the cortisol/albumin ratios used to adjust for protein binding are probably invalid in this condition [6]. Moreover, the affinity of cortisol may be lowered in sepsis when albumin molecules are damaged [24]. Together with the reported lowered binding capacity of cortisol for CBG in febrile sepsis [8, 23], this may also explain why CBG rather than albumin affected free cortisol in sepsis and even less so than in non-sepsis. In any case, free cortisol was closely related to total cortisol levels, regardless of the underlying conditions, so that both free and total cortisol levels increased in relation to severity of illness, as reported before [4, 6, 16]. Nevertheless, total cortisol somewhat underestimated a higher free cortisol, as observed before [14, 15, 17], in sepsis as compared to non-sepsis. However, both free and total cortisol was directly related to APACHE II scores and endogenous ACTH levels, suggesting HPA axis activation according to the severity of disease, irrespective of underlying condition. Nevertheless, free cortisol correlated better with endogenous ACTH than total cortisol levels, showing the important feedback regulatory function of biologically active free cortisol.

Percentage increases in total cortisol upon ACTH underestimated those in free cortisol, since ACTH increased the free fraction, as described before [4, 13, 17–19]. Nevertheless, the increase in total cortisol was highly concordant with that of free cortisol and of similar diagnostic value for adrenal insufficiency, particularly in sepsis, during which CIRCI may be frequent [2–6, 8, 18, 22]. Indeed, the diagnostic similarity of total and free cortisol increases was less in non-sepsis than in sepsis, since binding proteins affected the former more. This again agrees with data in healthy volunteers [11] and in predominantly non-septic, critically ill patients and dogs [4, 19, 20]. In non-septic patients, increases in total cortisol have been suggested to underestimate increases in free cortisol and to overestimate the occurrence of adrenal insufficiency, even though differences between the increases in free vs. total cortisol decrease at low responses to ACTH [4]. The latter authors, however, did not formally compare diagnostic values. Conversely, our results agree with those in septic patients obtained by others [5, 15, 21], showing good concordance of free and total cortisol increases and diagnostic and prognostic values, even though our cutoff value for the increase in free cortisol was about 25% lower than suggested before in septic patients [15]. Finally, higher endogenous ACTH levels at relatively low versus normal exogenous ACTH-induced total cortisol increases argue in favor of low increases reflecting relative adrenal insufficiency, irrespective of baseline cortisol values.

Our data may partly explain the diagnostic and prognostic value of baseline and peak total cortisol levels, of increases upon ACTH and their combinations [1–3, 5, 6, 15, 16, 21]. The commonly observed superiority of ACTH-induced increases in cortisol in this respect can be explained by our data since the increases were less dependent on binding proteins than raw levels. Baseline total levels may also not contribute when inversely related to increases in cortisol upon ACTH [4, 6]. Total cortisol increases upon ACTH had somewhat greater prognostic value than free cortisol increases in our patients, in partial agreement with other observations [16]. Otherwise, the role of etomidate in depressing ACTH-induced cortisol responses has been described before, and the frequency of low responses is also in line with the literature [1–7].

In our relatively large and prospective study, evaluating the clinical significance of (definitions of) relative adrenal insufficiency, including response to hydrocortisone treatment, was not the primary aim, but we still included some clinical correlates of low cortisol responses to ACTH. The study was designed to evaluate proper assessment of adrenal insufficiency using an independent method of direct free cortisol measurements in two major critical conditions. We therefore did not attempt to redefine clinically significant relative adrenal insufficiency from these measurements and used values reported in the literature for total cortisol responses to the most commonly used dose of 250 μg of ACTH [1–3, 5, 6].

In conclusion, our data on ACTH tests to characterize the relative adrenal insufficiency in CIRCI suggest limited additive diagnostic and prognostic value of assessing free over more readily obtainable total cortisol levels, particularly when using increases in total cortisol upon ACTH, and in sepsis as compared to non-sepsis.

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