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Enhanced feedback sensitivity to prednisolone in chronic fatigue syndrome

Walid K. Jerjes^{a,*}, Norman F. Taylor^a, Peter J. Wood^b, Anthony J. Cleare^{c,d}

^aDepartment of Clinical Biochemistry, Guy's, King's and St Thomas' School of Medicine, Bessemer Road, London SE5 9RS, UK

^bRegional Endocrine Unit, Southampton General Hospital, Southampton SO16 6YD, UK

^cKing's College London, Institute of Psychiatry, Section of Neurobiology of Mood Disorders, Division of Psychiatry and Psychological Medicine, De Crespigny Park, London SE5 8AF, UK

^dNational Affective Disorders Unit, Bethlem Royal and Maudsley Hospitals, Monk's Orchard Road, Beckenham, Kent BR3 3BS, UK

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Summary

Objective: Enhancement of negative feedback control of the HPA axis in patients with chronic fatigue syndrome (CFS) has been reported using the low dose dexamethasone suppression test. We have developed the use of prednisolone (5 mg) as a more physiologically appropriate alternative to dexamethasone in the investigation of mild degrees of glucocorticoid resistance or supersensitivity. The objective of the study was to use this test to look for alterations in negative feedback control of the HPA axis in CFS patients.

Methods: Fifteen patients with CFS were recruited after fulfilling strict criteria including the absence of comorbid psychiatric diagnosis. They collected urine between 0900 and 1800 h and saliva at 0900 h pre-prednisolone. At midnight, they took prednisolone (5 mg) orally and then collected urine and saliva at the same intervals the following day.

Results: Salivary cortisol was lower in CFS subjects pre-prednisolone than controls. Urinary cortisol metabolites were lower in CFS subjects pre-prednisolone, but did not reach significance. Both measures were significantly lower in CFS subjects post-dose. Mean percentage suppression of both salivary cortisol and urinary cortisol metabolites was significantly higher in CFS compared to controls.

Conclusion: There is enhanced sensitivity of the HPA axis to negative feedback in CFS as demonstrated using the prednisolone suppression test. This provides further evidence of alterations in the control of the HPA axis in patients with established CFS.

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*Corresponding author. Tel.: +44 20 7346 4131; fax: +44 207 737 7434.

E-mail address: w_jerjes@yahoo.co.uk (W.K. Jerjes).

1. Introduction

Chronic fatigue syndrome (CFS) is characterised by persistent debilitating fatigue and exhaustion, together with a number of other characteristic symptoms, unexplained by identifiable organic disease (Fukuda et al., 1994). Since many of the symptoms of CFS can also be associated with glucocorticoid deficiency states, evaluation of HPA axis activity in CFS has been undertaken in numerous studies. As a result of this, it has been hypothesised that some features of CFS are a result of moderate hypocortisolism. The majority of the well designed studies in this area have demonstrated low levels of cortisol in blood, urine or saliva (reviewed by Cleare, 2003). More recently, we have also found low levels of urinary and salivary free cortisol in a well characterised group of 15 CFS patients free from medication or comorbid psychiatric disorders (Jerjes et al., 2005, 2006a). However, the research in this area is not entirely consistent, and some other studies have shown no differences in serum cortisol levels (Racciatti et al., 1998; Altemus et al., 2001), and we have found no difference in 24 h urinary cortisol metabolite excretion between CFS and control subjects (Jerjes et al., 2006c).

A second hypothesis is that CFS symptoms result from alterations in central neuroendocrine pathways underlying the control of the HPA axis. Specifically, it has been suggested that there is deficient suprahypothalamic drive, reflected in reduced hypothalamic output of corticotropin-releasing hormone (CRH, Demitrack et al., 1991). Being the principal modulator of the stress response, CRH not only modulates endocrine and autonomic responses but also influences nociception and behaviour (Clauw and Chrousos, 1997). Several symptoms such as lethargy, pain and fatigue have been associated with a deficiency of hypothalamic CRH secretion (Gold and Chrousos, 1999).

Third, enhancement of negative feedback control of the HPA axis has also been proposed as a cause of a hypofunctional HPA axis in CFS (DeKloet et al., 1998). Preliminary reports suggested a heightened negative feedback in CFS subjects using dexamethasone (Poland et al., 1996) or hydrocortisone infusion (Lavelle and Dinan, 1996), while Gaab et al. (2002) examined salivary cortisol levels before and after low dose dexamethasone (0.5 mg), finding greater suppression in CFS, although levels of salivary cortisol in CFS were not different from controls pre-medication. However, dexamethasone may not be physiologically appropriate, since it has much higher relative affinity for the glucocorticoid receptor over the mineralocorticoid receptor (DeKloet et al., 1998), does not bind to corticosteroid binding globulin (Pugeat et al., 1981), and has a much longer half life compared with cortisol (Cassidy et al., 2000).

We have recently reported the use of prednisolone (5 mg) for investigation of mild degrees of glucocorticoid resistance or hypersensitivity, based on cortisol assay in a convenient pre and post dose saliva sample at 0900 h or on a urine sample collected 0900–1800 h (Jerjes et al., 2006b). Suppression was found to be approximately 50% for both saliva and urine in healthy subjects, but correlation between these measures was not established. Thus, the objective of the study was to explore alterations in negative feedback control of the HPA axis in CFS patients, using the prednisolone suppression test and assessing both saliva and

urine approaches. We hypothesised that there would be enhanced negative feedback sensitivity in patients with CFS in comparison to healthy subjects.

2. Materials and methods

2.1. Subjects

Fifteen patients with CFS (7 males and 8 females) were recruited via the CFS clinic at King's College Hospital (KCH), which sees secondary and tertiary care referrals from the south of the United Kingdom. Subjects were interviewed by experienced psychiatrists who used the semi-structured interview for CFS of Sharpe et al. (1997) and DSM-IV to determine the presence of any psychiatric diagnoses. Subjects were eligible for inclusion if they fulfilled the 1994 Centers for Disease Control (CDC) criteria for CFS (Fukuda et al., 1994) without any exclusionary psychiatric disorder as per these criteria. Further inclusion criteria stipulated an age range of 25–60 years and the absence of any history of neurological, endocrine or cardiovascular disorder. In order to obtain as pure a measure of the HPA axis as possible, we tested only patients who had never taken any psychotropic medication or had been abstinent from such medication for at least 2 months. Furthermore, although the modification in 1994 of the original CDC diagnostic criteria permitted inclusion of patients with comorbid major depression or anxiety disorders, patients with a current major depressive episode or anxiety disorder as defined by DSM-IV criteria were excluded from this study because of their potential impact on the HPA axis. Patients were recruited consecutively over about 6 months. We have reported previously on these CFS patients in terms of the diurnal rhythm of salivary, urinary cortisol and cortisol metabolites, and found that they had lower salivary and urinary cortisol across the day without any differences in cortisol metabolites (Jerjes et al., 2005, 2006a).

Twenty healthy volunteers (10 males and 10 females) as described in Jerjes et al. (2006b) were recruited from among the staff and student body at KCH and were matched for age and BMI with CFS patients. They were all assessed to be in good health without any serious medical illness or history of psychiatric disorder. Subjects were all studied during wintertime hours, between October 2002 and March 2003. All subjects had normal dietary habits, taking breakfast, lunch and dinner at about the same time. All subjects were asked to limit their intake of caffeine and alcohol during the collection period. All subjects were instructed to carry out sample collections at weekends to avoid possible increase of cortisol levels, which might result from stress on working days. No female subjects were on the oral contraceptive or pregnant. All subjects habitually went to bed between 2300 and 0100 h and got up between 0700 and 0800 h. All subjects gave written, informed consent and ethical approval for the study was obtained from our local committee.

Patients and healthy controls collected urine between 0900 and 1800 h and provided saliva at 0900 h only (Day 1). At midnight, all subjects took prednisolone (5 mg) and collected urine and provided saliva at the same times the

following day. Percentage suppression of these measures was calculated as described below.

2.2. Questionnaires

All subjects completed the Hospital Anxiety and Depression scale (HADS) (Snaith and Zigmond, 1986) for symptoms of anxiety and depression; and the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) for sleep disturbance. Patients completed further questionnaires to characterise their illness: the Chalder Fatigue Scale (Chalder et al., 1993) for fatigue severity; and the Work and Social Adjustment Scale (Marks, 1986) for disability.

2.3. Urinary collections

Subjects were provided with standard containers for urine and given clear instructions on how to complete the collection. They were asked to empty their bladder normally at 0900 h and to collect in the container all urine passed between 0900 and 1800 h on Day 1. Prednisolone (5 mg) was taken orally at midnight at the end of Day 1. On Day 2, subjects were again asked to empty their bladder at 0900 h and to collect all urine between 0900 and 1800 h. Upon receipt of the specimen at the laboratory, the volume was noted, and after vigorous shaking, two 20 ml aliquots were taken for freezing at -40°C prior to subsequent analysis.

2.4. Saliva collections

Saliva was collected using plain Salivettes (Sarstedt, Leicester, UK). Subjects were asked not to take a sample after brushing their teeth in order to avoid falsely high cortisol values due to plasma exudates from minor bleeding in the oral cavity. For the 10 min prior to each sample, subjects were asked to remain sitting, not to drink anything, smoke or chew gum. During saliva collections, subjects were instructed not to touch the samples with their hands. In association with urine collections, subjects provided one 0900 h sample on the first morning and provided another saliva sample at 0900 h the following morning after taking prednisolone (5 mg) at midnight. Subjects then kept the samples in their refrigerator overnight and returned them to the laboratory the next day.

2.5. Saliva cortisol measurements

Upon receipt at the laboratory, saliva samples were centrifuged and the clear fluid stored at -20°C until analysis. After defrosting, salivary cortisol was analysed by direct immunofluorimetric assay using the "DELFI" system. Microtitre strip format plates were coated with goat anti-rabbit antiserum, and samples were incubated with rabbit anti-cortisol antiserum 2330-5105 (Biogenesis Ltd, Poole, UK) or rabbit anti-cortisone antiserum N-137 and the corresponding steroid-3-CMO-biotin tracers. Europium-labelled neutralite avidin was used to detect bound tracer (Wood et al., 1997). Inter-assay imprecisions (CV%) were less than 12% for salivary cortisol results over 2 nmol/l.

2.6. Cortisol metabolite measurements

Urinary steroid profile analysis was carried out by high-resolution gas chromatography of methyloxime-trimethylsilyl ether (MO-TMS) derivatives as previously described (Taylor, 2006). The intra- and interassay CVs were between 7.1–21.1%, and 11.2–21.9%, respectively for different metabolites. Cortisol metabolites were calculated as $\mu\text{g}/9\text{ h}$ period. Derived sums of total urinary cortisol metabolites were as previously reported (Trainer et al., 2001). Total cortisol metabolites were determined from the sum of tetrahydrocortisone (THE), tetrahydrocortisol (THF), allo-tetrahydrocortisol ($5\alpha\text{THF}$), α -cortolone, β -cortolone, α -cortol, and β -cortol.

2.7. Statistical analysis

Analysis was undertaken in two ways, as per the prior literature. First, we performed a repeated measures analysis of variance for both total urinary cortisol metabolites and salivary cortisol using before and after prednisolone administration as the within group factor and subject group (CFS or control) as the between group factor. Since not all of the raw data were normally distributed, the data were log transformed prior to this analysis. Second, we calculated a summary measure for the percentage suppression by prednisolone using the following formula:

$$\frac{((\text{pre-prednisolone value} - \text{post-prednisolone value}) / (\text{pre-prednisolone value})) * 100.$$

Since the percentage suppression data were normally distributed, group comparisons were made by the independent *t*-test (using SPSS for windows V 11). Raw data and demographic data were also analysed using *t*-tests, with log transformation for data that was not normally distributed as indicated.

Data are expressed as mean \pm standard deviation.

3. Results

3.1. Demographic and questionnaire data

There was no difference in mean age and BMI between the 15 patients with CFS and the 20 controls (mean age (yrs); 35 ± 7.9 and 32 ± 11.4 , $p = 0.4$; and BMI (kg/m^2); 24.4 ± 5.0 and 23.7 ± 4.3 , respectively, $p = 0.6$). Patients with CFS had disrupted sleep compared to controls; total PSQI scores were 9.8 ± 3.3 and 3.1 ± 2.1 , respectively, $p < 0.01$). The CFS subjects had higher levels of self-rated symptoms of anxiety and depression, compared to controls. Scores for the HADS scale were 7.3 ± 5.6 and 3.5 ± 3.0 , $p < 0.01$ for anxiety and 8.0 ± 3.9 and 2.3 ± 2.6 , $p < 0.01$, respectively for depression. The CFS subjects reported high mean scores of fatigue on the Chalder fatigue scale: all scored above the "caseness" cut off score of 4 using the categorical scoring method, while the mean using the Likert scoring method was 25.1 ± 3.0 . CFS patients also reported high levels of disability on the Work and Social Adjustment Scale (22.5 ± 4.7). The mean duration of illness for CFS patients was 2.7 ± 0.6 years.

3.2. Salivary cortisol

Mean levels of cortisol in saliva at 0900 h were lower in patients with CFS than controls at baseline (6.9 ± 3.3 nmol/l and 11.5 ± 5.2 nmol/l, respectively, $t = -3.0$, $p = 0.005$). After prednisolone (5 mg) administration, mean levels of salivary cortisol were again significantly lower in CFS compared to controls (3.3 ± 1.7 and 6.7 ± 3.2 nmol/l, respectively, $t = -4.4$, $p < 0.001$ on log transformed data).

The repeated measures analysis of variance showed: a significant overall effect of group, cortisol being lower in CFS subjects ($F_{1,33} = 15.2$, $p < 0.001$); a significant effect of prednisolone in lowering cortisol ($F_{1,33} = 344$, $p < 0.0001$); and a significant group by prednisolone interaction, with a larger effect seen in the CFS group ($F_{1,33} = 9.5$, $p = 0.004$).

3.3. Total urinary cortisol metabolites

The baseline levels of urinary cortisol metabolites (0900–1800 h) did not differ significantly between CFS patients and controls (1860 ± 432 and 2208 ± 908 $\mu\text{g}/9$ h, respectively, $t = 1.0$, $p = 0.32$ on log transformed data). After taking prednisolone (5 mg), urinary cortisol metabolite levels were significantly lower in patients with CFS compared to controls (301 ± 160 and 959 ± 356 $\mu\text{g}/9$ h, respectively, $t = 6.6$, $p < 0.0001$).

The repeated measures analysis of variance showed: a significant overall effect of group, total cortisol metabolites being lower in CFS subjects ($F_{1,33} = 29.7$, $p < 0.001$); a significant effect of prednisolone in lowering total cortisol metabolites ($F_{1,33} = 253$, $p < 0.0001$); and a significant group by prednisolone interaction, with a larger effect seen in the CFS group ($F_{1,33} = 42.4$, $p < 0.001$).

3.4. Percentage suppression by prednisolone

Mean percentage suppression of salivary cortisol at 0900 h and urinary cortisol metabolites (0900–1800 h) was significantly higher in CFS compared to controls. For salivary cortisol, suppression was $52 \pm 10\%$ in patients and $41 \pm 12\%$ in controls ($t = -3.0$, $p = 0.005$). For Urinary cortisol metabolites, suppression was $82 \pm 11\%$ in patients and $56 \pm 8\%$ in controls ($t = -8.5$, $p < 0.0001$). These results are shown graphically in Fig. 1.

Thus, analysis by percentage suppression indicated the same findings as the repeated measures analysis of variance: for both salivary cortisol and total urinary cortisol metabolites, there was a larger suppressive effect of prednisolone in CFS subjects than controls.

4. Discussion

This is the first study to examine response of the HPA axis to prednisolone in CFS. The findings support suggestions that there is enhanced negative feedback on the HPA axis in CFS. The timing of sample collection was optimised for normal subjects (Jerjes et al., 2006b) but it is appropriate for our study group of CFS patients since we have demonstrated that they have a normal circadian rhythm for both saliva (Jerjes et al., 2005) and urine (Jerjes et al., 2006a) The

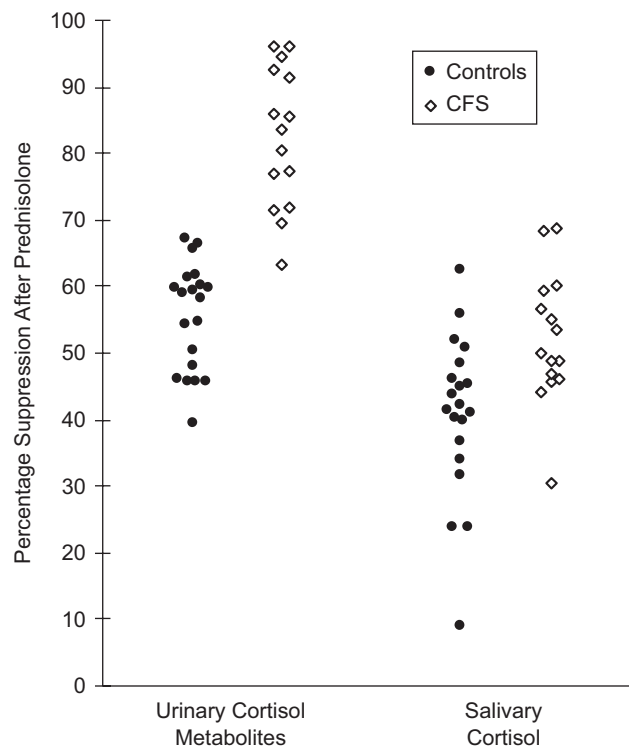


Figure 1 Scatter plot showing each individual's percentage suppression after the administration of prednisolone at 12 midnight, measured by urinary cortisol metabolites (0900–1800 h) and salivary cortisol (0900 h) the following day. Healthy controls are shown with closed circles and Chronic Fatigue Syndrome (CFS) patients with open diamonds. CFS patients showed a significantly higher mean suppression after prednisolone with both measures (see text).

percentage suppression of urinary cortisol metabolites was higher than for saliva in both groups, and the difference between CFS and controls was larger, probably because urinary cortisol metabolites show a long period of suppression post dose (up to 18 h) (Jerjes et al., 2006b). This might be because salivary levels only provide a snapshot at 0900 h, which might not reflect the overall suppression as accurately.

This study has several advantages over previous work. First, an increased negative feedback has been confirmed using two biological measures, salivary free cortisol and urinary cortisol metabolites. Second, patients in whom there was comorbid psychiatric disorder or had prescribed medication that may have affected the HPA axis have been excluded. Third, prednisolone was used in place of dexamethasone. This has a more physiological action, with, for example, a relative potency at glucocorticoid and mineralocorticoid receptors similar to that of the main endogenous corticosteroid cortisol, and having privileged CNS penetration (DeKloet et al., 1998). Dexamethasone has poor CNS penetration and thus only activates glucocorticoid receptors and may miss the important physiological interactions between mineralocorticoid and glucocorticoid receptors. The poor CNS penetration is due to the effect of multiple drug resistance (mdr1a) P-glycoprotein (Bourgeois

et al., 1993) at the level of the endothelial cells of the blood-brain barrier (Cordon-Cardo et al., 1989). It is possible, for example, that changes in the glucocorticoid receptor might be compensated by changes in the mineralocorticoid receptor. Given this more physiological effect of prednisolone, our finding of enhanced suppression in response to prednisolone is strong evidence that the response of the HPA axis to endogenous negative feedback is enhanced in CFS.

These findings extend those of Gaab et al. (2002) who found heightened HPA sensitivity in CFS, measuring salivary cortisol before and after low dose dexamethasone (0.5 mg). In vitro studies of feedback provide further support for enhanced GR sensitivity in CFS. Visser et al. (1998) found in CD4T lymphocytes from a small group of patients with CFS, that inhibition of CD4 proliferation occurred with a lower concentration of dexamethasone than in controls. (Visser et al., 2001) and in a further study of 10 CFS and 14 controls showed that this was not due to increased GR affinity or number. Two studies do not support the presence of enhanced GR function in CFS. One study reported only in abstract (Majeed et al., 1995) reported a decreased GH response to dexamethasone, but some patients in the sample were also suffering with irritable bowel syndrome and fibromyalgia. Also, this study did not measure feedback sensitivity directly, and the validity of the Dexamethasone-GH response as an index of GR function is not well established, especially as GH function itself may be disturbed in CFS (Moorkens et al., 1998). The second study (Kavelaars et al., 2000) found a reduced effect of dexamethasone on white cells, using T cell proliferation as a marker, but this was in adolescent patients, not in adults, and there is little understanding of how CFS or the HPA axis compares between adolescents and older subjects with CFS.

The suggestion that there is enhanced negative feedback and upregulated GR function in CFS is consistent with the observed reports of mild hypocortisolism in CFS (Cleare, 2003). Although the temporal link between the two findings cannot be tested, it is plausible that the enhanced negative feedback is one of the aetiological factors in the development of hypocortisolism in CFS. It has previously been noted that enhanced negative feedback in CFS is similar to the findings in a number of other conditions that may be stress related, such as posttraumatic stress disorder (Yehuda et al., 1995), women with histories of childhood sexual abuse (Stein et al., 1997), adolescents exposed to earth quake-related trauma (Goenjian et al., 1996), burnout syndrome (Pruessner et al., 1999), and chronic pelvic pain (Heim et al., 1998). Gaab et al. (2002) hypothesised that precipitating or chronic stress may be the common factor underlining the similar findings in each of these conditions. Supporting this, a recent experimental study by Zarkovic et al. (2003) examined the effect of prolonged psychological stress on cortisol secretion induced by continuous air raids, after elimination of the stress-inducing factor, in five healthy adults. They found a transient suppression of the HPA axis, manifested by low morning cortisol and reduced cortisol response to ACTH.

It also appears that enhanced GR receptor function may be of clinical relevance. We have found that low doses of hydrocortisone to replace the hypothesised deficiency are

effective in some patients with CFS (Cleare et al., 1999) and that those with higher GR sensitivity were most likely to respond to therapy (Cleare et al., 2001).

The present study has some potential limitations. First, it is possible that this is a cohort with low HPA axis function and thus the findings require replication elsewhere in a separate cohort. However, in the literature, half of well-designed studies measuring cortisol in CFS using well-validated methods do show hypocortisolism. Second, exact awakening time was not recorded in the current study, although all subjects got up between 0700 and 0800 h. Data presented by Gaab et al. (2002) indicate a post-awakening rise in salivary cortisol up to 30 min in both CFS and control subjects. Since our subjects were sampled at least 60 min post awakening the interval between awakening and sampling should not have influenced the results. Third, the menstrual cycle phase in female subjects was not controlled. Altemus et al. (1997) have demonstrated an effect of menstrual cycle phase on dexamethasone response, with reduced expression in lymphocytes of GR mRNA in the luteal phase. Another study has suggested that women in the luteal phase show a significantly higher salivary cortisol in response to a psychosocial stressor compared to women in the follicular phase (Kirschbaum et al., 1999). Several others have not found such an association between the HPA axis and menstrual cycle phase (Kudielka and Kirschbaum, 2003; Cevik et al., 2004). It is thus possible that these results are confounded by influence of menstrual cycle phase on prednisolone-induced suppression of the HPA axis. Fourth, it has been noted that HPA axis changes in CFS are apparent late in the course of the illness (Cleare, 2003) but do not accompany the early stages of chronic fatigue (Candy et al., 2003). The patients in this study had been ill for a relatively short duration (mean 2.7 ± 0.6 years) in the context of CFS. Thus they might not have developed HPA axis changes to the extent that might be found in patients ill for longer periods. Nevertheless, they did exhibit lower salivary cortisol levels and numerically (but not statistically) lower urinary cortisol metabolite levels (Jerjes et al., 2005, 2006a).

It has been reported that hypocortisolism in CFS is likely to be the result of a number of interacting influences, including current or previous stressors, but also psychiatric status and medication (where not controlled for), nutritional status, sleep disturbance, physical activity levels and deconditioning (reviewed by Cleare, 2003). It is therefore likely that any observed alteration to negative feedback in CFS would have a multifactorial origin.

In conclusion, these findings of enhanced HPA axis suppression in CFS by prednisolone suggests that there is enhanced physiological negative feedback in CFS and upregulated GR and/or MR receptors. This provides further evidence of alterations to the control of the HPA axis in patients with established CFS. This state of enhanced physiological negative feedback could be a factor underlying the observed hypocortisolism in some patients (including the group studied here), or could be a response to stress or other effects of the illness such as sleep and activity levels. Further work assessing the status of both GR and MR receptors at various stages of the genesis of CFS may be helpful in understanding the role of the HPA axis in this condition.

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