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Cortisol Response to Critical Illness: Effect of Intensive Insulin Therapy

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Context: Both excessive and insufficient activation of the hypothalamic-pituitary-adrenal axis in response to critical illness is associated with increased mortality.

Objective: The objective of the study was to study the effect of intensive insulin therapy, recently shown to reduce mortality and morbidity of critically ill patients, on the cortisol response to critical illness.

Design: This was a preplanned subanalysis of a large randomized, controlled study measuring serum total cortisol, cortisol-binding globulin, and albumin and calculating free cortisol levels.

Setting: The study was conducted at a university hospital surgical intensive care unit.

Patients: Four hundred fifty-one critically ill patients dependent on intensive care for more than 5 d and 45 control subjects matched for gender, age, height, and weight participated in this study.

Intervention: The intervention was strict blood glucose control to normoglycemia with insulin.

Results: Total and calculated free cortisol levels were equally elevated upon admission in both patient groups and thereafter were lower in intensive insulin-treated patients. Lower cortisol levels statistically related to the outcome benefit of intensive insulin therapy. Cortisol-binding globulin levels and structure were affected by critical illness but not insulin therapy, and neither were albumin levels. Administration of hydrocortisone in so-called replacement dose resulted in severalfold higher total and free cortisol levels, indicating that reevaluation of the doses used is warranted.

Conclusions: Lower serum cortisol levels in critically ill patients receiving intensive insulin therapy statistically related to improved outcome with this intervention. The lower cortisol levels were not related to altered cortisol-binding capacity. **(*J Clin Endocrinol Metab* 91: 3803–3813, 2006)**

AN APPROPRIATE ACTIVATION of the hypothalamic-pituitary-adrenal axis and cortisol response to critical illness is essential for survival because both high and low cortisol levels have been associated with increased mortality (1–8). High cortisol levels likely reflect more severe stress, whereas low levels may point to an insufficient response to stress, labeled relative adrenal insufficiency. The effects of cortisol are directed toward acute provision of energy, protection against excessive inflammation, and improvement of the hemodynamic status (9, 10). Several studies investigated glucocorticoid therapy in critically ill patients, but only so-called replacement doses of hydrocortisone and not high doses of synthetic glucocorticoids appeared beneficial (11).

CRH and ACTH mediate cortisol release in the acute phase (12), whereas non-ACTH-mediated pathways are involved during prolonged critical illness (9, 13, 14). In line with loss of hypothalamic ACTH control, the diurnal variation in cortisol secretion disappears (12, 15). Several cytokines modulate cortisol production as well as glucocorticoid receptor number and/or affinity (16–20).

Most often, cortisol measurements in critically ill patients are reported as total serum cortisol levels. Recently evaluating the free hormone has been suggested to be more appropriate in these patients (21). Indeed, only the free hormone is biologically active, whereas more than 90% of circulating cortisol is bound to proteins, predominantly corticosteroid-binding globulin (CBG) but also albumin (15). Severely reduced CBG levels have been observed in critically ill patients (22). Moreover, cleavage of CBG by elastase from activated neutrophils has been proposed as a mechanism for cortisol delivery to sites of inflammation (23, 24).

Hyperglycemia, in part evoked by the glucose-counterregulatory cortisol, is detrimental to critically ill patients as recently shown by a randomized, controlled clinical trial in a large group of surgical intensive care patients. Blood glucose control to normoglycemia with insulin significantly reduced mortality and morbidity (25). We already demonstrated a multifactorial origin of the clinical benefits of intensive insulin therapy, in which an effect on innate im-

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Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; CBG, corticosteroid-binding globulin; c-free-cortisol, free cortisol level; CRP, C-reactive protein; HDL, high-density lipoprotein; ICU, intensive care unit; IQR, interquartile range; m-free-cortisol, measured free cortisol.

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munity (26), an antiinflammatory effect (27), partial correction of the lipid profile (28), and protection of the mitochondrial compartment (29) and the endothelium (30) play a role. Literature data indicate that insulin therapy may also affect the cortisol response at multiple levels, which in turn could play a role in the outcome benefit observed with this intervention. First, insulin has been shown to suppress CBG levels, although this may be counteracted by insulin resistance (31, 32), from which a stimulatory effect of intensive insulin therapy on free cortisol levels could thus be hypothesized. On the other hand, antiinflammatory effects of insulin may alter levels of cytokines, which can directly drive cortisol secretion and metabolism. We therefore studied the effect of intensive insulin therapy on circulating cortisol, CBG, and albumin levels on CBG structure and an extensive series of cytokines in prolonged critically ill patients in relation to outcome.

Patients and Methods

Patients and study samples

All adult, mechanically ventilated patients from the large randomized, controlled trial on the effects of intensive *vs.* conventional insulin therapy (25), who were dependent on intensive care for more than 5 d (n = 451), were included in this study. The conventionally treated patients received insulin only when glucose concentrations exceeded 215 mg/dl with the aim of keeping concentrations between 180 and 200 mg/dl (hyperglycemia). Intensive insulin therapy targeted blood glucose levels between 80 and 110 mg/dl (normoglycemia). Written informed consent was obtained from the closest family member. The Institutional Review Board of the Leuven University had approved the study protocol. The baseline characteristics of the selected patients are shown in Table 1. A subgroup of patients were treated with exogenous glucocorticoids during intensive care unit (ICU) stay, 79 (33%) in the conventional and 70 (34%) in the intensive insulin group ($P = 0.8$). Hydrocortisone was given after diagnosis of relative adrenal insufficiency, based on clinical suspicion together with baseline total cortisol levels less than 15 μg/dl with an increment of less than 7 μg/dl in response to 250 μg ACTH. Systematic analyses of cortisol levels for study purposes were not available to the clinicians. Doses of 300 mg in a continuous infusion on the first 24 h of hydrocortisone treatment (not necessarily coinciding with a single day of intensive care) were tapered to 150 mg over the second 24 h and 90 mg per 24 h on maintenance dose. Methylprednisolone was administered for pharmacological indications, *e.g.* immune suppression for solid organ transplantation or as a treatment in the chronic phase of acute respiratory distress syndrome (33).

For this study, analyses were performed on morning serum samples (0600 h) obtained on the day of admission to the ICU, d 5, and the last day of intensive care and also on d 15 for those patients who required intensive care for at least 15 d. Sera were kept frozen at −80 C until assay. In addition, sera from 45 control subjects (healthy volunteers from the community or patients on hospital admission before elective surgery) matched for gender ($P = 0.8$), age ($P > 0.9$), height ($P = 0.6$), and weight ($P = 0.8$) (data not shown) were analyzed for establishment of reference ranges.

Levels of total cortisol, albumin, CBG, and free cortisol in serum

Total serum cortisol levels were measured using chemoluminescence assays on an Immulite 2000 (Diagnostic Products Corp., Los Angeles,

TABLE 1. Baseline characteristics and clinical outcome of patients who remained in the ICU for more than 5 d

	Conventional insulin therapy (n = 243)	Intensive insulin therapy (n = 208)	*P* value
Baseline characteristics			
Demography and anthropometry			
Gender, no. (% male)	164 (67)	144 (69)	0.7
Age, yr (mean ± SD)	61 ± 16	62 ± 15	0.8
BMI, kg/m^2 (mean ± SD)	25.6 ± 5.6	25.7 ± 4.6	0.8
History			
Diabetes, no. (%)	25 (10)	21 (10)	0.9
Malignancy, no. (%)	54 (22)	43 (21)	0.7
Reason for admission or type of surgery (n)			0.7
Complicated vascular	15	20	
Complicated abdominal	32	30	
Complicated cardiothoracic	116	90	
Multiple trauma and cerebral injury	52	46	
Solid organ transplant-hemato-other	28	22	
Clinical scores, median (IQR)			
APACHE II during first 24 h	12 (8–15)	11 (7–15)	0.7
TISS-28 during first 24 h	39 (33–45)	40 (35–45)	0.5
Admission glycemia			
Blood glucose upon admission, mg/dl (mean ± SD)	147.6 ± 55.6	144.2 ± 52.9	0.5
Hyperglycemia (≥200 mg/dl) upon admission, no. (%)	34 (14)	27 (13)	0.8
Clinical outcome			
Death in ICU, no. (%)	49 (20)	22 (11)	0.005
Cause of death (n)			0.5
Acute hemodynamic collapse	6	3	
MOF with a proven septic focus	25	7	
MOF with SIRS	16	11	
Severe brain damage	2	1	
Bacteremia, no. (%)	60 (25)	32 (15)	0.01
Acute renal failure requiring CVVH, no. (%)	58 (24)	31 (15)	0.02
Critical illness polyneuropathy, no. (%)	110 (45)	46 (22)	<0.0001
Days on mechanical ventilation, median (IQR)	12 (7–23)	10 (6–16)	0.006
Days in ICU, median (IQR)	15 (9–27)	12 (8–21)	0.003

The APACHE II score denotes the severity of illness, with higher scores for more severely ill patients (37). TISS-28 is a simplified Therapeutic Intervention Scoring System, with higher values reflecting more invasive treatments (38). BMI, Body mass index; MOF, multiple organ failure; SIRS, systemic inflammatory response syndrome; CVVH, continuous venovenous hemofiltration. 1 mg/dl glucose = (1/18) mmol/liter.

CA). The intraassay coefficient of variation amounted to 5.6%. The reported cross-reactivity of the assay with 100–200 μg/dl methylprednisolone is 21–23%. No cross-reaction was observed with 400 μg/dl cortisone.

At the time of the clinical study, serum albumin levels were only measured when the patients presented with clinical indications. We now quantified albumin levels in the d 5 serum samples using the bromocresol green method (Bioassay Systems, Hayward, CA).

CBG levels were determined by radial immunodiffusion using an in-house polyclonal antibody raised against purified human CBG as previously described (34), with an interassay coefficient of variation of 2.1%. The same antibody was used for Western blot analysis of CBG structure in the d 5 sample of a random subselection of 217 patients and the control sera. Protein bands were visualized by enhanced chemiluminescence (PerkinElmer, Boston, MA) captured on Hyperfilm (Amersham Biosciences, Aylesbury, UK) and quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Free cortisol levels (c-free-cortisol) were calculated from the total cortisol and CBG levels (35). The validity of applying the formula for critically ill patients was verified by measuring the percentage of free cortisol by centrifugal ultrafiltration for a subgroup of the patients and control individuals to determine measured free cortisol (m-free-cortisol) (35). Before use, [1,2,6,7-^{3}H(N)]cortisol tracer (PerkinElmer) was purified by HPLC on a Zorbax CN column (4.6 × 150 mm; Agilent, Palo Alto, CA) with 2-propanol/*n*-heptane [11/89 (vol/vol)] as mobile phase at a flow rate of 1.5 ml/min. The assay was scaled down for analysis of 250 μl serum.

Cytokine and C-reactive protein (CRP) levels in serum

Circulating levels of the cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, TNFα, TNFβ, regulated on activation normal T cell expressed and secreted, brain-derived neurotrophic factor, monocyte chemoattractant protein-1, and interferon-γ were quantified by a multiplexed microbead suspension ELISA as previously described (30, 36). CRP levels were measured by an immunoturbidimetric assay (Roche/Hitachi-Modular-P; Roche, Mannheim, Germany).

Statistical analysis

Two group comparisons were performed by the χ^2 test for proportions, Student's *t* test for normally distributed data (presented as mean ± SD), and the Mann-Whitney *U* test for data that were not normally distributed [presented as median (interquartile range, IQR)], unless indicated otherwise. Main effects between groups were analyzed by repeated measures ANOVA with two levels of the between subjects factor (randomization to conventional or intensive insulin therapy) and four levels of the repeated factor (days) when appropriate. Total and c-free-cortisol were first log transformed to obtain a normal distribution. Time effects for CBG, which were significant in repeated-measures ANOVA, were further analyzed by paired *t* tests with Bonferroni correction of the resulting *P* value for multiple comparisons. For comparisons between patients who received hydrocortisone *vs.* those who did not receive glucocorticoids, Mann-Whitney *U* test was used, also followed by Bonferroni correction for multiple testing. Similar tests were performed to analyze the effect of intensive insulin therapy in patients who received exogenous glucocorticoids. Linear or second-order polynomial regression analysis (for the relation between total and c-free-cortisol) was performed, and the corresponding Pearson correlation coefficients were calculated to assess the significance of correlations between parameters.

Multivariate logistic regression analysis was used to assess the relative relation of the effects of intensive insulin therapy on the studied variables with patient outcome. The baseline risk factors age, type, and severity of illness [Acute Physiology and Chronic Health Evaluation (APACHE) II score (37)], hyperglycemia on admission, history of diabetes, and malignancy as well as the level of blood glucose control and insulin dose related to the intervention were entered into the model, in addition to the studied variables of which univariate analysis revealed a significant difference between the conventional and intensive insulin groups. All continuous variables entered in the model were linearly related with the studied outcome parameter. Results are reported as odds ratio and 95% confidence interval.

Differences were considered statistically significant when two-sided *P* values were below or equal to 0.05. Statistical analyses were performed by StatView 5.0.1 for Macintosh (SAS Institute, Cary, NC).

Results

Blood glucose control and clinical outcome of the patients

Upon admission blood glucose levels were comparable for both treatment groups. Implementation of the study protocol resulted in significantly lower morning blood glucose levels in the intensive than the conventional treatment group at all further time points (Fig. 1A), which were achieved by administration of higher insulin doses (Fig. 1B). Intensive insulin therapy significantly increased the survival of these prolonged critically ill patients and reduced morbidity (Table 1), as was observed for the whole study population (25, 39).

Total cortisol, albumin, CBG, and free cortisol in serum

Upon admission to the ICU, total cortisol levels were elevated in the whole group of critically ill patients in com-

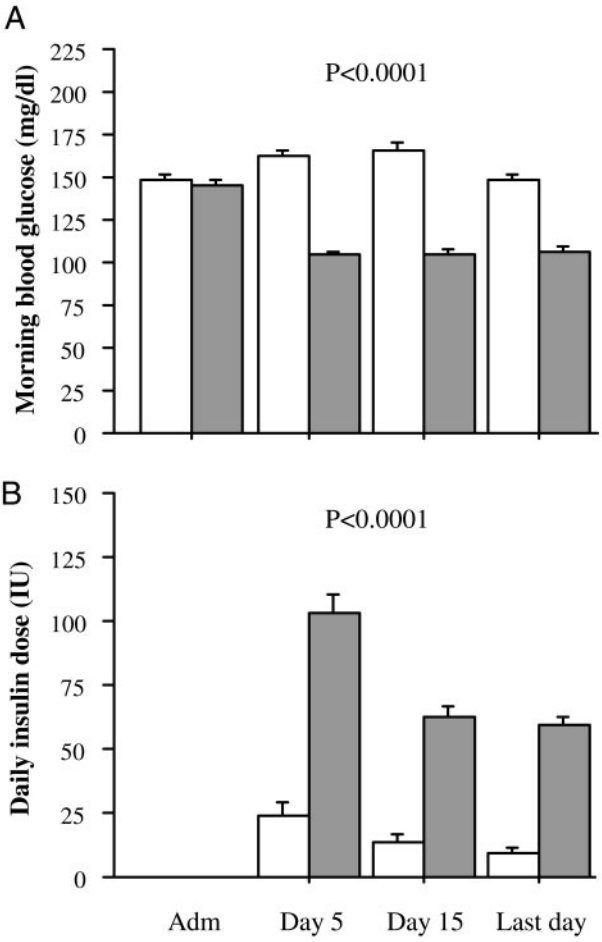
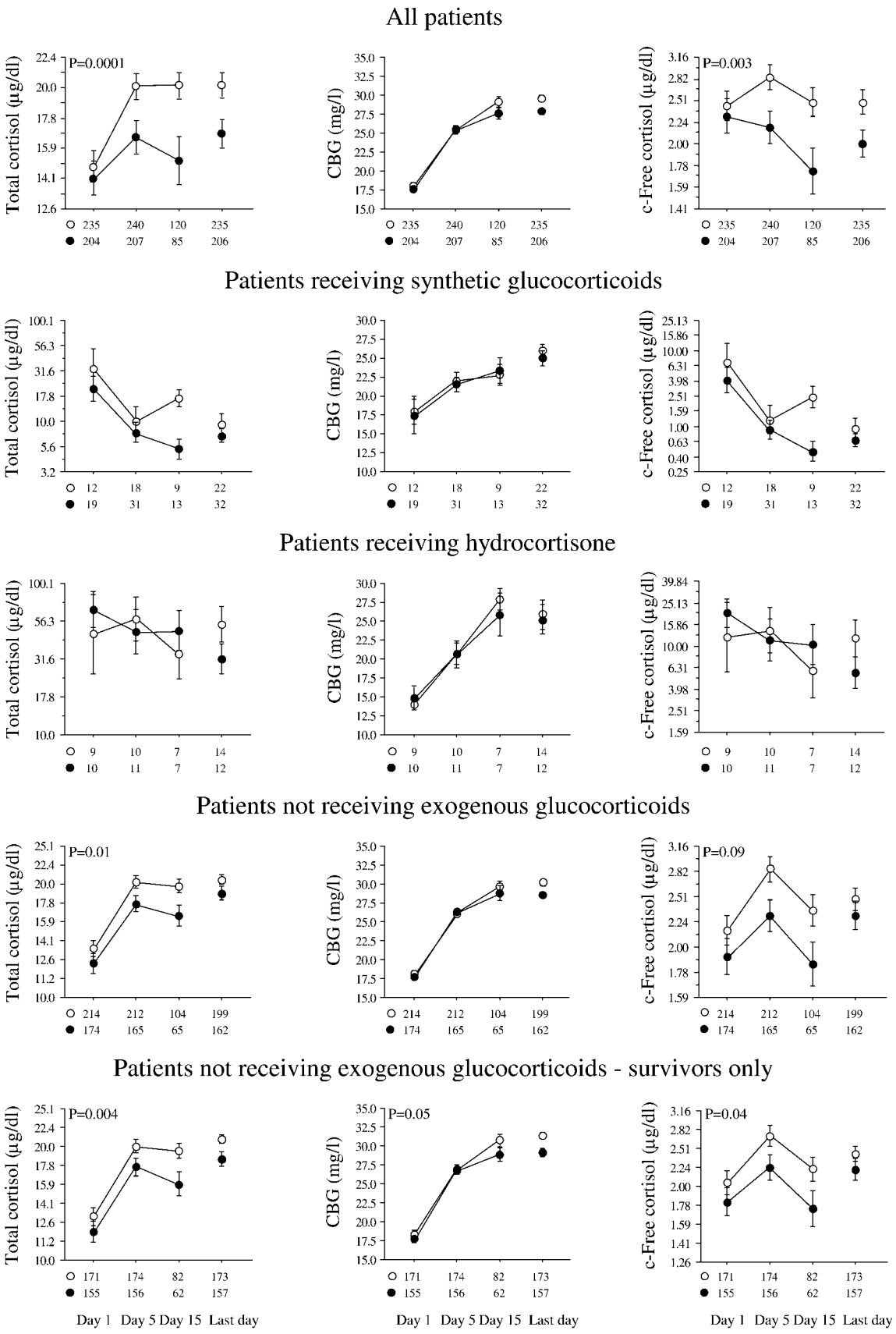


FIG. 1. Blood glucose control and insulin doses of patients randomized to conventional and intensive insulin therapy. Blood glucose levels were controlled with insulin to normoglycemia (80–110 mg/dl) in the intensive insulin therapy group (*gray bars*, n = 208, except for d 15, n = 87), whereas insulin administration in the conventional treatment group (*white bars*, n = 243, except for d 15, n = 123) aimed to maintain blood glucose levels between 180 and 200 mg/dl (1 mg/dl = (1/18) mmol/liter). Glucose levels (A) and insulin doses (B) are depicted as means and SEM. Adm, Admission.



parison with the levels measured in control subjects, similarly in the two insulin treatment groups, and they remained elevated throughout ICU stay (Fig. 2). Total cortisol levels in the intensive insulin treatment group were lower than in the conventional group on d 5, d 15, and the last day of intensive care. Albumin levels at d 5 were not affected by intensive insulin therapy [median (IQR), 3.8 (3.2–4.5) and 3.8 (3.3–4.6) g/dl in the conventional and intensive insulin patient groups, $P = 0.6$]. At this time point 106 patients (24%) had hypoalbuminemia (<3.3 g/dl), of which only five (1%) had extreme hypoalbuminemia (≤2.0 g/dl), equally distributed between both patient groups ($P = 0.8$). CBG levels were approximately 50% lower than in controls on the first day of intensive care, independently of randomization (Fig. 2). CBG levels gradually and equally increased in both groups ($P < 0.0001$) but remained in the subnormal range until the last study day. The corresponding c-free-cortisol levels were approximately 3-fold higher in patients, compared with normal, for all studied days (Fig. 2). Upon admission, c-free-cortisol levels were comparable in the two insulin therapy groups. c-Free-cortisol was lower on d 5, d 15, and on the last day in the intensive insulin patient group. Analyzing the data separately for patients who received synthetic glucocorticoids, hydrocortisone, or no exogenous glucocorticoids also revealed lower total cortisol levels and a trend toward lower c-free-cortisol levels in the intensive insulin-treated patients not receiving glucocorticoids (Fig. 2). Among survivors only, both total and c-free-cortisol levels were significantly lower in the intensive insulin patient group (Fig. 2), whereas no difference was seen among the nonsurvivors (both $P = 0.3$, data not shown).

A good overall correlation was found between c-free-cortisol and m-free-cortisol levels in a subgroup of 30 critically ill patients (15 in each treatment group) and 10 control subjects ($y = 1.15x$, $R = 0.99$) as well as for the individual groups (Fig. 3A) for albumin levels ranging from 2.3 to 7.4 g/dl. Together, these data support the validity of using the formula to calculate free cortisol levels in this study.

A good correlation was also present between total and c-free-cortisol levels in patients not receiving exogenous glucocorticoids ($R = 0.985$; Fig. 3B). The corresponding trend line was shifted upward, compared with the theoretical trend line, assuming normal CBG levels, in line with the suppressed CBG levels. For total cortisol levels up to 10 μg/dl, a narrow variation was seen in c-free-cortisol levels. Above 10 μg/dl total cortisol levels were no longer representative/predictive for c-free-cortisol because a wide scattering in free cortisol was observed for a given total cortisol level. Importantly, no increased risk of death was observed for patients with total and c-free-cortisol levels in the range below median control levels.

No relevant correlations were found between total or c-free-cortisol and the previously reported (28) total cholesterol, high-density lipoprotein (HDL)-cholesterol, or low-density lipoprotein cholesterol levels (data not shown).

Cytokine and CRP levels

Circulating cytokine levels were hardly affected by intensive insulin therapy (data not shown). Only for IL-10, a significant difference ($P = 0.05$) was found, with lower levels in the intensive insulin group [d 1 of critical illness 61.1 (32.6–131.5) *vs.* 65.1 (27.6–133.6) pg/ml; d 5, 33.7 (12.5–73.1) *vs.* 43.8 (19.6–101.0) pg/ml; d 15, 31.6 (12.6–72.3) *vs.* 60.0 (20.9–101.1) pg/ml; and last day, 37.7 (12.2–79.0) *vs.* 39.4 (13.1–91.1) pg/ml]. The effect was present in the patients who did not receive exogenous glucocorticoids ($P = 0.04$) but disappeared with glucocorticoid administration ($P = 0.6$) (data not shown). We previously described that intensive insulin therapy reduced CRP levels on d 5, d 15, and the last day in this group of prolonged critically ill patients (27). No clinically relevant correlation was found between any of the studied cytokines or CRP and the levels of total cortisol, CBG, or c-free-cortisol (data not shown). Patients who received exogenous glucocorticoids (hydrocortisone or synthetic glucocorticoids) had lower IL-6 and CRP levels than those who did not (data not shown).

Relative relation of the effect of intensive insulin therapy on serum cortisol with its outcome benefits in critical illness

In univariate analysis, total and c-free-cortisol levels were significantly higher in nonsurvivors than survivors ($P < 0.0001$), whereas CBG levels were lower ($P = 0.002$) (data not shown). Moreover, a positive linear relationship was observed between cortisol levels and risk of death.

In multivariate logistic regression analysis on d 5 of critical illness, c-free-cortisol ($P = 0.046$) and IL-10 ($P = 0.009$) levels independently predicted mortality, together with the preexisting risk factors age and severity of illness as indicated by the APACHE II score (Table 2). c-Free cortisol was also an independent predictor of mortality on d 15 ($P = 0.049$). Consequently, nonsurvivors of both treatment groups had identical cortisol levels as also shown in univariate analysis. Similarly, c-free-cortisol independently predicted the development of acute renal failure ($P = 0.03$) together with APACHE II score and IL-10 (data not shown), inherently resulting in equal cortisol levels in patients of both groups with renal failure.

FIG. 2. Effects of intensive insulin therapy on total cortisol, CBG, and c-free-cortisol. Results are presented as mean and SEM. Total and c-free-cortisol are shown on a logarithmic scale. *Open circles*, Patients in the conventional group; *filled circles*, patients in the intensive insulin therapy group. *P* values for the effect of intensive insulin therapy are shown if lower than 0.1. Values in control subjects (n = 45) were 12.2 ± 0.7 μg/dl for total cortisol (1 μg/dl = 27.6 nmol/liter), 35.9 ± 1.2 mg/liter for CBG, and 0.9 ± 0.1 μg/dl for c-free-cortisol. The percentage of c-free-cortisol was 6.3% of total cortisol levels in these control subjects. For the total group, CBG levels gradually increased over time ($P < 0.0001$), with all pairwise comparisons between days being significant ($P < 0.0004$) except between d 15 and last day ($P = 0.4$). A gradual CBG increase was also seen in the patient subgroups depicted in the figure. The patients to whom glucocorticoids were administered received 240 ± 94, 158 ± 84, 78 ± 22, and 116 ± 82 (mean ± SD) mg on admission, d 5, d 15, and last day, respectively, in the conventional insulin group *vs.* 130 ± 89, 149 ± 67, 93 ± 57, and 103 ± 83 mg in the intensive insulin therapy group. These doses were not significantly different between the groups ($P = 0.08$, > 0.9, 0.8, and 0.6, respectively). Numbers of observations are indicated *below each graph*.

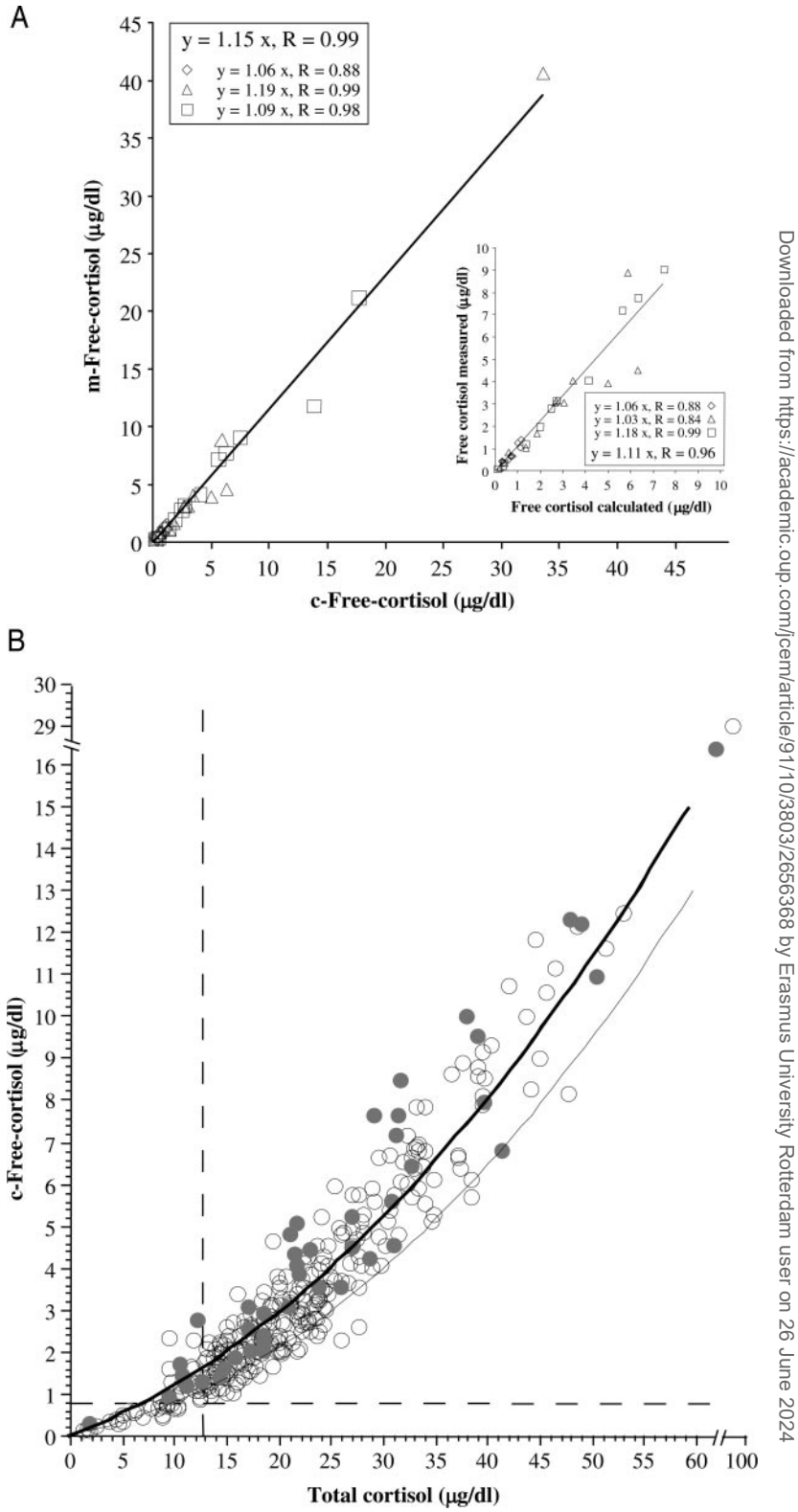


FIG. 3. Relation between total and free cortisol levels. A, Comparison of c-free-cortisol with m-free-cortisol levels. Free cortisol levels were measured in 10 control subjects (*diamonds*), 15 patients from the conventional insulin therapy group (*triangles*), and 15 patients from the intensive insulin therapy group (*squares*) and plotted against the calculated levels. The 15 patients in each of the critically ill patient groups were selected for glucocorticoid treatment on d 5 (no glucocorticoids n = 10, hydrocortisone n = 5). The trend line shows the overall correlation between both values. The correlations for each of the individual subgroups are indicated by the corresponding equations of the trend lines and the respective correlation coefficients. The portion of the figure representing free cortisol levels less than 10 μg/dl is shown in the *inset* (1 μg/dl cortisol = 27.6 nmol/liter). B, Correlation between total and c-free-cortisol levels. A good correlation was found between total and c-free-cortisol levels on d 5 of patients who did not receive exogenous glucocorticoids during intensive care (*thick black line*, R = 0.985). *Open circles*, Survivors; *gray circles*, nonsurvivors; *thin black line*, theoretical relation between c-free-cortisol levels and variable total cortisol concentrations for a hypothetical normal subject with a mean CBG level of control subjects (35.9 mg/liter) instead of the individual patient's values. *Dashed lines*, Median total and c-free-cortisol levels in the control subjects. Similar results were obtained for the other days (1 μg/dl cortisol = 27.6 nmol/liter).

Occurrence of clinical complications within 24 h of sampling for cortisol measurements

We studied the occurrence of the most severe clinical complications, expected to induce an increase in cortisol levels, within 24 h of the sampling times for cortisol measurements. In the conventional and intensive insulin treatment groups on d 5, d 15, and last day, the number of patients with acute renal failure (20, 23, and 20 *vs.* 16, 10, and 10; $P = 0.8$, 0.06 and $P = 0.3$, respectively) or bacteremia (3, 2, and 3 *vs.* 4, 0, and 2; $P = 0.6$, 0.2, and 0.8, respectively) were comparable.

TABLE 2. Relative relation of the effect of intensive insulin therapy on serum cortisol with its outcome benefits in critical illness

	Odds ratio	95% Confidence interval	*P* value
Preexisting risk factors			
Noncardiac surgery	1.043	0.539–2.017	0.9
Age, per year added	**1.032**	**1.008–1.057**	**0.009**
Admission hyperglycemia ≥200 mg/dl	1.085	0.504–2.334	0.8
Positive history of diabetes	0.452	0.157–1.301	0.1
Positive history of malignancy	1.628	0.824–3.215	0.2
APACHE II score (first 24 h), per 1 added	**1.083**	**1.022–1.146**	**0.007**
Randomized intervention			
Randomization to intensive insulin therapy	0.635	0.220–1.836	0.4
Mean morning blood glucose, per 10 mg/dl added	1.101	0.970–1.248	0.1
Mean daily insulin dose, per 10 U added	1.037	0.975–1.102	0.3
Studied variables			
CRP on d 5, per 10 mg/ml added	1.033	0.999–1.069	0.06
c-free-cortisol on d 5, per μg/dl added	**1.024**	**1.000–1.047**	**0.046**
IL-10 on d 5, per 10 pg/ml added	**1.025**	**1.006–1.044**	**0.009**

The variables studied on d 5 that upon univariate analysis were significantly different between patients in the conventional and intensive insulin therapy groups were entered into the multivariate logistic regression model for mortality as outcome parameter, together with known preexisting risk factors as well as the intervention. 1 mg/dl glucose = (1/18) mmol/liter; 1 μg/dl cortisol = 27.6 nmol/liter.

A subanalysis of the data showed that insulin therapy lowered total and c-free-cortisol levels in patients who did not require renal replacement therapy ($P = 0.0009$ and $P = 0.02$, respectively) but not in those who needed this therapy ($P = 0.2$ and $P > 0.9$, respectively). Likewise, the effect of insulin therapy was also present in patients who did not develop bacteremia ($P = 0.01$). Because patients were selected for an ICU stay of more than 5 d, no patients died before the end of d 5. At d 15 one patient in the conventional group had died. On the last day, fewer patients in the intensive insulin group had died as described in Table 1, inherent to the treatment benefit. However, also among survivors only, cortisol levels were lower in the intensive insulin group (Fig. 2). Fewer patients in the intensive insulin therapy group developed severe clinical complications. However, the patients in the intensive insulin group who developed renal failure presented with the complication earlier [5 (2–8) d)] than those in the conventional group [8 (4–13) d; $P = 0.03$]. There was no difference in the time to development of bacteremia [10 (5–19) d in the conventional *vs.* 7 (4–22) d in the intensive insulin group; $P = 0.9$], whereas there was a trend toward earlier death in the intensively treated patients [14 (10–25) *vs.* 18 (10–39) d; $P = 0.1$]. There was no relation between cortisol levels and ICU length of stay or duration of mechanical ventilation.

CBG structure

To investigate possible elastase-induced CBG cleavage in the circulation of critically ill patients, Western blots were performed. For comparison, purified CBG was first cleaved by elastase *in vitro*. This cleavage appeared to detrimentally affect the stability of the protein as revealed by reduced staining intensity with prolonged incubation (Fig. 4A). A relatively weak protein band of similar molecular weight as the elastase-cleaved CBG was detected in virtually all patients on d 5 as well as in 80% of the control subjects (Fig. 4, B and C). Semiquantitative scoring of the intensity showed that the band was more frequently of higher intensity in critically ill patients (40%) than the control subjects (15%, Fig. 4C), without an effect of insulin therapy. Quantification of the major, intact CBG doublet bands that are normally present in healthy serum revealed that critical illness is associated with a shift in the relative abundance of these two bands. We observed a lower intensity of the upper band relative to the lower band in critically ill patients, compared with control subjects (Fig. 4D, $P < 0.0001$). The ratio of the two bands was not affected by insulin therapy.

Glucocorticoid replacement therapy and cortisol levels

Administration of hydrocortisone in replacement doses to patients with relative adrenal insufficiency was associated with 5-fold higher median total cortisol levels on admission day, 2.5-fold on d 5, 1.9-fold on d 15, and 1.7-fold higher levels on the last day of intensive care, compared with patients not receiving exogenous glucocorticoids (Fig. 5), the decrease with time likely explained by tapering of the hydrocortisone dose. The intervention was also associated with lower CBG levels, reduced by 18% on admission, 21% on d 5, 11% on d 15, and 7% on the last day. As a result, c-free-cortisol levels in the hydrocortisone-treated patients were even more dramatically elevated, with 9-fold higher levels on admission, 5-fold on d 5, 4-fold on d 15, and 3-fold higher levels on the last day, *i.e.* approximately twice higher percentages of unbound to total cortisol levels at all time points.

Discussion

Intensive insulin therapy was associated with an attenuated rise in total and c-free-cortisol in prolonged critically ill patients, which statistically related to the outcome benefit of this therapy. This effect on cortisol was not mediated by cytokines. Critical illness reduced serum CBG concentrations and induced a structural change in CBG, but intensive insulin therapy did not affect CBG concentration or its structure. The excessively high cortisol levels measured after hydrocortisone therapy suggest that reevaluation of the glucocorticoid doses administered as replacement therapy for relative adrenal insufficiency is warranted.

Total and c-free-cortisol levels were elevated above normal in both patient groups, as described for a wide variety of severe insults (5, 15, 40). However, this hypercortisolism was attenuated by intensive insulin therapy. Insulin therapy did

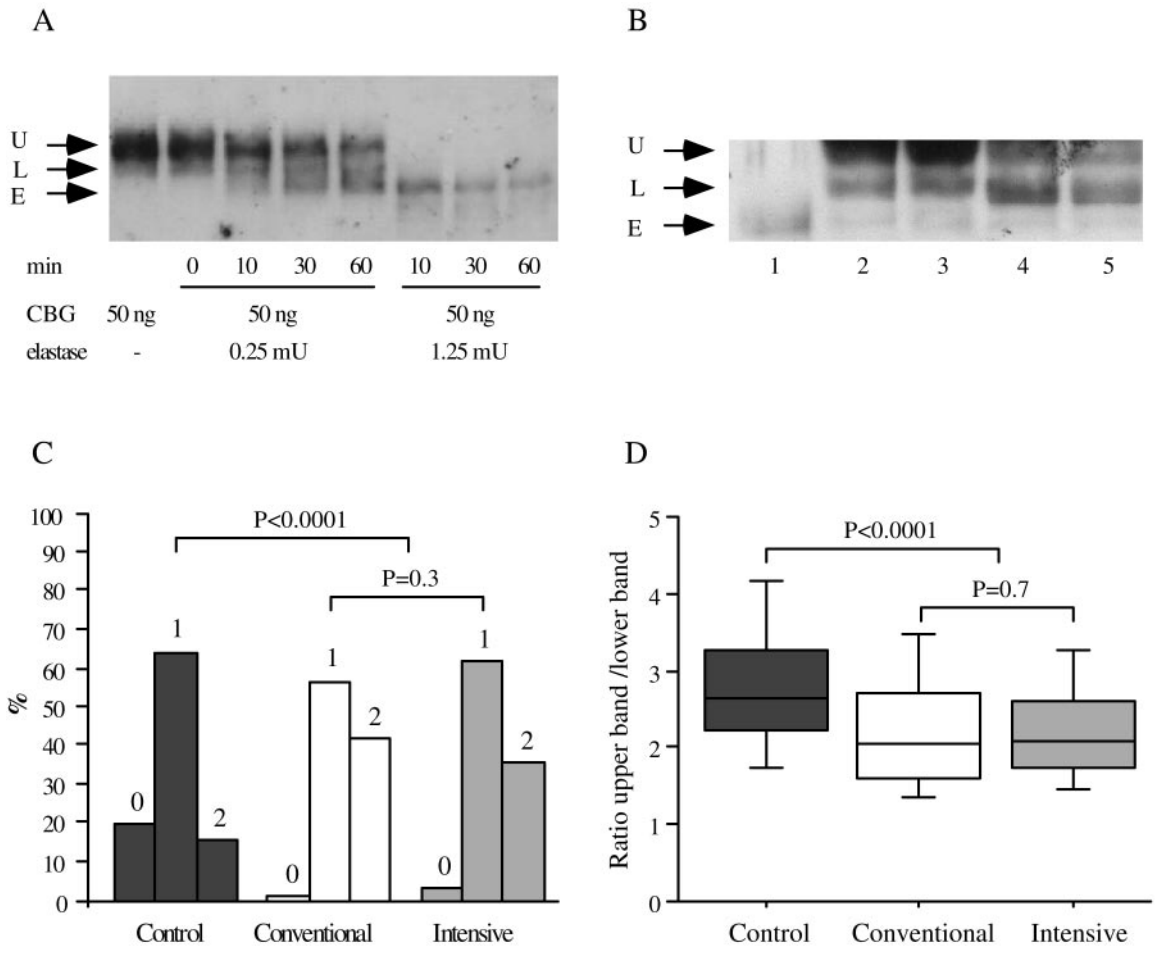


FIG. 4. Western blot analysis of CBG. A, *In vitro* cleavage of CBG by neutrophil elastase (Sigma, Bornem, Belgium). Lane 1, Purified CBG; lanes 2–5, incubation of 50 ng purified CBG with 0.25 mU elastase in 33 mM Tris and HCl (pH 7.5) for 0, 10, 30, and 60 min, respectively; lanes 6–8, incubation of 50 ng purified CBG with 1.25 mU elastase in 33 mM Tris and HCl (pH 7.5) for 10, 30, and 60 min, respectively. U, Upper band of CBG doublet; L, lower band of CBG doublet; E, CBG band appearing after cleavage by elastase. B, Presence of elastase-cleaved CBG in serum of critically ill patients. Lane 1, Elastase-cleaved CBG; lanes 2 and 3, serum samples of control subjects; lane 4, serum sample of patient in conventional insulin therapy group; lane 5, serum sample of patient in intensive insulin therapy group. U, Upper band of intact CBG doublet; L, lower band of intact CBG doublet; E, band appearing after cleavage by elastase. C, Scoring of elastase-cleaved CBG. Score 0, no band detectable; score 1, band present; score 2, band present with relatively high intensity. The frequency of each score is given for control subjects (n = 45, *dark gray bars*), patients from the conventional insulin therapy group (n = 116, *white bars*), and patients from the intensive insulin therapy group (n = 101, *light gray bars*). D, Quantification of the relative abundance of the upper and lower intact CBG bands in control subjects (n = 45, *dark gray box plot*) and patients in the conventional (n = 116, *white box plot*) and intensive (n = 101, *light gray box plot*) insulin therapy groups. Box plots represent median, IQR and the 10th and 90th percentiles.

not affect the circulating level or structure of CBG, and albumin levels were also similar in both patient groups, suggesting that cortisol binding capacity was not altered. These observations, together with the strong correlation between c-free-cortisol and m-free-cortisol levels for a broad range of albumin concentrations, also confirmed in a recent study on free cortisol in septic shock and sepsis (41), indicated that our results are not biased by unjustified use of c-free-cortisol levels (35). Because we could not further investigate the effect of insulin therapy on cortisol production or clearance due to technical limitations of our study, we are unable to speculate about which factor is responsible for the differences in circulating cortisol.

Several studies demonstrated a positive correlation between serum cortisol and severity of illness as well as the risk of death (1, 3, 5, 6, 8, 15, 40). Statistically the reduction of serum cortisol in our study independently explained part of the survival benefit of intensive insulin therapy, which leads to the question of whether this is actually cause or consequence. Subgroup analysis showed that the effect on cortisol was present only in the surviving patients and not in the patients who died. This lack of effect in the nonsurvivors may suggest that the lowering of cortisol levels with insulin therapy is not a consequence of the improved survival. The observation that the lowering of cortisol is present in the survivors only also explains why there is only a trend toward lower free cortisol levels in the intensive insulin therapy group and no statistically significant difference when survivors and nonsurvivors not receiving exogenous glucocorticoids are combined because in this way the effect is partially evened out.

Lower serum cortisol with intensive insulin therapy could,

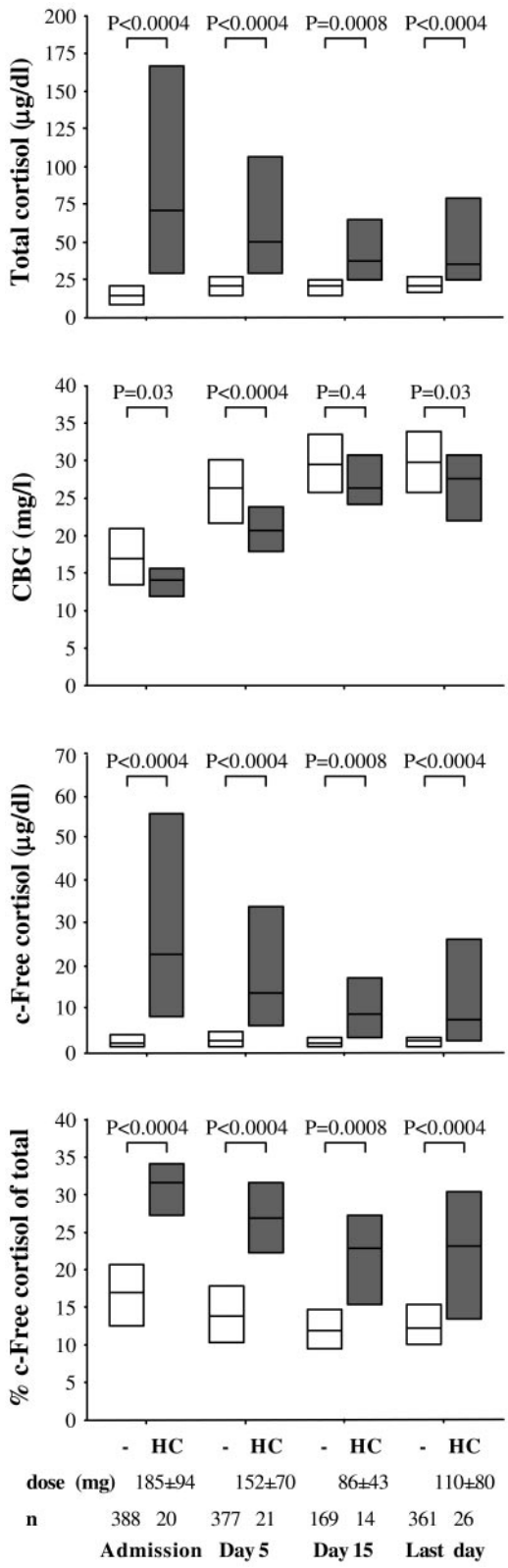


FIG. 5. Total cortisol, CBG, and c-free-cortisol levels in patients receiving hydrocortisone. Patients not receiving glucocorticoids (*open box plots*, n = 388, 377, 169, and 361 for admission day, d 5, d 15, and last day, respectively) were compared with patients who received hydrocortisone on the day of analysis (HC, *gray box plots*, n = 20, 21, 14, and 26 for admission day, d 5, d 15, and last day, respectively) for total cortisol, CBG, and c-free-cortisol levels as well as the percentage of c-free-cortisol relative to total cortisol levels. Means and SD values of the hydrocortisone doses administered on the days of analysis are indicated. The initiation of hydrocortisone therapy does not necessarily coincide with the start of a particular day in intensive care, implying that a day of hydrocortisone therapy may be spread over 2 d of intensive care. This explains why the doses on admission day are different from 300 ± 0 mg. Box plots represent median and IQR (1 μg/dl cortisol = 27.6 nmol/liter).

alternatively, also reflect attenuation or prevention of the development of severe complications by this treatment. The most important outcome measures were studied in detail. These included mortality, bacteremia, and acute renal failure requiring dialysis. The effect of insulin therapy on cortisol levels was present in all patients analyzed together but also in the subgroup of patients who did not have the respective complications, *i.e.* patients who survived, who did not develop bacteremia or acute renal failure requiring dialysis. Consequently, the lower cortisol levels were not due to the prevention of these major complications. Because our findings were independent of the prevention of these major complications, it is unlikely that they would be explained by very small differences in clinical condition such as differences in blood pressure, temperature, or oxygenation. Moreover, these are parameters for which measures are taken by the intensivist to obtain normal values because it would be unethical not to intervene, and thus they are not different between groups.

Another possible explanation for the lowering of cortisol could be an antiinflammatory action of insulin. CRP levels were indeed significantly lower in the intensive insulin therapy group. However, circulating cytokine levels were hardly affected, the unexpected decrease of IL-10 being the only one reaching significance. This argues against a major impact of cytokines, although local differences at tissue level cannot be excluded. Local differences may be important in view of cytokine effects on the equilibrium point of the cortisone/cortisol shuttle (18, 19) and glucocorticoid receptor expression and its binding to glucocorticoid response elements (20), besides their impact on cortisol production (16, 17). Nonsurvivors in both the conventional and intensive insulin therapy groups had identical cortisol levels. Hence, although tissue samples were available, these were from nonsurvivors only, which did not allow further investigation of mechanisms and physiological impact of the lower serum cortisol levels at the tissue level.

The free rather than total cortisol response to critical illness is clinically most relevant. Hamrahian *et al.* (21) demonstrated that total cortisol is not an accurate indicator of cortisol activity. In a subgroup of patients with low albumin levels, they found normal free serum cortisol despite a subnormal total cortisol response. Importantly, CBG levels are substantially decreased in the early phase of critical illness, resulting in proportionally much higher increases in free cortisol levels in comparison with total levels (21, 22). CBG levels recover in the chronic phase of critical illness, reaching normal levels by d 7 (22). We also observed such a biphasic CBG response to critical illness, but even on the last day of intensive care, CBG levels remained subnormal. One of the

mechanisms put forward to explain the CBG depletion is its specific cleavage by elastase expressed by activated neutrophils at sites of inflammation (24), which was found to regulate cortisol availability in target cells. This cleavage induces a conformational change in CBG, which results in a 10-fold decrease in affinity for cortisol (23). In turn, this allows release of large amounts of cortisol directly to inflammatory cells. Our Western blot analyses suggested that elastase-cleaved CBG is more profoundly present in the circulation of critically ill patients as compared with controls, without an effect of insulin therapy. Intact CBG is a glycoprotein that migrates as doublet bands in gel electrophoresis, the heterogeneity being due to differences in carbohydrate structure as well as the protein moiety (42). Interestingly, critical illness induced a change in the relative abundance of these two bands, which was not affected by insulin therapy. Carbohydrate structures are important for CBG survival in the circulation and proper folding and acquisition of steroid-binding capacity (43, 44). Importantly, the carbohydrate chains are also essential for the specific interaction between CBG and binding sites on cell membranes of various target tissues, which seems to be a crucial step in the guided delivery of cortisol into these tissues and hormonal signaling (45). Because different cortisol affinities have been reported for the two CBG bands (46), the shift in the abundance of these two bands in critical illness may also point to altered cortisol delivery.

Patients with poor cortisol rise in response to severe stress, labeled relative adrenal insufficiency, appear to have a high risk of death (1, 2, 4, 47). This deficiency can, for instance, result from anatomical damage to the hypothalamic-pituitary-adrenal axis or administration of interfering drugs (10, 48, 49). On the other hand, a causal relationship has been suggested between low HDL-cholesterol levels and the cortisol response in critically ill patients (50). However, our observation that intensive insulin therapy was associated with increased (HDL and low-density lipoprotein) cholesterol (28) but concomitantly decreased cortisol does not support this concept. The benefit of treating patients with glucocorticoids depends on the dose (11). Administration of high doses of glucocorticoids to critically ill patients has now shown to be ineffective if not harmful (11, 51). In contrast, so-called low-dose glucocorticoid replacement therapy for relative adrenal failure improved survival and led to earlier restoration of hemodynamic stability (7, 11, 52–54). As indicated by several authors, further optimization of the dose and duration of treatment and the accuracy of the diagnosis of relative adrenal failure is necessary (7, 55, 56). The latter is also illustrated by our data on relatively low mortality in patients with low cortisol levels and not receiving hydrocortisone (Fig. 2B). Importantly, these patients did not present with clinical indications for hydrocortisone therapy, and the results of the cortisol measurements for study purposes were not available to the clinicians at that time. In our study, hydrocortisone treatment was more often needed in nonsurvivors than survivors, inherent to the higher severity of illness and regardless of insulin therapy, and 32% of patients treated with hydrocortisone during intensive care died in contrast to 13.5% of patients not receiving this treatment ($P = 0.0004$). The administration of hydrocortisone in so-called replacement dose for presumed adrenal failure resulted in an up to 5-fold higher median total cortisol and 9-fold higher c-free-cortisol level than in patients with normal adrenal function. Thus, a large fraction of the administered hydrocortisone becomes actively available, as illustrated by c-free-cortisol fractions of more than 30%. Similarly high cortisol levels have been reported previously for critically ill patients receiving comparable doses of hydrocortisone (54, 57), although still called low dose. However, the dramatically elevated cortisol levels, compared with adrenal sufficient patients, clearly show that the term low-dose hydrocortisone therapy is not justified. These data suggest that a reevaluation of the doses used for replacement therapy is warranted, particularly because high cortisol levels are associated with worse outcome (1, 3, 5, 8, 40).

In conclusion, intensive insulin therapy was associated with lower serum cortisol levels in critical illness. This effect, which was independent of binding capacity, related to the improved outcome with this intervention.

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