

## Sick adrenal or sick eoadrenal?

Bala Venkatesh and Jeremy Cohen

The annual incidence of septic shock in the adult Australian population is 0.77 per 1000, corresponding to 15 700 new cases each year.<sup>1</sup> Frustratingly, despite significant investment of resources, mortality is still about 37%. A topic that has been widely investigated, without clear consensus, is the role of glucocorticoid supplementation in reducing mortality in septic shock. The uncertainty in this area arises from the inability of current tests to clearly identify who is truly corticosteroid "deficient" at a cellular level, and hence requires administration of supplemental glucocorticoids. Here, we propose that the changes in adrenocortical function observed in sepsis may not reflect a sick adrenal gland but a "sick eoadrenal syndrome".

### Glucocorticoid physiology

Activation of the hypothalamic-pituitary-adrenal axis is an essential part of the host response to critical illness and results in an increase in plasma cortisol concentrations in most patients with sepsis or septic shock. Most circulating cortisol is bound to corticosteroid-binding globulin (CBG).<sup>2</sup> At normal levels of total plasma cortisol (375 nmol/L [13.5 µg/dL]), less than 5% exists as free cortisol in the plasma; however, it is this free fraction that is biologically active. In healthy subjects, CBG can bind about 700 nmol/L cortisol (25 µg/dL). At higher concentrations, the increase in plasma cortisol is largely in the unbound fraction. CBG is a substrate for a polymorphonuclear enzyme, elastase, which cleaves CBG, markedly decreasing its affinity for cortisol.<sup>3</sup> This results in the liberation of free cortisol at sites of inflammation. CBG levels have been documented to fall during critical illness,<sup>4,5</sup> and these changes are postulated to increase the amount of circulating free cortisol. However, currently available routine assays measure only total cortisol levels.

Cortisol passes through cell membranes to bind with glucocorticoid receptor, a cytoplasmic protein found in nearly all nucleated cells. Once the receptor has bound with cortisol, its ligand-binding domain undergoes a conformational change, shedding heat shock factors, and migrating to the nucleus, where it binds to glucocorticoid response elements and influences gene transcription.<sup>6,7</sup> The primary effects of cortisol on the immune system are anti-inflammatory and immunosuppressive.<sup>8</sup> The production of cytokines is inhibited, an effect that is mediated by nuclear factor κB (NF-κB).<sup>9</sup> This occurs through both induction of NF-κB inhibitor and direct binding of cortisol to NF-κB, thus

### ABSTRACT

The role of glucocorticoid supplementation in septic shock remains contentious. In septic shock, the driver for steroid therapy is the premise that there is relative adrenal insufficiency (based on reduced plasma cortisol and blunted cortisol response to corticotropin). The uncertainty arises from the inability of current tests to clearly identify patients who are truly corticosteroid "deficient" at a cellular level, and hence require supplemental glucocorticoid administration. We hypothesise that plasma measurements (total plasma cortisol level and the response to corticotropin) do not consistently reflect the functional adrenal response to stress. Published evidence indicates that there are cellular adaptations in stress, such as pre-receptor upregulation of cortisol, altered receptor density and gene transcription changes, none of which are reflected by plasma cortisol level. This leads us to postulate that the lack of a clearly defined plasma response in severe stress and the presence of an adequate response at the cellular level suggest it is a "sick eoadrenal state", analogous to the sick euthyroid state, and not a sick adrenal indicating adrenal insufficiency.

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preventing its translocation to the nucleus. However, once cortisol is inside the cell, its concentration is profoundly affected by the activity of the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme system.<sup>10,11</sup> This system has two isozymes that have been extensively studied: 11β-HSD1 acts in vivo primarily as a reductase, generating active cortisol from inactive cortisone; in contrast, 11β-HSD2 has dehydrogenase action, inactivating cortisol by conversion to cortisone. The 11β-HSD2 enzyme is found primarily in mineralocorticoid target tissues, such as kidney, sweat glands and colonic mucosa, where it prevents illicit activation of the mineralocorticoid receptor by cortisol. The 11β-HSD1 enzyme has a wide distribution, including liver, adipose and vascular tissues and, although it is primarily a reductase, there is evidence its directionality may be tissue specific. The system is able to regulate the intracellular glucocorticoid concentration irrespective of the circulating concentration, thus rendering circulating glucocorticoid levels highly problematic as an indicator of tissue glucocorticoid action.

In septic shock, the driver for steroid therapy is the premise of relative adrenal insufficiency (RAI) — based on reduced plasma cortisol and a blunted cortisol response to corticotropin. The concept of RAI is not new; it was proposed in 1940 by Selye et al.<sup>12</sup> However, the idea has gained momentum in the past decade following publications by several groups that showed evidence of adrenal insufficiency from plasma testing.

Below, we provide data that refute the concept that plasma measurements (total plasma cortisol and the response to corticotropin) are reliable indicators of the functional adrenal response to stress. The concept of RAI was based on the premise of the "cortisol cascade": that is, changes in plasma cortisol reflect those in the interstitium, which in turn reflect the functional adrenal response at the cellular level.

We hypothesise that plasma measurements (total plasma cortisol and the response to corticotropin):

- do not consistently reflect severity of stress in septic shock; and
- do not consistently reflect the functional adrenal response to stress.

As part of this evidence base, we introduce the concept of "cortisol cascade failure", whereby we demonstrate a dissociation between:

- total versus free plasma cortisol;
- total and free plasma cortisol versus interstitial cortisol;
- total plasma cortisol versus pre-receptor changes; and
- plasma response versus functional response of the tissues to stress.

### Plasma measurements do not consistently reflect severity of stress in septic shock

The use of total plasma cortisol and the response to corticotropin to diagnose adrenal insufficiency is a time-honoured approach. The diagnosis of RAI is based on reduced total plasma cortisol and blunted cortisol response to corticotropin in septic shock. However, in the critically ill, each of these criteria has significant limitations.

#### Variability of total plasma cortisol

Studies have demonstrated a wide range of elevated total plasma cortisol levels in stressed ICU patients (400–1400 nmol/L)<sup>13–24</sup> compared with healthy volunteers (200–450 nmol/L), making it impossible to define a normal reference range for the critically ill. Critically ill patients display a wide range of responses, the nature of which is determined by several factors:

**Plasma cortisol rhythm.** The typical circadian rhythm of plasma cortisol level seen in healthy volunteers is absent in critically ill patients. Our study of hourly cortisol measure-

ments in 20 patients with septic shock showed that total plasma cortisol level varies so markedly that a random value has limited diagnostic utility.<sup>18</sup> The individual mean plasma cortisol concentrations ranged from 286 nmol/L (SD, 59 nmol/L) to 796 nmol/L (SD, 83 nmol/L), with marked hourly variability (coefficient of variation, 8%–30%). There was no correlation between total plasma cortisol level and outcome.

**Sex-based differences in response.** It is well recognised that adrenocortical responses to stress differ between males and females,<sup>25</sup> and between premenopausal and postmenopausal women.<sup>26</sup> In the various septic shock studies, the male to female ratio has varied from 0.8 to 3.4. This sex-based difference in response adds another layer of complexity to understanding the adrenocortical function during stress.

**Time of assessment.** A review of the published literature shows that patients have been studied from as early as 8 hours after the onset of shock until as late as 61 days after illness,<sup>27</sup> with their data pooled to define the optimal adrenal response to critical illness. However, the former time defines the acute adrenal response, while the latter identifies the response during chronic critical illness. In other studies, the time from admission to adrenal assessment was not clearly stated.<sup>28</sup>

**Significant variability between cortisol assays.** Cohen et al<sup>19</sup> were the first to report that there is a high degree of variability between cortisol assays, which may potentially confound the diagnosis of RAI, resulting in erroneous labelling of an individual's adrenal status.

### Use of the corticotropin stimulation test for diagnosing RAI

Criticisms of the corticotropin stimulation test include:

- lack of consensus on the appropriate change in cortisol after corticotropin stimulation that indicates RAI — 250 nmol/L or 400 nmol/L,<sup>20–24</sup> and
- lack of consensus on the dose of corticotropin to be used — 1 µg or 250 µg.<sup>29</sup>

In the face of so many confounding variables, attempts to develop robust criteria for the diagnosis of RAI based solely on baseline plasma total cortisol and results of the corticotropin stimulation test have proven unsuccessful.

### Cortisol cascade breakdown

#### Breakdown between total and plasma free cortisol

Plasma free cortisol (PFC) provides a better assessment of adrenal function than total cortisol because:

- PFC is the biologically active hormone;
- CBG and albumin levels decrease in critical illness, leading to increases in PFC;<sup>4,5</sup>

**Table 1. Pilot study of HSD1 RNA expression in a rat model of septic shock (mean [SD])**

	CLP group	Control group	P
Liver	28.4 (19.5)	6 (3)	<0.001
Adipose tissue	4.8 (2.6)	2.9 (2.5)	<0.01

CLP = caecal ligation and perforation model of septic shock.

- PFC increments correspond to severity of illness in septic shock, whereas total cortisol does not;<sup>30</sup> and
- PFC concentrations cannot be predicted from total cortisol because of the non-linear relationship.

Hamrahian et al clearly outlined the limitations of total cortisol and the superiority of PFC for assessing adrenal insufficiency in a heterogeneous cohort of critically ill patients.<sup>5</sup>

Beishuizen et al<sup>4</sup> provided evidence of a strong relationship between the inflammatory response in septic shock and PFC. They showed marked elevation of PFC in a group with sepsis compared with the control group. They also showed a strong inverse correlation of CBG (and therefore by extrapolation, direct correlation of PFC) with interleukin-6 in sepsis. Evidence of the ability of PFC to predict outcome as compared with total cortisol was also shown in a study of meningococcal sepsis in children:<sup>31</sup> those who died had lower levels of PFC than those who survived shock and sepsis. Total cortisol level had no significant predictive value.

#### Breakdown between plasma and interstitial cortisol

Cohen and colleagues measured interstitial cortisol in 13 subjects (10 patients with burns and three volunteers) using microdialysis: mean total plasma cortisol and PFC concentrations ( $\pm$  SD) were  $8.8 \pm 3.9$  and  $1.7 \pm 1.1$   $\mu\text{g/dL}$ , respectively ( $P < 0.001$ ) (unpublished observations). Mean cortisol concentrations ( $\pm$ SD) measured by microdialysis in tissue of the burn (MDB) and non-burn groups were  $0.80 \pm 0.31$  v  $0.74 \pm 0.41$   $\mu\text{g/dL}$ , respectively ( $P = 0.8$ ). Similarly, the correlation between PFC and MDB was poor ( $r = 0.2$ ). A fifth of the MDB concentrations were higher than the corresponding PFC values, highlighting the limited reliability of plasma measurements as an indicator of tissue values.

#### Breakdown between plasma and pre-receptor cortisol

As noted, there is pre-receptor regulation of intracellular glucocorticoid concentration through the  $11\beta$ -HSD 1 system. This enzyme locally regenerates active cortisol from inactive cortisone, amplifying glucocorticoid-receptor activation in the context of normal circulating plasma cortisol levels. Cortisol to cortisone ratios provide evidence of the global activity of the HSD system. Venkatesh et al showed

evidence of  $11\beta$ -HSD system activation in septic shock:<sup>32</sup> 52 critically ill patients (with septic shock, trauma and burns) were studied serially over 10 days, and their plasma cortisol to cortisone ratios were measured. These ratios were raised in all three cohorts and bore no consistent relationship to plasma cortisol level.

Further evidence of HSD upregulation has been shown at a tissue level by Cohen et al. Pilot data on HSD1 RNA expression in rats subject to caecal ligation and perforation as a model of septic shock clearly indicated exaggerated upregulation of HSD1 mRNA in the liver and adipose tissue as compared with controls (unpublished observations) (Table 1).

#### Dissociation between plasma cortisol and functional cellular response to cortisol

Further evidence of dissociation at a cellular level appears from studies on receptor binding and analysis of gene transcription changes in response to glucocorticoids in stress.

**Altered receptor binding.** At the cellular level, the physiological response to glucocorticoids involves a network of responsive genes. There is evidence of altered glucocorticoid receptor occupancy in infections. A study of these receptors in leukocytes in septic shock showed a reduction in receptor density compared with healthy controls, with increases in plasma cortisol level.<sup>33</sup>

**Gene transcription changes.** There is also evidence of activation of glucocorticoid receptor genes by the various inflammatory cytokines. A recent study clearly exemplified the dissociation between global and tissue indices of adrenal assessment.<sup>34</sup> In family caregivers of patients with brain cancer, the patterns of cortisol secretion were similar to those in healthy volunteers. However, in the caregivers, monocytes showed diminished expression of transcripts bearing response elements for glucocorticoids, and heightened expression of transcripts with response elements for the key pro-inflammatory transcription factor, NF- $\kappa$ B. Caregivers also showed relative elevations in the inflammatory markers, C-reactive protein and interleukin-1 receptor antagonist.

#### Conclusions

These data suggest evidence of cellular adaptations in stress, such as pre-receptor upregulation of cortisol, altered receptor density and altered gene transcription changes, none of which are reflected by plasma cortisol level. Changes in plasma cortisol may have some relationship with these cellular changes, but the prediction of high levels of adrenal insufficiency in septic shock based on plasma data are not borne out by evidence at the cellular level.

This leads us to postulate that the lack of a clearly defined plasma response in severe stress and the presence of an adequate response at the cellular level suggest it is a "sick eadrenal state", analogous to the sick euthyroid state, and not a sick adrenal indicating adrenal insufficiency.

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