

Plasma leptin in chronic fatigue syndrome and a placebo-controlled study of the effects of low-dose hydrocortisone on leptin secretion

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Summary

OBJECTIVE Previous studies have suggested that chronic fatigue syndrome (CFS) is associated with changes in appetite and weight, and also with mild hypocortisolism. Because both of these features may be related to leptin metabolism, we undertook a study of leptin in CFS.

DESIGN (i) A comparison of morning leptin concentration in patients with CFS and controls and (ii) a randomized, placebo-controlled crossover study of the effects of hydrocortisone on leptin levels in CFS.

PATIENTS Thirty-two medication free patients with CFS but not comorbid depression or anxiety. Thirty-two age, gender, weight, body mass index and menstrual cycle matched volunteer subjects acted as controls.

MEASUREMENTS We measured basal 0900 h plasma leptin levels in patients and controls. All 32 patients were taking part in a randomized, placebo-controlled crossover trial of low dose (5 or 10 mg) hydrocortisone as a potential therapy for CFS. We measured plasma leptin after 28 days treatment with hydrocortisone and after 28 days treatment with placebo.

RESULTS At baseline, there was no significant difference in plasma leptin between patients [mean 13.8, median 7.4, interquartile range (IQR) 18.0 ng/ml] and controls (mean 10.2, median 5.5, IQR 11.3 ng/ml).

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Hydrocortisone treatment, for both doses combined, caused a significant increase in leptin levels compared to placebo. When the two doses were analysed separately, only 10 mg was associated with a significant effect on leptin levels. We also compared the hydrocortisone induced increase in leptin between those who were deemed treatment-responders and those deemed nonresponders. Responders showed a significantly greater hydrocortisone-induced rise in leptin than nonresponders. This association between a clinical response to hydrocortisone and a greater rise in leptin levels may indicate a greater biological effect of hydrocortisone in these subjects, perhaps due to increased glucocorticoid receptor sensitivity, which may be present in some patients with CFS.

CONCLUSIONS We conclude that, while we found no evidence of alterations in leptin levels in CFS, low dose hydrocortisone therapy caused increases in plasma leptin levels, with this biological response being more marked in those CFS subjects who showed a positive therapeutic response to hydrocortisone therapy. Increases in plasma leptin levels following low dose hydrocortisone therapy may be a marker of pretreatment physiological hypocortisolism and of response to therapy.

Chronic fatigue syndrome (CFS) is an operationally defined condition requiring the presence of prolonged fatigue with marked disability, together with several somatic symptoms, in the absence of organic illness or severe psychiatric disorder which would explain the fatigue (Fukuda *et al.*, 1994). The aetiology of the condition is not fully understood, although contributions from both biological and psychosocial factors are likely (Wessely *et al.*, 1998). One such biological factor that has emerged as having a possible role is mild hypocortisolism. Studies using a variety of methods have suggested the presence of hypocortisolism and hypothalamic-pituitary-adrenal (HPA) axis dysfunction in CFS (Demitrack & Crofford, 1998). Furthermore, supplementation with low dose oral hydrocortisone seems to be effective in relieving fatigue in some subjects (McKenzie *et al.*, 1998; Cleare *et al.*, 1999). However, the underlying cause of this hypocortisolism, and whether it is

primary or secondary, remains unknown. Some have hypothesized that the hypocortisolism of CFS is a response to past chronic stress of some sort (Scott & Dinan, 1999), some that it might be secondary to CFS rather than primary, for example resulting from sleep disturbance (Leese *et al.*, 1996), and others that it may be suprahypothalamic in origin and represent a final common pathway for fatigue (Demitrack & Crofford, 1998).

Another possibility, however, is that disturbances in other physiological systems may underlie the HPA axis dysfunction. One mechanism could be through serotonergic mechanisms: several studies report upregulated central serotonergic receptors (Cleare, 1998), which may cause a lowering of cortisol levels. An unexplored factor at present is leptin. Leptin is a pleiotropic hormone with known roles in the regulation of body mass, satiety, immune responsiveness and reproduction. *Ob/ob* mice which are deficient in leptin protein production due to a nonsense mutation in the *ob* gene resulting in a premature stop codon (Zhang *et al.*, 1994) have unregulated appetite and become obese. Leptin acts via a class 1 cytokine receptor of which there are several isoforms. Its main actions appear to be hypothalamically mediated. Early studies showed high affinity binding of leptin in the rat and high levels of leptin receptor mRNA in the arcuate nucleus and slightly less in the dorso and ventromedial hypothalamic nuclei (Schwartz *et al.*, 1996). Hypothalamic neuropeptide Y (NPY) stimulates appetite and food intake (Longqvist, 1996); this action is inhibited by leptin through binding to receptors in the ventromedial hypothalamus. Other neuropeptides are important in appetite regulation, particularly those derived from the precursor pro-opiomelanocortin (POMC) such as alpha melanoocyte stimulating hormone. Colocalization of leptin receptors and POMC neurones (Inui, 1999) and the observation of increased *POMC* gene expression in response to exogenous leptin (Shwartz *et al.*, 1997) suggest a close relationship between leptin regulation and that of the *POMC* gene. Further evidence has accumulated to suggest that that leptin can inhibit HPA axis function either through a direct effect to inhibit cortisol release from the adrenal gland (Bornstein *et al.*, 1997), an inhibition of hypothalamic corticotrophin-releasing hormone (CRH) release (Heiman *et al.*, 1997) or through other mechanisms (Korbonits *et al.*, 1997; Licinio *et al.*, 1997; Torpy *et al.*, 1998). It is possible therefore that increased leptin levels could be implicated in the hypocortisolism of CFS.

On the other hand, glucocorticoids themselves impact on many physiological processes, including energy metabolism. Glucocorticoids are known to increase leptin levels in normal humans (Miell *et al.*, 1996). Thus, it is also plausible that changes in leptin levels may result from the probable hypocortisolism in CFS. Moreover, changes in appetite and

weight, primarily increases, have been reported frequently in CFS (Terman *et al.*, 1998), both of which could be accounted for by alterations in leptin metabolism.

This study examined leptin levels in a group of CFS patients, selected to be free from comorbid depression or anxiety, compared to a group of healthy matched controls. Additionally, in order to provide more information on the relationship between leptin and the HPA axis, we investigated the effects of low-dose glucocorticoid supplementation on leptin levels in CFS patients.

Patients and methods

Subjects

A total of 32 patients with CFS were recruited into this study from referrals to CFS clinics at King's College School of Medicine and Dentistry and Addenbrooke's NHS Trust, Cambridge. All patients had undergone thorough medical screening to exclude a detectable organic cause for their fatigue including physical examination and relevant investigation, with a minimum of urinalysis, full blood count, urea and electrolytes, thyroid function tests, liver function tests, 0900 h cortisol and erythrocyte sedimentation rate. Frank disturbance of the adrenal axis was excluded using an insulin stress test, CRH test, 24-h urinary free cortisol and adrenal antibodies including 21-hydroxylase. All patients were interviewed using a semistructured interview for CFS and psychological disorder (Sharpe *et al.*, 1997). Included subjects had to fulfil both international consensus criteria for CFS (Sharpe *et al.*, 1991; Fukuda *et al.*, 1994) and be free from comorbid psychiatric disorder as defined in DSM-IV (American Psychiatric Association, 1995). All were drug free for a minimum of 2 months prior to endocrine testing, with the exception of three patients using oral contraception or hormone replacement. Because of these strict entry criteria, we screened 218 patients referred to our clinics with chronic fatigue to obtain the final 32 participants.

A group of 32 control subjects was recruited from volunteers and staff members in our institutions. Three of the female controls were matched for the same dose of oestrogen as the patients. None of the controls had a history of significant medical problems, CFS or DSM-IV psychiatric disorder. Because of effects of body mass index (BMI) (Considine *et al.*, 1996) and gender (Havel *et al.*, 1996) on plasma leptin levels, we attempted to recruit a sample matched for these characteristics with the patient group.

All procedures were approved by the hospital ethics committee. All patients and controls gave their written informed consent. All female subjects were tested during days 1–7 of their menstrual cycle.

Measurements

Leptin was measured from a single blood sample taken through a cannula that had been *in situ* for at least 30 minutes. Samples were taken at 1000 h into an EDTA bottle, immediately spun in a refrigerated centrifuge, and the plasma separated and frozen at -20°C.

All subjects completed a randomized, placebo-controlled crossover study of low dose hydrocortisone as described previously (Cleare *et al.*, 1999). They received active treatment or placebo in random order for 28 days each. The first 16 patients were allocated a 5-mg replacement dose of hydrocortisone, and the remainder 10 mg. Additional samples for leptin were taken on day 28 of each treatment period.

All subjects completed the fatigue scale of Chalder *et al.* (1993), the Medical Outcomes Survey short form 36 (SF-36; Stewart *et al.*, 1988) and Work and Social Adjustment Scale (Marks, 1986) to assess disability; the General Health Questionnaire (GHQ; Goldberg, 1972) to assess psychiatric symptoms; and the Symptom Checklist of 40 items (Wittenborn & Buhler, 1979) to assess somatic symptoms.

Assays

Leptin was assayed using an immunoradiometric assay from DSL Webster, TX, USA. Sensitivity was 0.10 ng/ml. Intra-assay coefficients of variance were 3.7% at 2.8 ng/ml, 4.9% at 13.5 ng/ml and 2.6% at 73.6 ng/ml. Inter-assay coefficients of variance were 6.6% at 2.8 ng/ml, 5.3% at 14.4 ng/ml and 3.7% at 73.9 ng/ml.

Statistical analysis

Leptin values were not normally distributed. Therefore, we used a natural log transformation in order to allow the use of parametric tests for the comparisons of means (independent or paired *t*-test as appropriate). When comparing change from baseline values, we could not log transform as many values

were negative; in these cases we used nonparametric tests (Wilcoxon and Mann-Whitney *U*-test). Correlations were calculated using Spearman's correlation coefficients.

Results

Subjects and controls did not differ significantly in age, weight, gender or BMI. Clinical and demographic details of the sample are given in Table 1. Baseline endocrine testing did not reveal frank hypocortisolism in any patients. Full results of the insulin stress tests and CRH tests are presented elsewhere (Cleare *et al.*, 1999; Cleare *et al.*, in press). In summary, in comparison to controls, the group as a whole had reduced urinary free cortisol and a reduced cortisol response to CRH testing, suggesting a mild hypocortisolism, but normal ACTH responses to CRH and normal ACTH and cortisol responses to insulin stress testing.

Basal values

Mean basal leptin values are shown in Table 1. Log transformed values did not differ significantly between patients and controls ($t = -0.49$, d.f. = 62, $P = 0.63$). There was a significant positive correlation between BMI and leptin concentrations in CFS patients ($r = 0.35$, $P = 0.035$), and in the group as a whole ($r = 0.26$, $P = 0.04$). In the 25 patients who completed the mental health subscale of the SF-36, there was a correlation between good mental health and high leptin levels ($r = 0.45$, $P = 0.025$).

Effect of hydrocortisone

Figure 1 shows the leptin values at baseline and after active and placebo treatments. Combining data from both doses of hydrocortisone revealed a significantly higher value during active treatment (paired *t*-test $t = 2.98$, d.f. = 30, $P = 0.006$). We also carried out these calculations separately for the two

Table 1 Basal leptin levels in subjects with CFS and controls

	Basal leptin (ng/ml) ^a	Age (years)	Gender (no. female)	Body mass index (kg/m ²)	Weight (kg)	Illness duration (months)	Disability (Work and Social Adjustment Scale; max = 40)	Physical Function (SF-36: 0 minimum, 100 maximum)	General Health Questionnaire (max = 36)	Chalder Fatigue Questionnaire (max = 33)
Patients (<i>n</i> = 32)	13.8 (med 7.4, IQR 18.0)	35.3 (SD 11.6)	20/32	23.4 (SD 4.0)	68.5 (SD 13.5)	36.3 (SD 23.9)	24.3 (SD 6.9)	47.4 (SD 19.8)	16.3 (SD 6.5)	25.1 (SD 3.8)
Controls (<i>n</i> = 32)	10.2 (med 5.5, IQR 11.3)	32.5 (SD 7.8)	18/32	24.2 (SD 3.3)	70.7 (SD 13.4)	N/A	0.7 (SD 1.5)	92.4 (SD 20.6)	9.4 (SD 4.7)	10.3 (SD 2.7)

^a Leptin values show mean with median and interquartile range in parentheses.

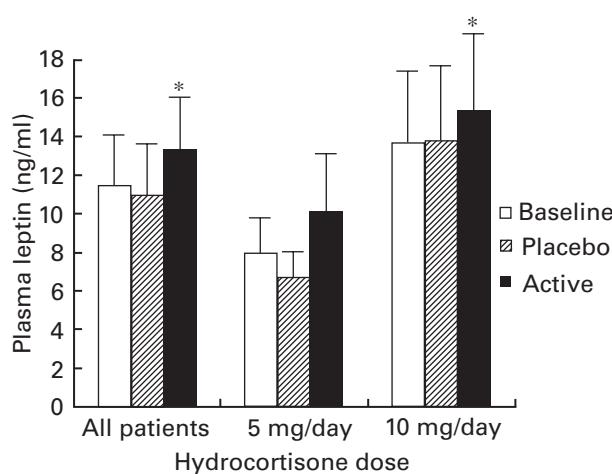


Fig. 1 Plasma leptin levels measured in 32 drug-free CFS patients without psychiatric comorbidity at baseline (white bars), after treatment with placebo (grey bars) and after treatment with hydrocortisone (black bars). The two doses of hydrocortisone were combined for the left hand set of bars, and are shown separately on the right. The asterisks denote increased levels after hydrocortisone treatment compared to those after placebo treatment ($P < 0.05$).

separate doses of hydrocortisone used. Whilst both doses appeared to result in higher leptin levels than placebo (Fig. 1), this was only significant for the higher dose (paired t -test 5 mg: $t = 1.65$, d.f. = 11, $P = 0.13$; 10 mg: $t = 3.22$, d.f. = 18, $P = 0.005$).

We also divided the subjects into responders ($n = 9$) and nonresponders ($n = 23$) to treatment, based on the results of the hydrocortisone trial (Cleare *et al.*, 1999). Briefly, responders were defined as having shown a fall in their fatigue ratings to a level equal or less than the population mean. Basal leptin values before any treatment were not related to subsequent treatment response: values were 15.2 (SD 14.2) ng/ml in subsequent responders and 11.0 (SD 13.3) ng/ml in subsequent nonresponders ($t = -0.84$, d.f. = 30, $P = 0.41$ on log transformed values). However, those who were responders to treatment showed a larger hydrocortisone-induced Δ leptin (calculated as the leptin level on placebo subtracted from the leptin level on active treatment) than nonresponders ($z = -1.97$, $P = 0.047$). The mean Δ leptin was 5.1 (SD 8.5) ng/ml in responders and 1.1 (SD 2.6) ng/ml in nonresponders.

Discussion

There are three main findings from this study. First, in a carefully selected group of medication-free CFS patients without psychiatric comorbidity, plasma leptin levels were

similar to matched controls. As in previous studies, leptin levels were positively correlated with BMI. Our findings suggest that it is unlikely that simple alterations in basal morning leptin levels are involved in the pathophysiology of CFS.

Second, our study helps understand further the links between leptin physiology and that of glucocorticoids. We found that a very low level of hydrocortisone supplementation leads to a small but significant rise in leptin levels after 28 days of treatment. The literature to date looking at the relationship between leptin and glucocorticoids has yielded conflicting results. Some studies have found inverse relationships between leptin and pituitary-adrenal function (Korbonits *et al.*, 1997; Licinio *et al.*, 1997) in the physiological state, while others have failed to find such links (Haffner *et al.*, 1997). Studies of administered glucocorticoids have generally used high doses. Thus, many studies have shown that short-term administration of the potent glucocorticoid dexamethasone can increase circulating leptin levels in normal or obese subjects (Larsson & Ahren, 1996; Miell *et al.*, 1996; Considine *et al.*, 1997; Dagogo Jack *et al.*, 1997; Elimam *et al.*, 1998; Halleux *et al.*, 1998). There are fewer studies of administered hydrocortisone. In one previous study, investigators administered supraphysiological doses of hydrocortisone (either 40 mg/day or 160 mg/day) and found a dose-dependent increase in leptin levels after 1 day of treatment, an attenuation of this effect after 4 days of treatment and a return to baseline levels of leptin by the 10 day follow-up (Newcomer *et al.*, 1998). On the other hand, another study administered an infusion of hydrocortisone for 48 h, designed to mimic normal diurnal levels of cortisol, to patients with adrenal insufficiency (Purnell & Samuels, 1999). They found no acute alterations in leptin levels during the second 24 h when compared to the effects of a control saline infusion on a separate occasion. Our study suggests that lower doses of hydrocortisone may produce modest increases in leptin release, and maintain this over a longer time frame.

The mechanism by which glucocorticoids effect the release of leptin has also come under scrutiny. Considine *et al.* (1997) suggested dexamethasone stimulates release of leptin from adipocytes, and indeed studies *in vivo* (Papaspyrou Rao *et al.*, 1997) and *in vitro* (Halleux *et al.*, 1998) have found that dexamethasone can increase the expression of leptin messenger RNA in adipose tissue. On the other hand, it has also been suggested that, in Cushing's syndrome, cortisol affects leptin release mainly through its effects on insulin and insulin sensitivity (Widjaja *et al.*, 1998). Cizza *et al.* (1997) followed up Cushing's patients after definitive treatment and found that leptin levels did not fall when the cortisol levels normalized, but were more directly linked to the BMI of patients.

Our third main finding is that the rise in leptin induced by hydrocortisone appears to be a biological marker of a

therapeutic glucocorticoid effect, i.e. those who reported an antifatigue effect of hydrocortisone showed significantly larger rises in leptin. This increased leptin rise in treatment-responders may be due to a pretreatment state of upregulated glucocorticoid receptor function. Such upregulation is associated with hypocortisolism which, as discussed previously, has been detected in CFS. However, not all subjects have profiles suggestive of hypocortisolism. Thus, it may be that the hydrocortisone-induced leptin rise is a marker of a pretreatment state of relative hypocortisolism: those who had suffered a relative hypocortisolism were both responsive to treatment and more susceptible to the biological effects of hydrocortisone. Few studies in CFS have yet looked at directly at glucocorticoid receptor sensitivity in CFS, although preliminary evidence does exist to suggest upregulated glucocorticoid receptors, including a hypersuppression of cortisol release (Poland *et al.*, 1996) and IL-4 production (Visser *et al.*, 1998) in response to dexamethasone.

An alternative explanation is that those who did not respond may have had downregulated glucocorticoid receptors. This would explain a lack of response to treatment and a relative lack of the physiological rise in leptin with glucocorticoids. Given the overlap between depression and CFS, and the association of depression with glucocorticoid receptor downregulation (Checkley, 1996), it would be tempting to suggest that the nonresponders may have been those with comorbid depression. However, this was not the case, since we specifically excluded overt DSM depression in these subjects. Notwithstanding this, we previously found that one pretreatment factor which distinguished nonresponders to hydrocortisone from responders was a higher depression/anxiety score on the GHQ, albeit outside of the range associated with clinical depression (Cleare *et al.*, 1999). It is possible that glucocorticoid receptor sensitivity is lower in those with subthreshold increases in GHQ scores associated with their CFS.

Another possibility is that the rise in leptin may be central to the antifatigue effects of hydrocortisone. However, given that CFS patients do not have lowered leptin at baseline, and given the absence of any literature suggesting that leptin can modify fatigue, this seems unlikely.

Limitations to our study include sampling leptin at only one time point on each occasion; thus we can not assess the diurnal changes or pulsatility of leptin release in CFS. More accurate determination of leptin status would be achieved by repeated sampling or by assaying pooled samples over a longer sampling period. Also, we only sampled after 28 days of treatment, and thus do not know what may have happened in the interim. We did not assess any short-term changes in BMI or body composition that may have occurred during the low-dose hydrocortisone regime, though this is unlikely to have been significant over a 28-day time period and with such low

doses of hydrocortisone. We did not assess glucocorticoid receptor sensitivity directly using *in vivo* or *in vitro* techniques; such methods would be needed in order to confirm or refute our hypotheses regarding the link between glucocorticoid receptor sensitivity and differential leptin responses to hydrocortisone. Furthermore, this was a crossover study using patients as their own controls. The effect of these doses of hydrocortisone in healthy individuals remains untested. Finally, our study was not powered to detect very small differences in leptin levels between CFS patients and controls, though such small differences are unlikely to be of clinical significance in the aetiology of the condition.

In conclusion, whilst we found no evidence of an alteration in leptin levels in CFS, we did find a small but significant effect of low-dose hydrocortisone in increasing leptin levels in these patients. The rise was most marked in those who had a positive therapeutic response to hydrocortisone. This may be a marker of a greater biological effect of the hydrocortisone in those who responded, perhaps due to increased glucocorticoid sensitivity.

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