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**Cortisol output in adolescents with chronic fatigue syndrome:
Pilot study on the comparison with healthy adolescents
and change after cognitive behavioural guided self-help treatment**

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Short running head: Cortisol in adolescents with chronic fatigue syndrome

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Abstract

Objective: This study examined cortisol in adolescents with chronic fatigue syndrome (CFS) compared to healthy adolescents and changes in cortisol after cognitive behavioural guided self-help treatment.

Exploratory analyses investigated the association between cortisol output and psychological variables.

Methods: Salivary cortisol was measured upon awakening, at 15, 30, 45 and 60 mins afterwards and at 12 noon, 4pm and 8pm, in adolescents with CFS and healthy controls (HC). Groups were matched for age, gender, menarche status, menstrual cycle and awakening time. Twenty-four adolescents with CFS provided saliva samples six months after treatment. The main outcome measure was total salivary output over the day, calculated by area under the curve (AUC). The salivary awakening response was also assessed.

Results: Cortisol output over the day was significantly lower in the CFS group (n=46) than in healthy controls (n=33). Within the CFS group, lower daily cortisol output was associated with higher self-reported perfectionist striving and prosocial behaviour. There were no significant group differences in the awakening response (n=47 CFS versus n=34 HC). After treatment, adolescents with CFS (n=21) showed a significant increase in daily cortisol output, up to normal levels.

Conclusion: The reduced daily cortisol output in adolescents with CFS is in line with adult findings. Associations between reduced cortisol output and two psychological variables - perfectionism and prosocial behaviour - are consistent with cognitive behavioural models of chronic fatigue syndrome. The mild hypocortisolism is reversible; cortisol output had returned to healthy adolescent levels by six months after cognitive behavioural guided self-help treatment.

Keywords: fatigue, youth, paediatric, hypothalamic-pituitary-adrenal axis, neuroendocrine, perfectionism.

INTRODUCTION

Chronic fatigue syndrome (CFS) is likely to be a multifactorial condition in which biological, psychological and social factors contribute and interact. Few biological changes have been reliably demonstrated in this condition, one of the exceptions being disturbed hypothalamic-pituitary-adrenal (HPA) axis dysfunction (Papadopoulos & Cleare, 2012). In adults with CFS, reduced cortisol output over the day has been demonstrated in salivary and urinary cortisol studies (Jerjes, Cleare, Wessely, Wood & Taylor, 2005; Jerjes, Peters, Taylor, Wessely & Cleare, 2006; Tak et al., 2011). In adults there is also evidence of an attenuation of the usual rapid increase in salivary cortisol levels after awakening (Roberts, Chalder, Papadopoulos & Cleare, 2004). The cortisol awakening response is not well understood but appears to be influenced by a number of factors in addition to HPA activation, such as sensitivity to light mediated by an extra-pituitary pathway (e.g. Clow, Hucklebridge, Stalder, Evans and Thorn, 2010). Therefore it is important not to rely only on measures of the cortisol awakening response to assess HPA axis activation.

Reduced cortisol may be a primary etiological factor in CFS and / or secondary to sleep disturbance, reduced activity or other factors commonly associated with CFS such as increased stress or distress. Stress is reported by some young people with CFS as a contributory factor to their condition (Gray & Rutter, 2007) and there are elevated rates of mood and anxiety problems in adolescents with CFS compared to young people with other chronic illness (e.g. Bould, Collin, Lewis, Rimes & Crawley, 2013; Rangel, Garralda, Hall & Woodham, 2003). Chronic stress is associated with hypocortisolism (Miller, Chen & Zhou 2007) and a study in adults with CFS found that blunted cortisol awakening responses were only present in those with a history of childhood trauma (Heim et al., 2009). It has been suggested that hypocortisolism can reflect a protective response following a history of repeated high cortisol responses (Fries, Hesse, Hellhammer & Hellhammer, 2005). Hypocortisolism is associated with symptoms such as fatigue and pain, so once it has developed it could act as a maintaining factor for CFS.

Three previous studies have reported serum cortisol levels in adolescents with CFS, with inconsistent findings (Kavelaars, Kuis, Knook, Sinnema & Heijnen, 2000; Wyller, Evang, Godang, Solhjell & Bollerslev, 2010; Segal, Hindmarsh & Viner, 2005). Each of these studies used a potentially stressful invasive testing

procedure in a hospital environment. Given that cortisol is a hormone that shows diurnal variation and is secreted in a pulsatile manner, another limitation is the use of a single blood cortisol measurement to assess basal HPA axis function. Studies are needed using repeated salivary measures of cortisol over the day, taken in a naturalistic home environment that eliminates the confounding stress associated with both intravenous cannulation and hospital attendance. Katz et al. (2013) found little evidence of reduced salivary cortisol levels in adolescents with CFS but this had a number of limitations including a very small sample size (nine adolescents with CFS), just two measurement points (morning and evening) and the inclusion of adolescents who developed CFS after infectious mononucleosis only. Nijhof et al. (2014) found lower cortisol awakening response in adolescents with CFS compared to healthy controls but they did not investigate cortisol output over the course of the day. This is the first salivary cortisol study to report both total daily cortisol output and the cortisol awakening response (CAR) in adolescents with CFS.

Nijhof et al. (2014) compared characteristics of adolescents with CFS dichotomised by below and above average AUCg levels and found that only sleep duration before CAR was significantly different. They did not find differences in terms of depression but did not compare the groups on other psychological characteristics that relate to stress vulnerability such as anxiety or high standards for performance or personal conduct. The present study undertook exploratory analyses investigating associations between cortisol output and questionnaire measures of perfectionism and prosocial behaviour (as indications of high standards for performance and personal conduct) as well as other characteristics such as anxiety, depression, emotional and behavioural difficulties and fatigue severity.

Cognitive behavioural treatments are evidence-based interventions for CFS (Chalder, Deary, Husain & Walwyn, 2010; Stulemeijer, de Jong, Fiselier, Hoogveld & Bleijenberg, 2004). These interventions are based on a multifactorial approach to CFS in adolescents (e.g. Lievesley, Rimes & Chalder, 2014; Surawy, Hackmann, Hawton & Sharpe, 1995) in which it is hypothesised that certain premorbid characteristics, including high standards for performance or personal conduct, conscientiousness and stress vulnerability (e.g. Rangel, Garralda, Levin, & Roberts, 2000) increase the risk for developing fatigue in the context of challenges such as physical illness or extra environmental demands. For example, perfectionist or conscientious individuals may strenuously attempt to keep up with their usual standards despite the extra

challenges, and end up more stressed and fatigued as a result (e.g. Dittner, Rimes & Thorpe, 2011). It is suggested that acute fatigue can become chronic via a number of mechanisms including level of physical activity (e.g. too high or too low; ter Wolbeek et al. 2008), fearful beliefs about fatigue or the effects of fatigue (e.g. Stahl, Rimes & Chalder, 2014) or emotional difficulties such as anxiety or depression (Rangel, Garralda, Hall and Woodham, 2003; Rimes et al., 2007). Cognitive behavioural interventions typically aim to improve symptoms and functioning by a) encouraging patients to achieve a balance between activity and rest, b) gradually increasing activities, c) establishing a sleep routine, d) addressing unhelpful beliefs (e.g. about fatigue or high standards) and e) paying attention to relapse prevention. Although initial studies focused on face-to-face treatment, travelling to the clinic can cause extra fatigue, and more recent studies have found evidence to support the use of internet-based CBT (Nijhof et al., 2012) and telephone-based guided self-help (Lloyd, Chalder, Sallis and Rimes, 2012).

Cognitive behavioural interventions address a number of variables that influence cortisol levels including activity patterns and deconditioning, stress, sleep and personality characteristics associated with stress vulnerability. A previous study in adults with CFS found that daily cortisol output normalized after cognitive behavioural treatment (Roberts, Papadopoulos, Wessely, Chalder & Cleare, 2009). Nijhof et al. (2014) reported that adolescents who recovered in their trial after either CBT or “treatment-as-usual” (which might have been CBT or graded exercise therapy or a rehabilitation programme) had a significant rise in their cortisol awakening response. That paper did not report change in the cortisol awakening response after a single type of treatment such as CBT. There have been no previous studies reporting the cortisol awakening response after a single type of treatment such as CBT, or changes in cortisol output *over the day* before and after treatment. In the present study we investigated both cortisol output over the day and the cortisol awakening response before and after a telephone-based guided self-help cognitive behavioural intervention, which was the standard treatment on offer in our clinic at that time. We hypothesized that cortisol output would be significantly higher at six months after the end of cognitive behavioural guided self-help than at pre-treatment.

METHODS

Participants

Participants were 49 adolescents with CFS, and 36 healthy adolescents who were selected to match for age and gender. The CFS group was comprised of patients attending a specialist CFS Unit in London who met CDC (Fukuda et al., 1994) and Oxford (Sharpe et al., 1991) criteria for CFS. Assessment at the CFS clinic included the exclusion of depression or other psychiatric problems as the primary diagnosis. They had all been assessed by paediatricians who had conducted appropriate tests to exclude other possible diagnoses causing their fatigue. Self-reported mean duration of CFS was 25.3 months (SD=16.1). Saliva samples were taken prior to treatment. The healthy adolescents were recruited via local schools and were required to have no history of CFS and no current medical condition likely to cause excessive fatigue. Of the CFS sample, 24 returned post-treatment questionnaires and cortisol samples; of the remainder, three did not complete treatment and the others did not return usable post-treatment cortisol samples or questionnaires or both. Independent t-tests indicated that there were no significant differences between participants for whom post-treatment questionnaire and cortisol data were or were not available, in terms of age, duration of CFS, baseline fatigue (Chalder Fatigue Scale; Chalder et al., 1993) or any of the pre-treatment main cortisol measures ($t_s < 1.9$).

Procedure

The study was approved by the Institute of Psychiatry (King's College London) Ethics Committee, reference 011/00. Written informed consent was obtained from the young people and their parents. Questionnaire completion and saliva collection was undertaken at home. Participants were asked to collect the samples on a Saturday when they were able to wake up between 06:00 – 09:00. Samples were taken using untreated Salivettes. Participants were instructed to provide a sample of saliva immediately after waking, 15 mins, 30 mins, 45 mins and 60 mins after awakening, then at 12:00, 16:00 and 20:00 hours. Standard collection instructions were given, e.g. not to eat or brush teeth in the hour prior to collection. Participants were asked to note the time they took the samples and what they had been doing the previous hour before each sample, including anything eaten or drunk. Participants not complying with the collection instructions were not included. Samples were kept in the refrigerator overnight before being returned to the hospital. Cortisol was

measured for a second time at six months after the end of treatment, to allow time for consolidation of the treatment response and for any effects on the HPA axis to occur. The same saliva collection procedure was used at Time 2.

Treatment

Treatment consisted of telephone-based guided self-help (described in the Introduction). After face-to-face assessment, patients received a self-help manual and up to 6 fortnightly telephone sessions of 30 minutes. The primary clinical outcome was school attendance.

Clinical measures

School attendance, the primary clinical outcome, was calculated as a percentage of the time that they should be attending; hours at school and hours they should have attended were self-reported by the young person. Questionnaires completed were the Chalder Fatigue Scale (Chalder et al., 1993), the Birleson Depression Inventory (Birleson, 1981), the Spence Children's Anxiety Scale (Spence, 1998) and the Child and Adolescent Perfectionism Scale (Flett, Hewitt, Boucher, Davidson & Munro, 2000). The perfectionism questionnaire was scored in three subscales in line with previous research findings (O'Connor, Dixon & Rasmussen, 1990): self-oriented - striving perfectionism (e.g. "I try to be perfect in everything I do"); self-oriented - critical perfectionism (e.g. "I get mad at myself when I make a mistake" and socially-prescribed perfectionism (e.g. "Other people always expect me to be perfect"). The CFS patients also completed the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) which provides subscale scores for Difficulties (emotional and behavioural) and Prosocial Behaviour (e.g. "I try to be nice to other people"). This latter scale was used as a way of assessing high standards for personal conduct. All questionnaires except the perfectionism scale were repeated at Time 2.

Cortisol analysis

Saliva samples were collected as previously described (Roberts, Chalder, Papadopoulos & Cleare, 2004) in plain Sarstedt Salivettes which were stored at -40 °C until analysis in the hospital laboratory. Saliva cortisol concentrations were determined using the "Immulite" Siemens's Immunoassay System; www.diagnostics.siemens.com (Mondelli et al., 2010).

Statistical Analysis

Analyses were undertaken using the Statistical Package for Social Sciences (SPSS) version 15. Two-tailed tests were used. For the salivary cortisol day curve (the primary outcome), the total output from waking to 2000 hours was assessed by calculating the area under the curve (AUCday), using the trapezoidal method (Pruessner, Kirschbaum, Meinlschmid & Hellhammer, 2003). The mean cortisol over the day (8am, noon, 4pm and 8pm) was also calculated. To assess the cortisol awakening response, the area under the curve with respect to the increase (AUCi) and with respect to the ground (AUCg) were calculated. A third measure of the awakening response was calculated by subtracting the immediate awakening value from cortisol at 30 mins after waking. Change in cortisol responses was calculated by subtracting the values for the second time point from the first, e.g. change in AUCday = post-treatment AUCday minus pre-treatment AUCday.

For the pre-treatment group comparisons, AUCday, mean cortisol over the day and AUCg was not normally distributed, so were log-transformed prior to independent t-tests. AUCi, CAR as represented by the difference between cortisol at 30 mins after waking and immediate waking, the Chalder Fatigue Scale, Spence Anxiety Scale, Birleson Depression Inventory, Child and Adolescent Perfectionism Scale and Strengths and Difficulties Questionnaire (total difficulties and prosocial) were not normally distributed even after log transformation, so non-parametric analyses were used. Kolmogorov-Smirnoff tests were used for comparing two groups rather than Mann-Whitney tests as there were ties in the ranked data, and the Mann-Whitney is based on the assumption of no ties. Cortisol analyses were repeated excluding participants who may be depressed, to check whether any group differences could be accounted for by this factor. After excluding participants who scored 13 or above, the cut-off for likely depressive disorder on the Birleson Depression Inventory (Birleson, 1981), there were 20 CFS patients and 32 HC. Similar analyses involving the Birleson Depression Inventory were undertaken in which the item that referred to energy was excluded; the results showed a similar pattern and are not reported here. Between-group comparisons were also repeated excluding participants taking medications with the potential to affect cortisol production. These were doxycycline (n=1), propranolol (n=1), citalopram (n=1), paroxetine (n=1), amitriptyline (n=3), pizotifen (migraine medication), melatonin (n=3), and oral contraceptive (n=1). The medication exclusions

resulted in groups of 30 CFS and 33 HC participants for the AUCday analyses, and 29 CFS and 34 HC for AUCi and AUCg.

For the pre- to post-treatment analyses, AUCday and mean cortisol over the day were log-transformed prior to paired t-tests to compare scores before and after treatment. For AUCi and CAR delta (30 mins minus waking) pre-post comparisons, the non-normal distribution could not be corrected by transformation so non-parametric analyses were used (Wilcoxon Signed-Rank Tests).

RESULTS

Baseline demographic and clinical characteristics

The groups were not significantly different in age, gender or the proportion of female participants who had reached menarche (see Table 1). The mean time of waking was 0832h in the CFS group and 0835h in the HC group, which was not significantly different ($t(62)=-0.20$, $p=0.84$). The day of the menstrual cycle in the CFS females (mean=12.1, SD=9.0, if 1 is first day of menstrual bleeding) was not significantly differently different from the HC females (mean=14.0, SD=12.6; $t(28)=0.47$, $p=0.644$). The CFS participants had significantly higher fatigue and depression scores than the HC but were not significantly higher on anxiety or perfectionism (see Table 1).

Table 1. Sociodemographic, clinical characteristics and cortisol measures for adolescents with CFS and healthy controls

Variable	N	Adolescents with CFS		Healthy controls		Test statistics ^a	p
		CFS/HC	Mean SD	Mean SD	SD		
Age	49 / 36		14.9 1.7	15.0 1.7		t(83)=-0.32	0.748
Gender (female / male)	49 / 36	n = 31 / 18	63 / 37 %	n = 21 / 15	58 / 42 %	$\chi^2(1)=0.21$	0.645
Menarche reached (females only)	31 / 21	n=27	87%	n=18	86%	$\chi^2(1)=0.02$	0.886
		Median	IQR	Median	IQR		
Chalder Fatigue Scale (Likert)	49 / 36	23.0	21.0 – 23.0	11.0	10.3 – 12.8	Z=3.90	<0.0005
Spence Children's Anxiety Scale	47 / 36	16.0	9.0 – 34.0	16.5	8.0 – 22.8	Z=0.73	0.668
Birleson Depression Scale	45 / 35	13.0	9.0 – 17.0	5.0	2.0 – 7.0	Z=3.09	<0.0005
Child & Adol. Perfectionism Scale: Self-oriented - Striving	47 / 33	10.0	8.0 – 12.0	12.0	10.0 – 13.5	Z=1.12	0.106
Self-oriented -Critical	47 / 33	6.0	5.0 – 12.0	7.0	5.0 – 10.0	Z=0.91	0.374
Socially Prescribed	47 / 33	14.0	11.0 – 18.0	16.0	9.5 – 20.5	Z=0.50	0.962
Strengths & Difficulties Q: Difficulties	48 / -	11.0	9.0 -16.8				
Prosocial behaviour	49 / -	8.0	6.0 - 9.0				
Cortisol output over day: AUCday (nmol/L h)	46 / 33	Mean = 63.6	SD = 22.8	Mean = 71.7	SD = 19.5	t(77)=2.0	0.046
Mean cortisol over the day (8am, noon, 4pm, 8pm; nmol/L)	46 / 33	Mean = 5.6	SD = 2.0	Mean = 6.3	SD = 1.7	t(77)=1.8	0.078
Cortisol awakening response – AUCg (nmol/L min)	47 / 34	Mean =11.7	SD = 5.6	Mean = 12.0	SD = 6.2	t(79)=0.3	0.797
Cortisol awakening response – AUCi (nmol/L min)	47 / 34	Median =0.1	IQR= -1.7 - 4.0	Median= -0.3	IQR= -3.5 - 2.7	Z=0.76	0.608
Cortisol awakening response –30 mins minus wakening (nmol/L)	47 / 35	Median =0.8	IQR = -1.9 – 6.6	Median = 0.6	IQR = -3.3 – 5.4	Z=0.81	0.535

AUCday= area under the curve for the day; AUCg= area under curve with respect to ground; AUCi= area under curve with respect to increase; IQR = Interquartile Range.

^a Independent t-test, chi-square analysis or Kolmogorov-Smirnov test.

Cortisol excretion during the day

Saliva cortisol concentrations were numerically lower in the CFS group than the healthy participants at all time-points (see Figure 1) but significantly so at only 4pm ($Z=1.70$, $p=0.006$). The primary outcome, total cortisol output over the day (AUCday) was significantly lower in the CFS group than the HC group. (See Table 1. This group difference in AUCday remained if participants were excluded for having a score of 13 or higher on the Birleson Depression Inventory ($Z=1.56$, $p=0.016$) or for taking medications that may affect cortisol levels ($Z=1.38$, $p=0.044$). (Non-parametric analyses were used because logAUCday was not normally distributed for these samples). There was a non-significant trend for mean cortisol over the day to be lower in the CFS group (See Table 1).

Figure 1 about here

Associations between daily cortisol output (AUCday) and other characteristics

As the above results had indicated AUCday to be significantly reduced in the CFS group, exploratory investigations using Spearman's correlations were conducted to examine whether this cortisol measure was associated with clinical characteristics within this group. There was a significant association between AUCday and Self-Oriented Perfectionist Striving (Spearman's rho (r_s)=-0.298, $p=0.050$) and SDQ prosocial behaviour ($r_s = -0.425$, $p=0.003$). AUCday was not significantly associated with Socially Prescribed Perfectionism ($r_s=0.08$), Self-Oriented Perfectionism Critical ($r_s=-0.10$), SDQ Difficulties ($r_s=1.0$), Chalder Fatigue Scale ($r_s=0.06$), Birleson Depression Inventory ($r_s=0.05$) or Spence Children's Anxiety Scale ($r_s=-0.10$). There was no association between AUCday and duration of CFS ($r_s=-0.09$) or gender differences in AUCday ($Z=0.85$, $p=0.468$). For the healthy control group, there were no significant correlations between AUCday and questionnaire scores; the largest value for r_s was 0.29 ($p=0.120$) for the association for Self-Oriented Perfectionist Striving, which is in the opposite direction than in the CFS group.

Cortisol awakening response (CAR)

There were no significant differences between the two groups in terms of the area under the curve with respect to the increase (AUCi) or with respect to the ground (AUCg); see Table 1. Similarly, there were no

group differences if participants were excluded who scored 13 or above on the Birleson Depression Inventory or who were taking medication that might affect cortisol. There were no significant correlations between measures of CAR and questionnaire measures.

Change in clinical outcomes after treatment

There was a significant improvement in school attendance, the primary clinical outcome, from 24% to 49% ($Z(24)=-2.5$, $p=0.012$). The reduction on the Chalder Fatigue Scale (mean=24 (SD=5) to mean=21 (SD=21)) did not reach significance ($t(23)=1.5$, $p=0.14$). There was a significant reduction on the Spence Children's Anxiety Scale (mean=22 (SD=17) to mean=17 (SD=14); $t(21)=2.1$, $p=0.005$). The Birleson Depression Scale (excluding Q7 about energy) also showed a significant decrease (mean=11 (SD=6) to mean=8 (SD=5); $t(23)=2.1$, $p=0.044$). There were no significant changes on the SDQ scales ($Zs<2.0$).

Cortisol levels after treatment

The primary cortisol outcome was the AUCday and a paired t-test indicated that this had significantly increased (by 24%) after treatment (see Table 2). Post-treatment AUCday for the CFS patients was not significantly different from the AUCday for HC reported in Table 1 ($Z=0.786$, $p=0.567$). The cortisol awakening response showed no significant change after treatment; see Table 2.

Table 2. Salivary cortisol measures before and after treatment in adolescents with CFS

	n	Pre-treatment	Post-treatment	Test statistic ^a	P value
		Mean (SD)	Mean (SD)		
Cortisol output: Area Under the Curve for the Day ^b (nmol/L h)	21	58.0 (14.9)	72.0 (28.2)	$t=-2.2$	0.039*
Mean value across the day ^b (8am, noon, 4pm, 8pm; nmol/L)	21	5.3 (1.4)	6.4 (2.7)	$t=-1.7$	0.109
Cortisol Awakening Response - Area Under the Curve with respect to the ground (nmol/L min)	24	12.2 (4.5)	12.7 (5.4)	$t=-0.5$	0.613

Cortisol Awakening Response – Area Under the Curve with respect to the increase (nmol/L min)	24	Median (IQR) -0.14 (-1.6, 5.1)	Median (IQR) 0.74 (-1.6, 4.0)	Z= -0.5	0.648
Cortisol Awakening Response – delta (30 mins minus waking; nmol/L)	24	1.00 (-1.98 – 7.2)	1.55 (-1.78 – 5.8)	Z= -0.386	0.700

^a Paired t-test or Wilcoxon Signed-Rank Test ^b Log transformed prior to paired t-test

Associations between changes in cortisol output and clinical measures

Exploratory correlational analyses were undertaken to investigate possible associations between change in AUCday and clinical variables. There were no significant associations between changes in AUCday and changes between pre-treatment and six-month follow-up on percentage school attendance, Chalder Fatigue Scale, Birlson Depression Inventory, Spence Children's Anxiety Scale, SDQ Difficulties or SDQ Prosocial scales (all correlation coefficients < 0.36).

DISCUSSION

This is the first study to report that adolescents with CFS have significantly lower saliva cortisol secretion over the course of the day than age and gender-matched healthy controls. This is in line with research in adults with CFS (Jerjes et al., 2005). Lower daily cortisol output was associated with two psychological characteristics in the CFS participants: perfectionist striving and prosocial behaviour (kind, considerate, helpful and sharing behaviours). As far as the authors are aware, this is the first demonstration of associations between daily cortisol output and these characteristics, in young people or adults, in relation to CFS or other populations. In adults in the general population there is some evidence that perfectionism may be associated with *higher* cortisol responses to acute stressors in the laboratory (e.g. Wirtz et al., 2007), but it is possible that chronic stress may lead to a longer-term blunting of the cortisol response (Miller, Chen & Zhou 2007). In the present study, the association between mild hypocortisolism and perfectionism or prosocial behaviour may reflect a chronic stress response related to attempts to keep up high standards of

performance or personal conduct, in line with cognitive behavioural approaches to CFS (e.g. Lievesley, Rimes & Chalder, 2014; Surawy, Hackmann, Hawton & Sharpe, 1995). Indeed, in adults there is evidence from a prospective study that perfectionism at the time of a virus is associated with increased risk for CFS (Moss-Morris, Spence & Hou, 2011). It is possible that cortisol disturbance is one of the mechanisms underlying this relationship. However, the associations between cortisol and perfectionism and prosocial behaviour in the present study were the result of exploratory analyses and require replication in a second, larger sample, in which perceived stress should also be assessed. In the healthy control group, although the association between AUCday and perfectionist striving was not quite significant, it was in the opposite direction to that observed in the CFS group, with greater striving being positively associated with AUCday in healthy adolescents. There have been previous findings of different patterns of associations with perfectionism in adults with CFS than in healthy controls (Deary and Chalder, 2008), highlighting the need for an improved understanding about how perfectionism may be helpful or unhelpful for different populations or in different contexts.

Daily cortisol output (AUCday) increased significantly after cognitive behavioural guided self-help for chronic fatigue syndrome in adolescents. This is the first time that post-CBT daily cortisol output changes have been reported in adolescents with this condition. The findings are consistent with a previous study in adults with CFS which also found an increase in daily cortisol output after cognitive behavioural treatment (Roberts et al., 2009). Post-treatment AUCday for the CFS patients was not significantly different to AUCday for the healthy controls measured in the first part of the study. As this change took place within the context of a non-randomised cohort study, it cannot be concluded that the intervention was necessarily responsible for the normalisation of the daily cortisol output. However, the improvement after the cognitive behavioural intervention is encouraging, especially because hydrocortisone treatment cannot be recommended; research in adults has found limited evidence of benefit, together with loss of effects after discontinuation, and adrenal suppression as a side-effect (McKenzie et al., 1998; Cleare et al., 1999; Blockmans, Persoons, Van Houdenhove, Lejeune & Bobbaers, 2003). Comparison with an untreated CFS group would be necessary to clarify whether cortisol changes could have been due to natural course, but it was not considered ethical to withhold treatment, particularly as CFS can have such profound effects on socioemotional, educational and other aspects of development. Future research should examine cortisol

changes before and after a CBT intervention compared to a control intervention. Another limitation was that the Strengths and Difficulties Questionnaire was not administered in the healthy control group. We found no association between changes in cortisol output and changes in school attendance, fatigue, depression, anxiety, general emotional / behavioural difficulties or prosocial behaviour. Future studies should investigate the role of other possible explanatory factors including activity levels and sleep disturbance.

In contrast to the findings for daily cortisol output, there were no significant differences between adolescents with CFS and healthy controls for the salivary cortisol response to waking, unlike Nijhof et al. (2014). The reason for this is unclear but there were several differences between that study and the current one. For example, in the previous study, participants had been instructed to stay lying in bed for an hour after waking, and data was collected on a week day rather than the weekend. In the present study participants were asked to collect data on a day on which they were able to wake up between 06:00 and 09:00 whereas no time instructions were given by Nijhof et al. (2014) and they found that sleep duration was longer in their CFS group than the healthy controls. Future studies need to examine further a wide range of factors that may be affecting both the cortisol awakening response and data collection.

Strengths of the study include the use of a healthy control group matched for age, gender, menarche status, day of menstrual cycle and awakening time. Other confounding and explanatory factors such as body mass index, perceived stress and trauma experiences should be measured in future research. The CFS patients were consecutive attendees at a specialist CFS clinic but may not be representative of adolescents with CFS in other health clinics or the community. The cross-sectional design cannot address the issue of whether cortisol disturbance is a primary etiological factor; to address this issue, prospective designs are required. Further limitations are the sample size, which meant that some of the changes on clinical questionnaires did not reach statistical significance, unlike in the previous study investigating this guided self-help intervention with a larger sample (Lloyd et al., 2011). Furthermore, not all adolescents with CFS provided a cortisol sample at follow-up. Analyses investigating the relationship between cortisol measures and clinical variables were not adjusted for multiple testing due to the exploratory nature of these investigations; this increases the risk of false positives, so the findings require replication in future research.

Conclusions

Following this evidence of reduced daily cortisol output in female adolescents with CFS who have passed the menarche, further research into the potential role of hypocortisolism in the development or maintenance of CFS in adolescence is needed. The association between daily cortisol output and two psychological variables, perfectionist striving and prosocial behaviour, is a novel finding that requires replication. It was encouraging to find that the reduced daily cortisol output in adolescents with CFS had normalised six months after a course of cognitive behavioural treatment.

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Figure 1. Mean salivary cortisol over the day in adolescents with CFS and healthy adolescents

Highlights

- Salivary cortisol was investigated in adolescents with chronic fatigue syndrome.
- Reduced salivary cortisol output over the day was found.
- Cortisol output was associated with perfectionism and prosocial behaviour.
- The adolescents received cognitive behavioural guided self-help treatment.
- By six months post-treatment, cortisol output had increased up to normal levels.