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Measurement of Salivary Cortisol Concentration in the Assessment of Adrenal Function in Critically Ill Subjects: A Surrogate Marker of the Circulating Free Cortisol

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Methods: Baseline and cosyntropin-stimulated serum (total and free) and salivary cortisol concentrations were measured, in the early afternoon, in 51 critically ill patients and healthy subjects. Patients were stratified according to their serum albumin at the time of testing: those whose serum albumin levels were 2.5 gm/dl or less *vs.* others whose levels were greater than 2.5 gm/dl.

Results: Baseline and cosyntropin-stimulated serum free cortisol levels were similar in the two groups of critically ill patients and were severalfold higher ($P < 0.001$) than those of healthy subjects. Similarly, baseline and cosyntropin-stimulated salivary cortisol concentrations were equally elevated in the two critically ill patient groups and were severalfold higher ($P < 0.001$) than those of healthy subjects. Salivary cortisol concentrations correlated well with the measured serum free cortisol levels.

Conclusions: Salivary cortisol measurements are simple to obtain, easy to measure in most laboratories, and provide an indirect yet reliable and practical assessment of the serum free cortisol concentrations during critical illnesses. The concentrations of the two measures of unbound cortisol determined in two different body fluids correlated very well, regardless of the serum protein concentrations. Measurements of salivary cortisol can serve as a surrogate marker for the free cortisol in the circulation. **(*J Clin Endocrinol Metab* 92: 2965–2971, 2007)**

APPROXIMATELY 90–93% OF cortisol in the circulation is protein bound (to transcortin and albumin), whereas the remaining 7–10% is free or unbound (1–5). The current paradigm dictates that the free or unbound pool of cortisol is responsible for its physiological function. Standard assays for serum cortisol measurements determine the total (*i.e.* the bound plus the free fractions) hormone concentrations. It becomes evident that, in light of the high degree of protein binding, measured serum cortisol levels would be greatly influenced by alterations in plasma binding protein concentrations. Whereas the impact of increased transcortin levels on measured serum cortisol is well documented (1–6), the importance of decreased binding proteins was only recently appreciated (7–12).

Recently published studies addressed the impact of low serum transcortin levels on measured serum cortisol concentrations (7–12). To correct for low transcortin concentrations, some studies recommended the use of a calculated index as a surrogate marker for adrenal function instead of relying on serum cortisol itself (7–10). The latter was referred to as the cortisol index and was calculated as the cortisol concentration divided by the transcortin level. Whereas the calculated cortisol index represented an improvement in appreciating the impact of low transcortin on measured serum cortisol concentrations, it did not provide direct measurements of free cortisol and did not take into account the impact of low serum albumin levels, which often accompany states of low serum transcortin concentrations. More importantly, the free cortisol index does not take into account the fact that cortisol binding to transcortin is saturable. A recent study (12) conducted in patients with sepsis used the previously published method of Coolens *et al.* (4) to calculate serum free cortisol concentrations. The latter study demonstrated that there was a good correlation between calculated and measured serum free cortisol in septic patients.

In a recent study conducted on critically ill patients at our institution (11), we demonstrated that critically ill patients have markedly increased serum free cortisol concentrations (7- to 10-fold). The latter impressive increase in glucocorticoid secretion was not discernible when only the total serum cortisol concentration was measured. The discordance between total and free cortisol concentrations was best appreciated in patients with low plasma proteins (albumin $\leq$ 2.5 gm/dl). In fact, even though they had normally stimulated adrenal function, 39% of critically ill patients with low serum albumin had low serum total cortisol levels that would have been interpreted to be consistent with adrenal insufficiency. However, serum free cortisol levels were consistently and similarly increased in all of these patients, irrespective of their serum binding protein (transcortin and albumin) concentrations. Thus, serum free cortisol levels are very valuable in the assessment of adrenal function in critically ill patients, particularly those with hypoproteinemia. A very recent study investigating adrenal function in patients with sepsis and others with septic shock confirmed the superiority of serum free cortisol measurements in evaluating adrenal function (12). Although measurements of serum free cortisol

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Abbreviations: HPA, Hypothalamus-pituitary-adrenal; ICU, intensive care unit.

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levels are available at specialty and research laboratories, they are not commonly used in routine clinical care.

Several research groups have investigated the use of salivary cortisol concentration as a surrogate marker for serum free cortisol levels. Studies over the past 15 to 20 yr have demonstrated that cortisol in the saliva is in equilibrium with, and correlate with the free (unbound) fraction of the hormone in the circulation (13, 14). Salivary cortisol measurements are frequently used in evaluating patients suspected to have Cushing's syndrome (14–16). Other studies have demonstrated predictable responses in salivary cortisol concentrations after known stimuli of cortisol release such as stimulation with cosyntropin (14, 16–20), CRH, or insulin-induced hypoglycemia (15–17) as well as after its suppression with dexamethasone (14–16).

In the current investigation, we examined the value of measuring salivary cortisol concentrations as surrogate markers for serum free cortisol levels in critically ill patients. We postulated that salivary cortisol concentrations will be increased in the critically ill in parallel to the elevation in serum free cortisol levels.

Patients and Methods

Patient population and study design

Fifty-four consecutive, critically ill patients with various illnesses and who had an Acute Physiology and Chronic Health Evaluation III score (21) of 15 or greater (see below) were recruited for the study from the medical, surgical, or cardiac intensive care units (ICUs). Of the 54 enrolled patients, three intubated subjects were later excluded because their saliva samples were inadequate. Thus, 51 were included and completed the study. Although some patients were admitted directly to the ICU, most were initially admitted to a general medical or surgical ward and then transferred to the ICU when their condition required intensive care. The five patients recruited from the cardiac ICU were there because the medical ICU was full at the time of admission. The majority (39 of 51) of patients were recruited and tested during the first 24 h of admission to the ICU. Testing of the remaining 12 subjects was delayed for an additional 12–24 h to minimize interference with their medical care and/or obtain consent for the study. Patients were excluded if they had any of the following: hypothalamic-pituitary or adrenal disease, glucocorticoids or estrogen use over the preceding year, intake of medications known to influence glucocorticoid secretion (*e.g.* ketoconazole) in the preceding 6 months, or liver disease. Patients who were pregnant or breast-feeding were excluded. Also excluded were patients who had anemia (hematocrit $< 25\%$), oral candida infections, and any visible bleeding in the oral cavity as well as others who were received chlorhexidine to prevent ventilator-associated pneumonia.

Patients were divided into two groups based on their serum albumin concentrations at the time of testing (Table 1). This stratification was based on our earlier studies in critically ill patients (11, 22). These studies demonstrated the importance of determining serum albumin concentrations in interpreting data used in the assessment of adrenal function (11, 22). Whereas serum total cortisol determinations provided reliable data on adrenal function when the serum albumin levels were greater than 2.5 gm/dl, discordance between serum total and free cortisol were noted when the albumin levels were 2.5 gm/dl or less (11, 22). Thus, in the current investigation, critically ill subjects were divided into two groups: group 1 included patients with a serum albumin of 2.5 gm/dl or lower, and group 2 consisted of patients with a serum albumin of greater than 2.5 gm/dl.

The primary underlying disease processes were similar in the two groups. Of the 22 patients in group 1, five had sepsis/infection, five had cardiovascular illnesses, five had postoperative complications, four had respiratory distress, and three had gastrointestinal bleeding. Similar illness severity (Table 1) and distribution were noted in the 29 group 2 patients (seven had sepsis/infection; seven had cardiovascular illnesses; six had postoperative complications; five had respiratory distress; and four had gastrointestinal bleeding). The physiological components of the Acute Physiology and Chronic Health Evaluation III scoring system were used to calculate a severity of illness score. Because albumin was used to define the two patient groups, the severity of illness score was determined with and without this variable and was similar.

Patients were managed as clinically necessary by the respective ICU team. Laboratory assays for total cortisol, free cortisol, salivary cortisol, transcortin, and ACTH were performed at the conclusion of the study. Thus, the data were not available for the treating ICU physicians and therefore did not influence patients' management. None of the patients in the study received glucocorticoids during their stay in the ICU.

Similar determinations of baseline and stimulated cortisol levels as described below were performed in 31 healthy subjects without known illnesses and who were not taking any medications. The healthy subjects

TABLE 1. Characteristics of the study groups

	Healthy subjects (n = 31)	Group 1, albumin ≤ 2.5 (n = 22)	Group 2, albumin > 2.5 (n = 29
Age (yr)	52.7 ± 15.4	60.8 ± 13.1	58.6 ± 16.5
P values as compared with healthy subjects		0.07	0.11
Plasma ACTH (ng/liter)	22.1 ± 9.5	38.1 ± 11.2	35.7 ± 16.5
P values as compared with healthy subjects		<0.001	<0.001
Transcortin (mg/liter)	39.4 ± 4.0	27.3 ± 6.8	33.3 ± 11.6
P values as compared with healthy subjects		<0.001	0.04
P values between the two patient groups		0.03	
Serum albumin (g/dl)	3.9 ± 0.3	2.1 ± 0.4	3.3 ± 0.5
P values as compared with healthy subjects		<0.001	<0.001
P values between the two patient groups		<0.001	
Total serum protein (g/dl)	6.7 ± 0.3	4.5 ± 0.7	6.2 ± 1.0
P values as compared with healthy subjects		<0.001	<0.01
P values between the two patient groups		0.01	
Duration of hospitalization	N/A	11.1 ± 7.0	4.4 ± 4.7
Before testing (d)		0.01	
Number of patients intubated	N/A	9/22	12/29
Severity of illness score	N/A	60.6 ± 24.1	48.9 ± 22.2
Number of patients died/survived	N/A	6/22	7/29

Data are shown as mean ± SD. The *P* values for the comparisons between the two groups with each other or for the comparisons of each group of patients with healthy subjects are shown in the table. Age, ACTH levels, severity of illness scores, number of patients intubated, and the ratio of those who died/survived were similar in the two groups. To convert plasma ACTH concentrations from nanograms per liter to picomoles per liter, multiply by 0.2202. N/A, Not applicable.

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were matched to patients with respect to gender and age distribution (within a decade).

Measurements

Published data on normal cosyntropin-stimulated serum cortisol concentrations vary in different published series but are generally 18 μg/dl or greater (23, 24). Our laboratory defines normal as 18.5 μg/dl or greater (25).

Cosyntropin stimulation tests (250 μg, iv) were performed in both patient groups and healthy subjects between 1200 and 1400 h. Preliminary data on the timing of the peak salivary as well as serum total and free cortisol response to cosyntropin indicated that to be at or after 45 min. Thus, serum cortisol total and free cortisol as well as salivary cortisol concentrations were measured before and 45 and 60 min after cosyntropin administration. The plasma concentrations of ACTH as well as the serum levels of albumin and transcortin were determined before cosyntropin administration.

The institutional review board approved the study, and informed written consent was obtained from healthy subjects and patients or their legal guardians.

Laboratory analysis

Baseline plasma ACTH concentration was measured using immunoradiometric assay kits (Quest Diagnostics, San Juan Capistrano, CA). Intra- and interassay coefficients of variation determined at different ranges in the assays were under 4.5 and 5%, respectively (11). Serum cortisol measurements were performed using standard RIA (11, 25). Serum free cortisol concentrations were measured by equilibrium dialysis of undiluted serum sample for 18 h followed by RIA (11). The intraassay variation at different concentrations (1 and 3 μg/dl) within the latter assay was less than 5%, whereas the interassay variation for the same range was 11.8 and 6.8%, respectively. Measurements of serum free cortisol concentrations were performed by Quest Diagnostics at the end of the study. Serum transcortin concentrations were measured by RIA using kits purchased from BioSource Europe. S.A. (Nivelles, Belgium), with an intra- and interassay coefficient of variation of less than 3.9 and 5.5%, respectively, as determined at various concentrations.

On each occasion a saliva sample was obtained, a cotton tube was placed in the mouth and the subject was asked to chew on it for 2–3 min. Saliva samples were obtained from intubated patients by placing the cotton tubing in the mouth and moving it repeatedly for 2 min. The cotton tube was then placed in the saliva-collecting device (Salivette) and centrifuged to obtain the saliva sample. The latter sample was frozen for later analysis. Salivary cortisol concentrations were determined using an enzyme immunoassay kit obtained from Salimetrics Inc. (State College, PA). The interassay coefficient of variation over the range of low to high values varied from 5.7 to 6.8%, whereas the respective intraassay coefficients of variation were 3.2 and 6.3%. The cross-reactivity for cortisone in the salivary cortisol assay was 0.13%.

Statistical analysis

Data are presented as mean ± SD. The data from the two patient groups and controls were first analyzed using the Kruskal-Wallis test as a nonparametric alternative to ANOVA test. Comparisons between groups were done using the Wilcoxon rank sum test for nonparametric measurements. Categorical data were compared using χ^2 and Fisher exact tests. Differences were considered significant when the two-sided P values were less than 0.05. Bonferroni's correction for multiple comparisons was used as appropriate. The correlation between serum free and salivary cortisol was examined in each of the three groups separately. To evaluate the relationship between salivary and serum free cortisol concentrations, linear, quadratic, and logarithmic equations were tested using standard regression techniques. Regression lines were compared between groups with the use of analysis of covariance or the mixed-model approach. Data were analyzed using the SAS (Cary, NC) and SPSS (Chicago, IL) statistical programs.

Results

The two patient groups had similar clinical characteristics except for their respective serum albumin, total proteins, and transcortin concentrations and the duration of hospitalization (Table 1). Baseline serum cortisol concentrations measured in the early afternoon in these 51 critically ill patients varied (Fig. 1 and Table 2), widely ranging from 5.3 to 40.7 μg/dl (140.9–1082.2 nmol/liter). Mean baseline serum total cortisol concentrations in the two patient groups were higher than that of healthy subjects (Fig. 1 and Table 2). Although hypoproteinemic patients (group I) had lower baseline serum total cortisol concentrations, compared with those with near-normal serum protein (group 2), their levels were higher ($P = 0.03$) than those of healthy subjects. Cosyntropin-stimulated serum total cortisol levels in patients with near-normal serum protein concentrations (group 2) were higher ($P < 0.001$) than those of the hypoproteinemic subjects (group I) and also higher than those of healthy subjects ($P < 0.001$). The cosyntropin-stimulated serum total cortisol levels in hypoproteinemic patients were similar to those of healthy subjects but significantly lower than the levels observed in patients with near-normal serum protein (Table 2).

Baseline and cosyntropin-stimulated serum free cortisol levels in critically ill patients were severalfold higher than the respective values of healthy subjects (Table 2 and Fig. 2). The two patient groups had similarly increased baseline and cosyntropin-stimulated serum free cortisol concentrations (Table 2 and Fig. 2). The percent-free cortisol in healthy subjects was 8.0% at baseline and increased to 11.9% after cosyntropin stimulation. In contrast, the mean percent free cortisol in the critically ill subjects was approximately 25% at baseline and did not increase appreciably after cosyntropin stimulation (Table 2). However, and as predicted, hypoproteinemic critically ill subjects had higher circulating percent free cortisol than either healthy subjects or other critically ill patients with near-normal serum albumin concentrations (Table 2).

Baseline salivary cortisol concentrations in the critically ill were severalfold higher than those of healthy subjects (Table

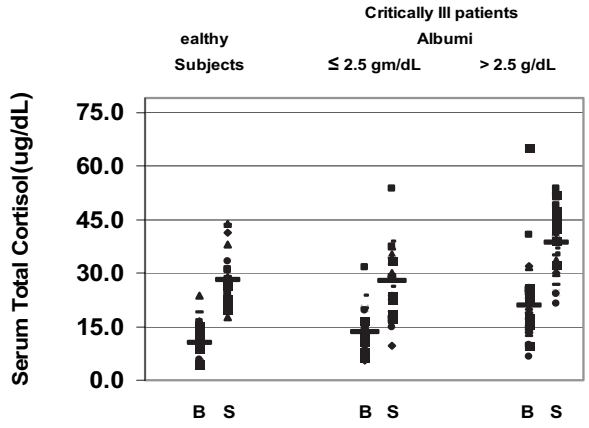

FIG. 1. Baseline and cosyntropin-stimulated serum total cortisol levels in healthy subjects and critically ill patients stratified according to their serum albumin concentrations (≤2.5 and >2.5 g/dl). In each of the three sets of data, the *left panel* represents the baseline (B) values, whereas the *right panel* represents the peak or stimulated (S) values for each group. The *horizontal lines* within each panel represent the mean for the respective data. To convert serum cortisol values from micrograms per deciliter to nanomoles per liter, multiply by 27.59.

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TABLE 2. Study groups divided according to their baseline serum albumin levels

	Healthy subjects (n = 31)	Critically ill patients	
		Group, 1 albumin ≤ 2.5 g/dl (n = 21)	Group 2, albumin > 2.5 g/dl (n = 29)
Baseline serum total cortisol (μg/dl)	8.1 ± 3.6	14.2 ± 6.7	21.1 ± 7.8
P values as compared with healthy subjects		0.011	<0.001
P values between the two patient groups		0.03	
Cosyntropin-stimulated serum total cortisol (μg/dl)	28.2 ± 6.0	27.8 ± 10.2	38.4 ± 9.5
P values as compared with healthy subjects		NS	<0.001
P values between the two patient groups		<0.001	
Baseline serum free cortisol	0.76 ± 0.40	3.72 ± 2.68	3.17 ± 1.75
P values as compared with healthy subjects		<0.001	<0.001
P values between the two patient groups		NS	
Cosyntropin-stimulated serum free cortisol (μg/dl)	3.29 ± 1.51	8.73 ± 5.27	8.18 ± 4.11
P values as compared with healthy subjects		<0.001	<0.001
P values between the two patient groups		NS	
Baseline salivary cortisol concentrations (μg/dl)	0.19 ± 0.11	1.47 ± 1.03	1.16 ± 0.89
P values as compared with healthy subjects		<0.001	<0.001
P values between the two patient groups		NS	
Cosyntropin-stimulated salivary cortisol concentrations (μg/dl)	1.49 ± 0.74	3.53 ± 1.75	3.84 ± 1.89
P values as compared with healthy subjects		<0.001	<0.001
P values between the two patient groups		NS	
Free/total cortisol at baseline (%)	8.0 ± 3.5	37.3 ± 26.8	14.2 ± 5.6
P values as compared with healthy subjects		<0.001	0.01
P values between the two patient groups		0.01	
After cosyntropin	11.9 ± 5.6	41.3 ± 23.3	21.7 ± 6.1
P values as compared with healthy subjects		<0.001	0.005
P values between the two patient groups		0.058	

To convert serum total, free cortisol, and salivary cortisol concentrations from micrograms per deciliter to nanomoles per liter, multiply by 27.59.

2 and Fig. 3). Similarly, the cosyntropin-stimulated salivary cortisol concentrations in the critically ill were higher than those of healthy subjects (Table 2 and Fig. 3). The baseline and cosyntropin-stimulated salivary cortisol concentrations were similar in the two groups of critically ill subjects (Table 2).

Subnormal cosyntropin-stimulated serum total cortisol concentrations (less than 18.5 μg/dl) were observed in five patients, all of whom had hypoproteinemia. Baseline and cosyntropin-stimulated serum free cortisol as well as salivary cortisol concentrations in these five patients were near or above the mean for the entire population of the critically ill subjects.

Baseline serum total cortisol levels in healthy subjects correlated with those of serum free cortisol ($r = 0.85$, $P < 0.001$), salivary cortisol ($r = 0.72$, $P < 0.001$), and serum transcortin ($r = 0.29$, $P < 0.05$) but not serum albumin concentrations. Baseline serum cortisol levels in the two patient groups cor-

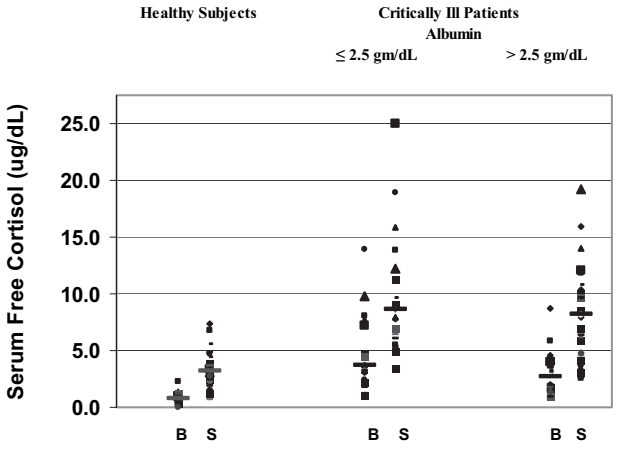

FIG. 2. Baseline and cosyntropin-stimulated serum free cortisol levels in healthy subjects and critically ill patients stratified according to their serum albumin concentrations (≤2.5 and >2.5 g/dl). In each of the three sets of data, the *left panel* represents the baseline values (B), whereas the *right panel* represents the peak or stimulated (S) values for each group. The *horizontal lines* within each panel represent the mean for the respective data. To convert serum free cortisol values from micrograms per deciliter to nanomoles per liter, multiply by 27.59.

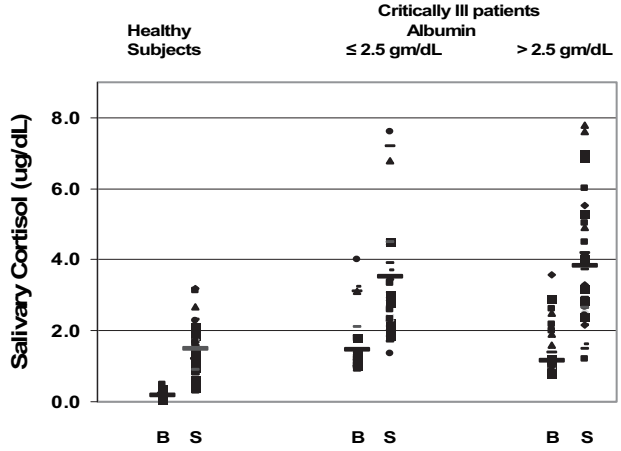

FIG. 3. Baseline and cosyntropin-stimulated salivary cortisol concentrations in healthy subjects and critically ill patients stratified according to their serum albumin levels (≤2.5 and >2.5 g/dl). In each of the three sets of data, the *left panel* represents the baseline (B) values, whereas the *right panel* represents the peak or stimulated (S) values for each group. The *horizontal lines* within each panel represent the mean for the respective data. To convert salivary cortisol concentrations from micrograms per deciliter to nanomoles per liter, multiply by 27.59.

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related with the respective serum free cortisol concentrations ($r = 0.33$, $P < 0.01$; $r = 0.55$, $P < 0.001$, respectively, for groups 1 and 2). The latter findings confirm our earlier findings in a similar group of critically ill patients (11). There was a strong correlation between serum free cortisol levels and the respective salivary cortisol concentrations. The latter was noted in each of the three groups. However, the relationship between salivary and serum free cortisol concentrations was different in each of the three groups. Although the relationship between salivary and serum free cortisol concentrations was linear in both the healthy subjects and critically ill patients with near-normal albumin, the regression lines for the relationship differed significantly ($P < 0.005$; Fig. 4, A and B). In contrast, the relationship between salivary and serum free cortisol concentrations in the hypoproteinemic, critically ill subjects was logarithmic (Fig. 4B).

Discussion

The data demonstrate that critically ill patients have an activated pituitary-adrenal axis characterized by elevated plasma ACTH concentrations, a 4- to 7-fold increase in serum free cortisol as well salivary cortisol concentrations. The degree of increase in glucocorticoid secretion could not be discerned from measurements of serum total cortisol concentrations. This was particularly evident in hypoproteinemic subjects. The latter observation confirmed our earlier report (11) and that of others (12), demonstrating the superiority of free cortisol measurements in determining glucocorticoid secretion during critical illness. Furthermore, the hypercortisolism during critical illness was not diagnosis specific, and it persisted in patients with prolonged severe illnesses.

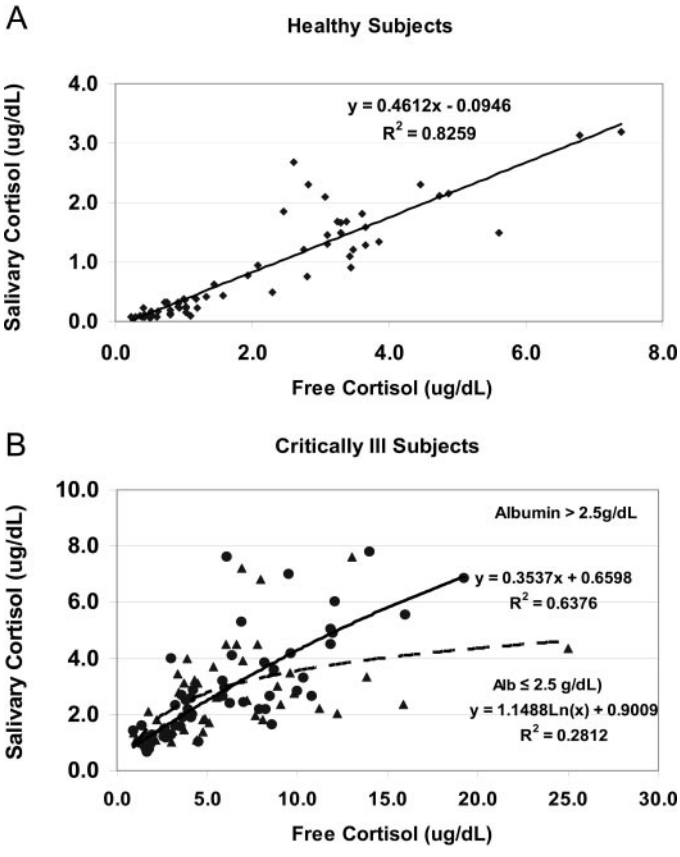

FIG. 4. A, Correlation between serum free cortisol and salivary cortisol concentrations in healthy subjects. B, Correlation between serum free cortisol and salivary cortisol concentrations in the two groups of critically ill patients. Data obtained in patients with an albumin of 2.5 g/dl or less are shown as *solid triangles*, whereas that of patients with and albumin greater than 2 g/dl are shown as *solid circles*.

Although the data on salivary cortisol concentrations in critically ill patients is limited by the number of subjects studied, we feel they, independently, demonstrate activation of the hypothalamus-pituitary-adrenal (HPA) axis. The salivary cortisol concentrations in these patients are severalfold higher than the respective levels in healthy subjects. None of the patients included in the study received glucocorticoids during their hospital stay, and the majority survived their critical illness. Importantly, the concentrations of salivary, serum total and free cortisol in the critically ill who survived were similar to those who did not. The free cortisol concentrations were severalfold higher than those of healthy subjects and similar to those reported in other critically ill subjects (11, 12). The total cortisol levels were also as elevated as reported in earlier studies (11, 12). Thus, even though the data are limited by the relatively small number of patients, they do indicate activation of the HPA axis. Additional studies involving a larger number of patients will need to be evaluated to establish the normal or appropriate levels during critical illness.

In this study, the mean baseline salivary cortisol concentration in healthy subjects was 0.19 μg/dl (5.1 nmol/liter) and was similar to that obtained in our laboratory in a larger sample ($n = 320$) of ambulatory healthy volunteers recruited for other studies. Published data on the normal afternoon salivary cortisol concentrations showed a similar range of 2–6 nmol/liter (14–16). The salivary cortisol concentrations observed herein in the critically ill population were severalfold higher than those of healthy subjects and even higher than those reported in most patients with established Cushing's syndrome (14–16). It would be interesting to obtain similar data on other groups of hospitalized patients who are not critically ill, even though adrenal function is not a common clinical concern in this setting.

The data demonstrate that salivary cortisol measurements are easy to obtain, even in the critically ill patients. The samples were inadequate in only three of the 54 patients. Earlier studies demonstrated that the concentration of cortisol in the saliva is not affected by the rate of saliva production (13). Furthermore, an increase in plasma-free cortisol level is reflected by a change in salivary cortisol concentration within a few minutes (13). Thus, obtaining a salivary sample over a 2- to 3-min period accurately reflects the circulating plasma levels of free cortisol at that time. It is important to point out that activity of the enzyme 11-β-hydroxy-steroid dehydrogenase was detected in the saliva by other investigators (13). Thus, some of the free cortisol entering the saliva can be converted into cortisone by that enzyme. There are no published data on the activity of the latter enzyme in critically ill subjects. However, *in vitro* studies have demonstrated that the activity of the enzyme can be altered by cytokines (26). Because critical illness is associated

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with increased cytokine production, it is possible that this might lead to alteration in the 11-β hydroxyl steroid dehydrogenase activity in the saliva. The physiological and clinical impacts of possible alteration in enzyme activity are not known.

Whereas most of the published data on salivary cortisol concentrations involved patients with Cushing's syndrome and psychiatric illnesses, some data are available on the use of salivary cortisol in defining normal adrenal function (13–20). Some studies emphasized the usefulness of this determination in women with elevated transcortin concentrations (13–20). However, only limited data are available on salivary cortisol concentrations in the critically ill (27). To the best of our knowledge, and with the exception of the limited data published by Cohen *et al.* (27), this is the first study demonstrating the use of salivary cortisol measurements in the setting of critical illness. In the latter brief communication, Cohen *et al.* (27) reported that only 12 of 30 samples obtained from 10 patients with severe sepsis contained enough saliva volume to determine cortisol concentrations. Interestingly, however, the mean baseline salivary cortisol concentration in their limited data (34.6 nmol/liter) was remarkably similar to that obtained in our population of critically ill subjects (36.1 nmol/liter). The authors used a surgical stitch to obtain the saliva in unconscious patients. It is not clear whether the latter technique contributed to poor sampling reported by the authors. There are obvious limitations for using salivary cortisol measurements in the critically ill population. Such limitations include mouth dryness (leading to poor specimen yield) and bacterial or candida infection (likely leading to underestimation of the concentration). Bleeding in the oral cavity can also be another limitation because it would lead to serum contamination. In this study, we excluded patients with oral bleeding and others with oral candidiasis. However, we did not perform bacterial cultures on the saliva to determine whether there was any clinically unrecognized bacterial infection that might have resulted in underestimating salivary cortisol concentration. Despite these limitations, we were able to obtain adequate samples in the majority of critically ill subjects including those who were intubated. Additional studies involving a large number of patients need to be conducted to assess the feasibility of using the salivary cortisol concentrations in defining the integrity of HPA axis in critically ill subjects.

We found a strong correlation between the concentrations of the two measures of unbound cortisol: the serum free and salivary cortisol concentrations. However, the relationship between salivary and serum free cortisol concentrations was significantly different among the three groups studied. The relationship between salivary and serum free cortisol concentrations was linear in healthy subjects and also the critically ill patients with near-normal protein levels. However, the slopes and intercepts of the regression equations in the latter two groups were significantly different. In contrast, the relationship between salivary and serum free cortisol concentrations in the hypoproteinemic critically ill patients was logarithmic, and the regression equation was different from the other two groups. It is not clear at this point why the relationship between salivary and serum free cortisol should be different in the two groups of patients with critical illness. It is important to emphasize that the difference between the groups of critically ill subjects becomes evident at high serum free cortisol levels (>10 μg/dl or 27.6 nmol/liter). Because only a few points were included in the curve beyond a free cortisol concentration of 10 μg/dl (27.6 nmol/liter), it would be difficult to discern whether this was a true trend or a sampling error. Additional studies including a larger number of patients will be necessary to investigate this issue more accurately. It is important to emphasize that within the range of serum free cortisol concentrations frequently observed during critical illness (2–10 μg/dl or 55.2–276 nmol/liter), the relationship between salivary and serum free cortisol was similar in the two groups of patients. It is also worthwhile emphasizing the fact that the baseline salivary cortisol concentrations observed in either of the two groups of critically ill patients was clearly elevated to levels near those seen after cosyntropin stimulation in healthy subjects.

Biochemical assessment of adrenal function during critical illness has depended primarily on the baseline and/or the standard cosyntropin stimulated serum total cortisol concentrations (28). Only limited data are available on the use of low-dose cosyntropin stimulation tests in the critically ill (29–31). Despite the known limitations of the standard dose cosyntropin test (22, 25, 32), it continues to be the most commonly used test in the assessment of adrenal function in this setting. It is, however, important to emphasize that the test be interpreted in the context of critical illness. In that respect, the current investigation confirms our previous finding (11, 22) as well as that of others (12), demonstrating that critically ill subjects have higher cosyntropin-stimulated serum total and free cortisol levels than those of healthy subjects of similar age and gender. In the current investigation, we also demonstrated the same finding when salivary cortisol was used as a marker of adrenal response. These findings call into question the common use of arbitrary cutoff points that were based on data from normal subjects in defining the adequacy of adrenal function.

In summary, the current investigation demonstrated that salivary cortisol concentrations are increased in critically ill subjects irrespective of their serum protein levels. The data also showed that the rise in salivary concentrations was paralleled by a concordant increase in serum free cortisol levels. The concentrations of the two measures of unbound cortisol determined in two different body fluids correlated very well, regardless of the serum protein concentrations. In contrast, serum total cortisol levels in hypoproteinemic patients (group 1) were lower than those with similar illnesses but whose binding proteins were near normal (group 2), even though both groups of critically ill subjects had similarly elevated serum free and salivary cortisol concentrations. Our current study confirms previous data suggesting that measuring baseline or ACTH-stimulated serum total cortisol levels in critically ill patients with hypoproteinemia (serum albumin under 2.5 gm/dl) can be misleading if criteria for adrenal insufficiency are based on levels from healthy subjects with normal serum binding proteins. In contrast, baseline and stimulated serum free cortisol as well as salivary cortisol concentrations in critically ill hypoproteinemic patients were not different from the levels observed in patients with near-normal serum albumin levels.

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