Value of Free Cortisol Measurement in Systemic Infection

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- septic shock
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Abstract

Systemic infection induces an increase in plasma cortisol which accords approximately with illness severity. However, both basal and synthetic ACTH stimulated cortisol levels are not strong predictors of mortality. Moreover, plasma cortisol levels do not readily define those patients who have been clinically observed to respond, with respect to blood pressure elevation, to exogenous hydrocortisone. It is likely that free cortisol, accounting for 6–20 % of circulating total (bound plus free) cortisol has most of the life-saving effects on circulation and metabolism in severe sepsis, as corticosteroid-binding globulin bound and albumin-bound cortisol have reduced access to tissues. In addition, sepsis reduces CBG and albumin levels, hence blunting the effect of increasing illness severity on total cortisol. Our recent studies suggest that free cortisol correlates more closely to sepsis severity than total cortisol and that free cortisol levels can be estimated using the plasma CBG and total cortisol, obviating the need for direct free cortisol measurement. Studies directed at determining if free cortisol is a better guide than total cortisol to the need for hydrocortisone supplementation may be of value.

Introduction

Septic shock, defined as sepsis from a microbial infection resulting in a systemic inflammatory response with severe hypotension, has a mortality of approximately 50 % despite treatment with antimicrobials, catecholaminergic vasopressors and ventilatory and other supportive measures [1]. Sepsis and septic shock represent a severe stress, or threat to homeostasis, and are accompanied by marked activation of the hypothalamic-pituitary-adrenal axis with cortisol production rates increasing approximately 10-fold. Cortisol is crucial in survival from systemic infection through its roles in re-directing and restraining immune function and elevating blood pressure through augmentation of the effect of catecholamines [2]. Cortisol elevation is thought to occur in large part through elevation of inflammatory cytokines which act predominantly at the hypothalamic-pituitary unit to increase cortisol secretion [2].

Given the role of cortisol in elevating blood pressure and anecdotal reports of benefit of cortisol supplementation in septic shock, and well known states of cortisol deficiency in sepsis such as the Waterhouse–Friedrichsen syndrome of adrenal hemorrhage and infarction first reported in children with meningococcal infection [3], it was not surprising that glucocorticoid supplementation was trialed to improve outcome in septic shock. Early attempts to supplement glucocorticoid were based on high dose, short term administration of synthetic glucocorticoids up to 40,000 mg hydrocortisone per day equivalent. Randomized controlled trials and meta-analyses showed no benefit from this therapy [4, 5]. In contrast, since 1997 septic shock studies of "low-dose" hydrocortisone (200–300 mg/day), designed to match maximum cortisol secretion given over a longer duration (up to 7 days) were effective in hastening vasopressor withdrawal and reducing mortality [6, 7].

Basal cortisol levels are relatively poor predictors of mortality in septic shock [8]. However, benefits from intravenous hydrocortisone in septic shock were only seen in those with a reduced total plasma cortisol increment after ACTH injection of ≤ 248 nmol/l [8, 9]. This cortisol increment cut-off was derived from earlier studies showing that a low cortisol increment after ACTH in septic shock patients was associated with an increased

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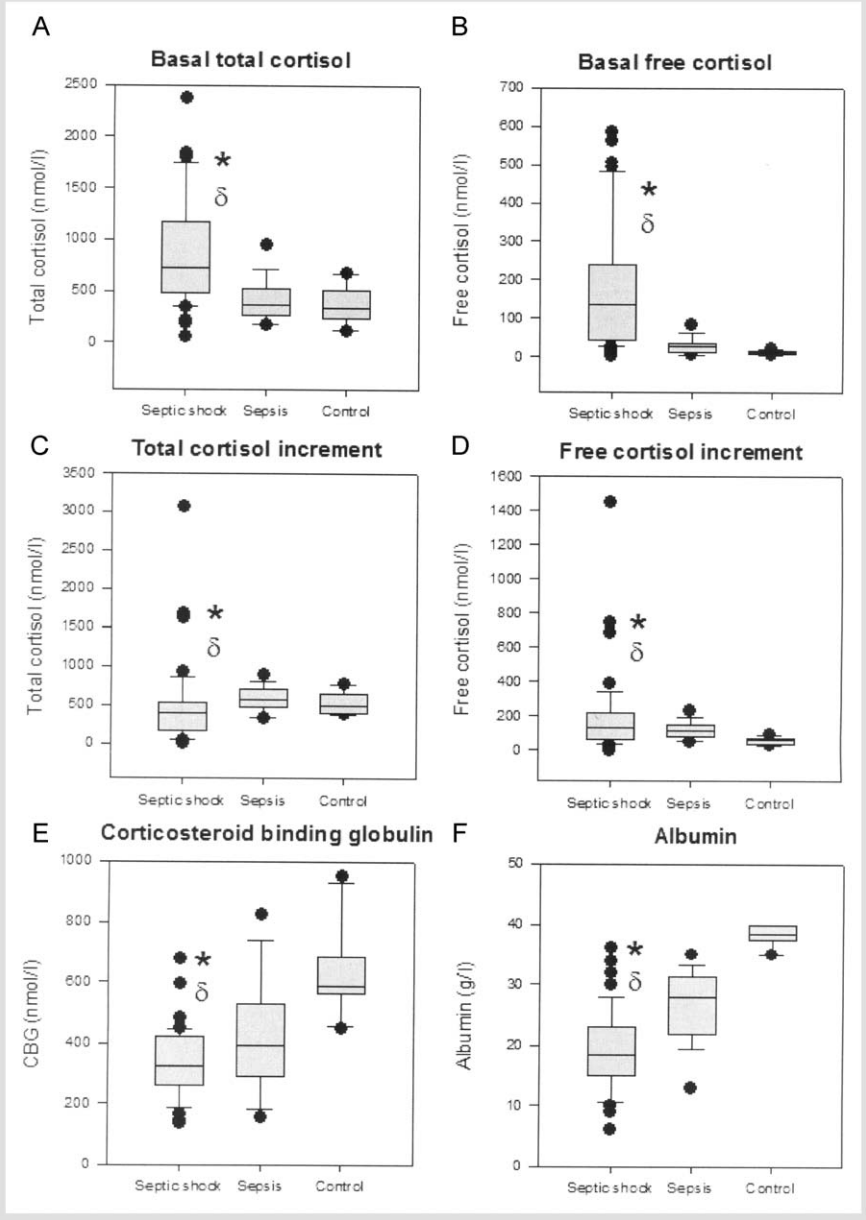

Fig. 1 Basal total and free plasma cortisol, and cortisol increments after tetracosactrin (ACTH 1–24), CBG and albumin levels in patients with patients with septic shock (n = 45), sepsis (n = 19) and in healthy controls (n = 10). The plots represent median, 25th and 75th percentiles as vertical boxes with error bars (10th and 90th percentiles); * denotes $p < 0.05$ for septic shock *vs.* sepsis and for septic shock *vs.* control. The basal total (Panel **A**) and free cortisol concentrations (Panel **B**) were significantly higher in the septic shock group than in the sepsis and control groups. Free cortisol increments (Panel **D**) corresponded more to sepsis and its severity than total cortisol increments (Panel **C**). Corticosteroid-binding globulin (CBG) and plasma albumin levels (Panel **E** and **F**) fell significantly with illness severity.

mortality and an enhanced effect of hydrocortisone on the pressor effect of noradrenaline [10–12].

Relative adrenal insufficiency (RAI) is conceptually defined as an inadequate cortisol response to severe illness associated with rapid clinical and hemodynamic improvement following stress-dose glucocorticoid therapy [10, 11, 13–15]. Empirically, RAI is defined by a total cortisol increment of 248 nmol/l or less, using current most validated criterion [11]. The RAI concept and particularly the definition based on synacthen response has been widely criticized, since it may be reasoned that a low cortisol response to synacthen may represent a circumstance where additional ACTH stimulation has no effect since maximum cortisol secretion for that individual has been reached. An alternative explanation for the responses to exogenous glucocorticoid seen in individuals with low responses to exogenous ACTH may be that of tissue resistance to cortisol, where a state of high basal cortisol and reduced responsiveness to ACTH may select that group of individuals who have reached maximum cortisol output but require still more cortisol to overcome cytokine-induced cortisol resistance [16, 17].

Free cortisol in sepsis/septic shock

Our studies were directed towards the investigation of free cortisol measurements in sepsis and septic shock. Cortisol circulates in three fractions, 80–90% is bound to corticosteroid-binding globulin (CBG), 5–10% is bound to albumin and 5–7% is free. In systemic infection CBG and albumin levels may fall by as much as 50% within 24 hours. CBG levels fall due to cytokine induced inhibition of hepatic synthesis and catabolism at inflammatory sites, where CBG may play a role in transporting cortisol to inflammatory cells [13, 14]. A recent study of critically ill patients suggested that low cortisol binding proteins, rather than low free cortisol levels, may underlie reports of reduced total cortisol in critical illness [15].

Our aims were to compare total and free plasma cortisol levels (basal and ACTH stimulated) in patients with septic shock, sepsis and in controls, in those who meet the current operational definition of RAI and non-RAI. In addition, we evaluated the use of the Coolens' equation [18], which estimates free cortisol from CBG and total cortisol levels. We also determined the rate of recovery from the operationally defined RAI since this may

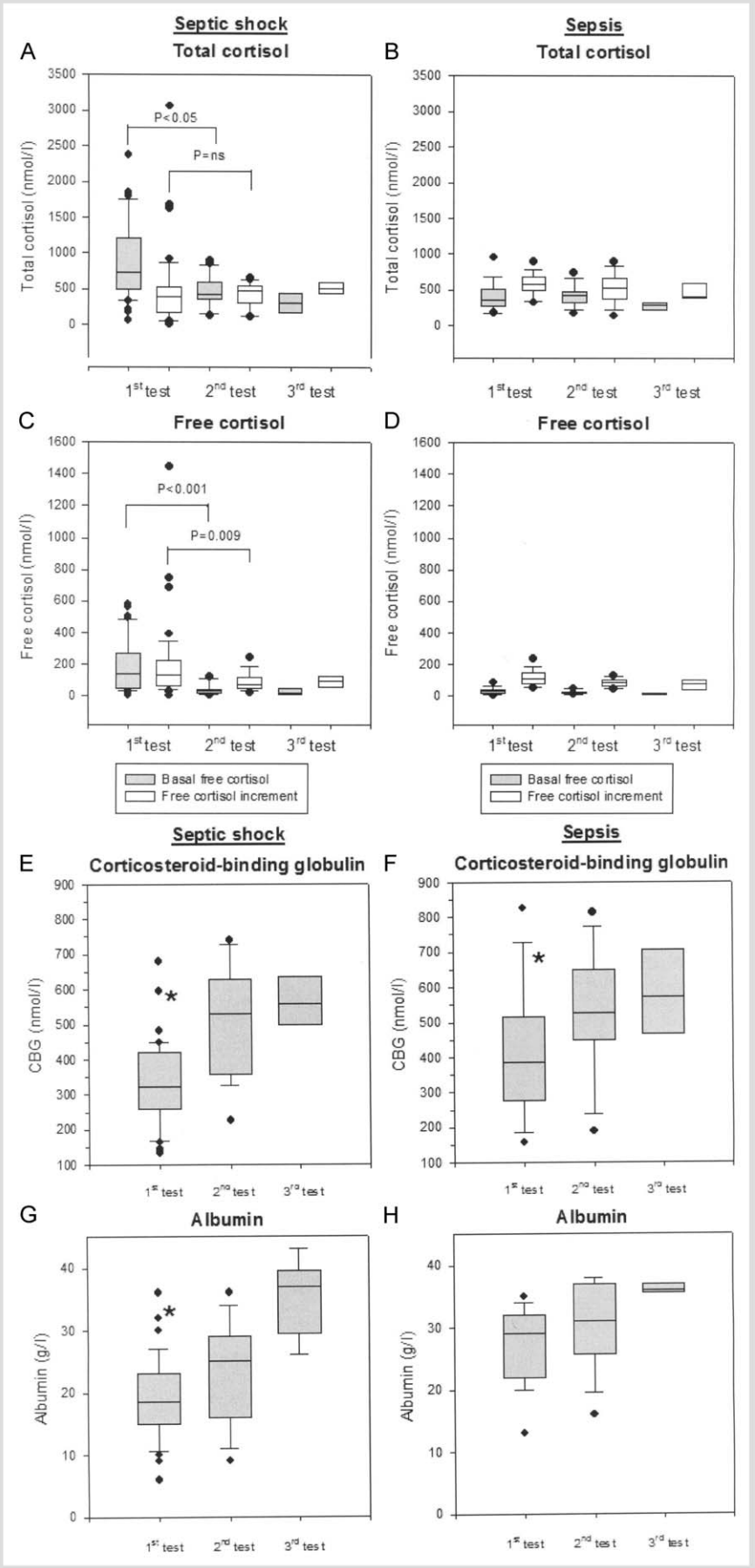

Fig. 2 Cortisol (total and free), CBG and albumin levels from serial tetracosactrin stimulation tests in septic shock patients. Results are shown as median, 25th and 75th percentiles with standard errors; *denotes $p<0.05$ for SS *vs.* S *vs.* HC. Tests were performed at the following times: 1st test: at the time of illness, 2nd test: immediately prior to hospital discharge and 3rd test: 6–12 weeks after discharge. Twenty SS and 10 S patients had the second test and 14 SS and 8 S patients had the third test. In the septic shock group basal total cortisol fell on the second test but free cortisol fell to baseline. Also, total cortisol increments after tetracosactrin did not change by the second test but the free cortisol had normalized. Differences in total and free cortisol in the sepsis group were smaller.

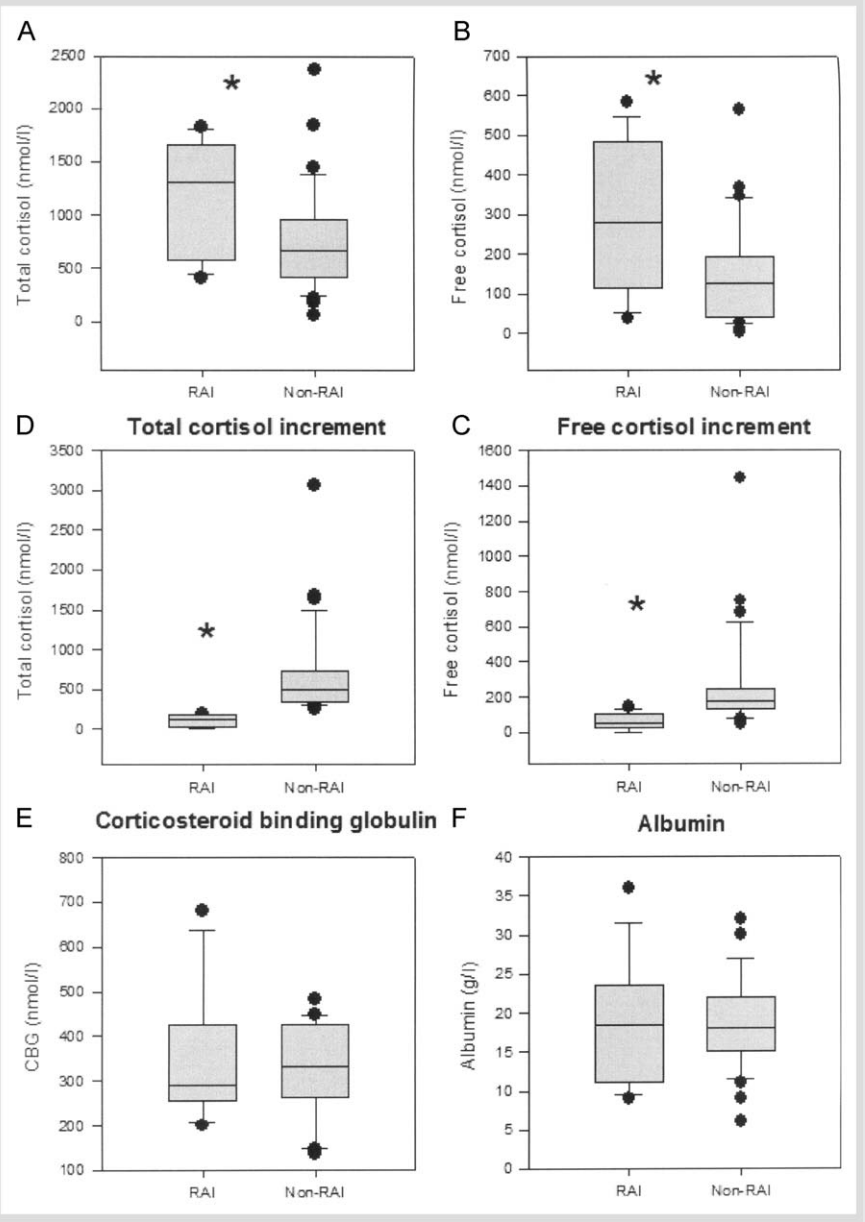

Fig. 3 Basal and tetracosactrin-stimulated total and free cortisol levels in septic shock patients with RAI (n = 15) and without RAI (n = 30). The plots represent median, 25th and 75th percentiles as vertical boxes with error bars (10th and 90th percentiles); *denotes *p*&lt0.05 for RAI *vs.* non-RAI. Patients with RAI had significantly higher basal total cortisol (Panel **A**) and free cortisol levels (Panel **B**), and lower total and free cortisol increment post tetracosactrin stimulation (Panel **C** and **D**). There was no difference in corticosteroid-binding globulin (CBG) and plasma albumin levels between the groups.

throw light on the question of structural *vs.* functional impairment of adrenocortical function in septic shock. Measurement techniques included standard commercial immunoassay for total plasma cortisol, an ultracentrifugation method for free cortisol, validated against the gold standard of equilibrium dialysis, and an in-house monoclonal ELISA for CBG, as described elsewhere [19].

Our results in 45 patients with septic shock, 19 with sepsis and 10 controls, suggested that free cortisol is a better guide to circulating glucocorticoid activity than total cortisol [19]. In support of this proposition we found that (1) ACTH stimulated free cortisol increments after ACTH were higher with greater illness severity than total cortisol increments (**Fig. 1**), and (2) free cortisol increments normalized more promptly after recovery in survivors than total cortisol increments (**Fig. 2**). Prompt and universal recovery from RAI in our studies suggests a functional defect rather than adrenocortical structural damage underlying the basis of RAI. There were no cases of RAI among the sepsis group but a 33% incidence in the septic shock group suggesting the presence of RAI is related to illness severity (**Fig. 3**). We also found that calculated free cortisol calculated by Coolens' method corresponds well with measured free cortisol by ultracentrifugation (**Fig. 4**).

Implications for future studies

The implications of our findings are that free cortisol is likely to better reflect true cortisolemia due to its closer correlation to illness severity. This is not surprising as illness tends to suppress plasma CBG and albumin and at the same time elevate cortisol secretion. The effect of illness on cortisol binding proteins tends to limit the extent of increase in total cortisol, but accentuate the increase in free cortisol. Our study supports Hamrahian's findings that free cortisol provides a better guide to circulating cortisolaemia than total cortisol in critical illness. Currently however, free cortisol measurement is cumbersome and expensive as it is non-automated and labor intensive. Our data, however, suggest that the Coolens' equation which requires total cortisol and CBG measurements which could both be performed

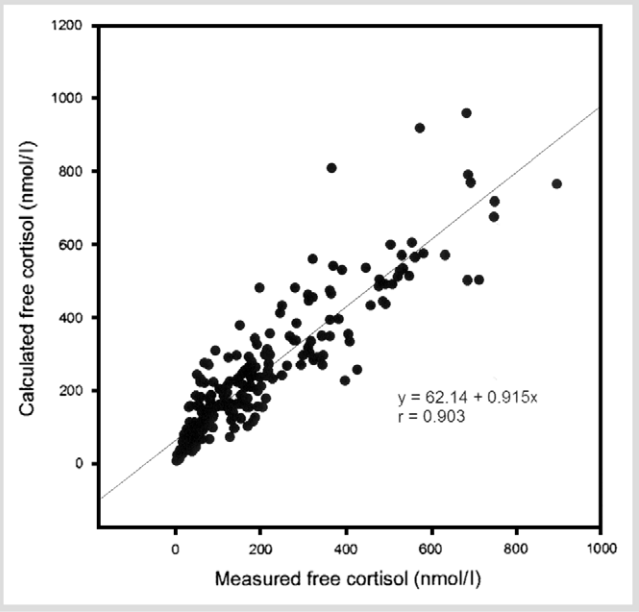

Fig. 4 Plasma free cortisol levels as measured by ultrafiltration method, validated by equilibrium dialysis, are compared with estimated free cortisol using the Coolens' equation: $U^2 X K(1+N)+U[1+N+K(G-T)]-T=0$, where T is cortisol, G is CBG, U is unbound cortisol, and K is the affinity for cortisol at 37C. Measured and calculated free cortisol levels correlate well.

by modern platform immunoassays, will allow reliable estimates of free cortisol in critical illness.

Hence, estimation of free cortisol in critically ill patients may lead to a better separation of patients into those that will and will not benefit from hydrocortisone therapy. This proposition, however, requires testing in a study large enough to examine patient outcomes and the effects of hydrocortisone treatment. This is important, as although published studies have not shown a negative effect of stress-dose hydrocortisone, they were not designed to show detrimental effects such as increased muscle atrophy and delayed rehabilitation and may not have been able to detect subtle hyperglycemia and hypokalemia, both of which may worsen outcome.

In this context it is relevant that current suggested stress-dose hydrocortisone regimens produce hypercortisolism in most individuals. For example, one regimen of 10 mg/hour hydrocortisone results in circulating mean total cortisol levels of around 3100 nmol/l which is well above the range we observed in 45 septic shock patients (95 % CI 500–1700 nmol/l) [19, 20]. Another potential problem with current stress-dose hydrocortisone regimens is that they are given on a "one-size-fits-all" basis. It is very likely that cortisol levels, if they do need to be increased at all in individual patients, may need to be increased to varying extents depending on individual illness and constitutional factors. Given that cortisol levels after hydrocortisone administration do not reflect underlying levels of inflammation-induced glucocorticoid resistance, it is likely that trials of hydrocortisone therapy will produce conflicting results until a suitable tissue marker of glucocorticoid activity can be found that may comprise a target for glucocorticoid therapy in individual patients.

Possible candidate tissue markers include markers of glucocorticoid receptor activity or more distal systems affected by glucocorticoids such as pro-inflammatory cytokine levels or nitric oxide which are elevated in systemic infection and can be suppressed by administered hydrocortisone [20]. Mean nitric oxide (NOx) levels are elevated 20-fold in septic shock and can be suppressed by intravenous hydrocortisone [20]. However, little is known about the stability of the NOx measurement within patients, or interindividual variability independent of sepsis severity. Factors such as these will be critical in assessing the potential value of NOx as a target for hydrocortisone therapy in septic shock. Pending results of the CORTICUS multicenter trial of hydrocortisone therapy in septic shock, there is a need to consider studies to address tissue markers of glucocorticoid sufficiency that may allow hydrocortisone therapy to be individualized in terms of application and dosage. In addition, a recent study suggesting that septic shock may be prevented in severe pneumonia suggests that glucocorticoids may be more effective in preventing than treating septic shock [21].

Conclusion

There remain many unanswered questions regarding hydrocortisone therapy in systemic infection. Currently trialed therapies remain relatively high-dose and have the potential to cause harm. Further, our capacity to individualize treatment using total cortisol measures seems flawed in concept and of limited proven advantage. Free cortisol may help with patient selection but are unlikely to overcome the problem of high variation in individual maximal cortisol secretory output and tissue resistance through a combination of genetic and acquired factors. Hydrocortisone therapy may be better targeted if a practical marker of glucocorticoid sensitivity could be used in combination with the best available measure of circulating cortisolemia which may be stimulated free cortisol.

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