The role of hypocortisolism in chronic fatigue syndrome

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KEYWORDS
Chronic fatigue; Adolescents; Cortisol; Awakening response; Recovery

Summary
Background: There is accumulating evidence of hypothalamic–pituitary–adrenal (HPA) axis hypofunction in chronic fatigue syndrome (CFS). However, knowledge of this hypofunction has so far come exclusively from research in adulthood, and its clinical significance remains unclear. The objective of the current study was to assess the role of the HPA-axis in adolescent CFS and recovery from adolescent CFS.

Method: Before treatment, we compared the salivary cortisol awakening response of 108 diagnosed adolescent CFS patients with that of a reference group of 38 healthy peers. Salivary cortisol awakening response was measured again after 6 months of treatment in CFS patients.

Results: Pre-treatment salivary cortisol levels were significantly lower in CFS-patients than in healthy controls. After treatment recovered patients had a significant rise in salivary cortisol output attaining normalization, whereas non-recovered patients improved slightly, but not significantly. The hypocortisolism found in CFS-patients was significantly correlated to the amount
of sleep. Logistic regression analysis showed that an increase of one standard deviation in the difference between pre- and post-treatment salivary cortisol awakening response was associated with a 93% higher odds of recovery (adjusted OR 1.93 (1.18 to 3.17), p = 0.009). Pre-treatment salivary cortisol did not predict recovery.

Conclusions: Hypocortisolism is associated with adolescent CFS. It is not pre-treatment cortisol but its change to normalization that is associated with treatment success. We suggest that this finding may have clinical implications regarding the adaptation of future treatment strategies.

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1. Background

Chronic fatigue syndrome (CFS) is characterized by unexplained persistent or relapsing disabling fatigue that lasts for at least 6 months and is accompanied by at least four out of eight possible symptoms (memory or concentration problems, sore throat, tender lymph nodes, muscle pain, multiple joint pain, headache, unrefreshing sleep, postexertional malaise) (Fukuda et al., 1994). CFS is found in adolescents as well as in adults. Its primary adverse impact in adolescents is extreme disability, associated with considerable school absence (Nijhof et al., 2011a,b).

Despite substantial research, a biological substrate for this syndrome has not yet been established. It is considered to be a multifactorial condition in which biological, psychological and social factors play a predisposing, precipitating or perpetuating role.

Hypofunction of the hypothalamic–pituitary–adrenal (HPA)-axis as manifested by a low salivary cortisol awakening response (CAR) (Tak et al., 2011) is the most replicated biological finding in CFS, but only through studies in adulthood (Cleare, 2003; Demitrack et al., 1991; Heim et al., 2000; Roberts et al., 2004; Tak et al., 2011). The exact role of this hypofunction remains unclear. It is not known whether this is a relevant biological factor in the aetiology of CFS. It has been hypothesized that lowered cortisol occurs, in part, secondarily to aspects of CFS such as disturbed sleep, inactivity or stress (Roberts et al., 2004; Tak et al., 2011).

In adolescents, CFS has to be identified and treated as soon as possible, to lower the risk of developmental and educational disturbances (Bell et al., 2001; Nijhof et al., 2011a,b). Cognitive behavioural therapy (CBT) is one of the most successful treatments for adolescents with CFS (Chalder et al., 2010; Nijhof et al., 2012; Stulemeijer et al., 2005). Perpetuating factors such as fatigue-related cognitions and behaviour, are addressed by CBT. However, not all patients suffering from CFS respond to CBT. It is of paramount importance to differentiate between responders and non-responders as early as possible in order to change content, duration, or choice of treatment.

The factors that influence treatment outcomes in adolescent CFS are largely unknown. A number of factors in young patient and parents have been revealed to be associated with an unfavourable outcome after treatment (van de Putte et al., 2006; van Geelen et al., 2010; Knoop et al., 2008; Nijhof et al., 2013). Most consistent factors are an older age at inclusion (van Geelen et al., 2010), longer disease duration before start of treatment (Nijhof et al., 2013), and maternal focus on fatigue or bodily symptoms (Knoop et al., 2008; Nijhof et al., 2013; van de Putte et al., 2006). However, none of these factors provide direct clues for pathophysiological treatment targeting and monitoring during treatment, while continued HPA-axis hypofunction monitoring might serve that purpose. Only in adults, was an association found between HPA-axis hypofunction and a poor response to CBT (Roberts et al., 2010), suggesting that hypocortisolism could be a factor in the persistence of CFS.

It has not been studied whether the change in HPA-axis after treatment is related to recovery from CFS in adolescents. We hypothesized that HPA-axis hypofunction is a relevant biological factor in adolescent CFS, and that an association exists between recovery from CFS and a change in cortisol levels after treatment.

The aims of our study were: to examine the association between salivary cortisol response to awakening and CFS, through (1) comparison between healthy adolescents and CFS adolescents, and (2) investigation of the change in salivary cortisol response to awakening in relation to recovery of CFS.

2. Methods

2.1. Subjects

One hundred and twenty-three adolescents (12–18 years) diagnosed with CFS participating in the FITNET (Fatigue In Teenagers on the internet) trial (Nijhof et al., 2011a,b, 2012) were invited to participate in this cohort study on cortisol between March, 2008 and February, 2010. 118 (96%) patients agreed to participate. They all complied with CDC-criteria for CFS diagnosis (Fukuda et al., 1994). FITNET, an internet-based CBT program for adolescents with CFS, was developed as an alternative to face-to-face CBT (Nijhof et al., 2011a,b, 2012). A detailed description of the FITNET study protocol, methodology, and program has been reported elsewhere (Nijhof et al., 2011a,b, 2012). In the original trial, all patients were randomly assigned to either FITNET or usual care (pre-treatment). Participants were reassessed after 6 months of treatment (post-treatment).

As a reference group, healthy participants to a previous CFS research were re-invited between March 2009 and February 2010 (Nijhof et al., 2011a,b). Of these, adolescents with neurological abnormalities, chronic illnesses, or under treatment by a psychiatrist or psychologist were excluded from participation. Thirty-nine of 58 eligible healthy adolescents (67%) completed the saliva-sampling protocol. In view of the demographic characteristics and fatigue levels, both the CFS-patients and healthy peers were unselected samples of former studies (Nijhof et al., 2011a,b, 2012).

The medical ethics committee of the University Medical Centre Utrecht (UMCU) approved this study. Written
informed consent was obtained from the adolescents and their parent(s).

2.2. Questionnaires

Questionnaires were used to assess pre-treatment characteristics and recovery from CFS post-treatment (Nijhof et al., 2011a,b, 2012). Participants were asked to fill out these questionnaires without assistance from their parent(s) or the researcher. Length of illness was derived from the paediatrician’s history.

Fatigue was assessed using the Checklist Individual Strength (CIS-20), subscale ‘fatigue severity’ (range 8–56). The CIS-20 has excellent internal consistency (Cronbach’s α = 0.93) and discriminative validity for CFS (Vercoulen et al., 1994). Physical functioning was measured using the Child Health Questionnaire (CHQ-CF87), subscale ‘physical functioning’ (range 0–100%). This scale has been validated as having a good internal consistency (Cronbach’s α = 0.86) (Raat et al., 2002). Actual physical activity was measured by an actometer: a motion-sensing device attached to the ankle and worn continuously for 12 days (Scheeres et al., 2009; van der Werf et al., 2000). The mean hours of sleep registered by the actometer were used to assess the sleeping pattern. During these twelve days, school attendance was registered and calculated as the proportion of classes attended, expressed as a percentage of the normal school schedule (Nijhof et al., 2012).

Depression in adolescents was measured pre-treatment with a validated Dutch translation of the Children’s Depression Inventory (CDI) (Kovacs, 1985). Cronbach’s α reliability coefficients range from 0.71 to 0.89. Finally, ‘self-rated improvement’ (SRI) was measured post-treatment using a 4-item tool in which patients indicate whether they have completely recovered, feel much better, have the same complaints or have become worse compared to the previous measurement (Nijhof et al., 2012).

2.3. Cortisol-sampling procedure and assay

The circadian rhythm of the HPA-axis develops within the first three to four years of life. After this period of time it reaches an adult, diurnal rhythm with the highest levels of cortisol in the morning and the lowest during the night (Jessop and Turner-Cobb, 2008). Awakening is a mild physiological stressor for the HPA-axis, resulting in a rapid increase in cortisol, referred to as the cortisol awakening response (CAR) (Clow et al., 2004). The CAR is indicative of the HPA-axis responsiveness (Wust et al., 2000).

Measurement of salivary cortisol in children has been standard practice for more than 20 years. It is a non-invasive technique allowing cortisol to be measured without possible interference with the stress of intravenous cannulation and hospital attendance (Jessop and Turner-Cobb, 2008).

Saliva was collected at home using the Salivette® sampling device (Sarstedt BV, The Netherlands). Participants were instructed to chew gently on sterile cotton wool swabs immediately after spontaneously awakening, and 15, 30 and 60 min thereafter while still lying in bed (Kupper et al., 2005; ter Wolbeek et al., 2007). They were asked to sample on a weekday. During collection, subjects were instructed not to touch the samples with their hands. They were not allowed to brush their teeth, nor to eat or drink, except for water, during the sampling period. Furthermore, if possible, they were requested not to take any medication during the week before testing and were asked not to collect saliva when they were ill. Participants reported bedtimes and exact times of saliva collection. The CAR is sensitive to various factors, such as use of oral contraceptive, BMI, smoking, physical activity, and sleep-related factors (Fries et al., 2009; Puressner et al., 1997; Steptoe and Ussher, 2006; Wust et al., 2000). Therefore, these factors were registered in order to check for potential influence on endocrine functioning.

Samples were stored in the refrigerator immediately after collection. Within two weeks they were returned to the laboratory of the UMCO, where they were stored at −20 °C and analysed using in-house radioimmunoassay, with all intra-individual samples together in the same batch. Cortisol is stable enough to endure these temperature shifts (Tornage, 2009). Previous studies have shown the long-term stability of cortisol samples stored frozen, even over decades, with little degradation or desiccation (Garde and Hansen, 2005; Kley and Rick, 1984; Stroud et al., 2007).

Laboratory personnel were blinded to the clinical status of the subject and the study design. The lower limit of detection was 1.0 nmol/L. Intra- and inter-assay variations were 4% and 5–9%, respectively. Reference values for salivary cortisol used by the endocrinological laboratory were 9–29 nmol/L for cortisol collected in the morning.

2.4. Cortisol response estimation

The total cortisol response to awakening (nmol/L/h) was measured as the integrated area under the curve with respect to the ground (AUCG). The trapezoidal method was used to calculate the AUCG (Fekedulegn et al., 2007; Puressner et al., 2003). Subjects with a negative slope in cortisol concentration were excluded from analysis because a negative slope indicates that the samples were taken after the initial physiological rise in cortisol (Kupper et al., 2005). At pre-treatment, 10 (8.5%) patients and one healthy peer showed a negative slope (2.6%). Post-treatment saliva specimens of 112 (95%) participants were obtained, of which five patients (4.5%) were excluded from analysis because of a negative slope. After exclusion of these cases with a negative slope, respectively, 108 CFS-patients and 38 peers were included in the pre-treatment cortisol analyses, and 107 CFS-patients post-treatment. The pre-treatment characteristics of excluded participants from analyses, did not differ from those who adhered to the study schedule.

If a cortisol measurement was missing from valid curve estimations, we imputed this missing value (19 missed sample points, 1.4%). No participant missed more than one individual cortisol measurement. With the assumption of randomly missing values, we applied the following imputation method: the mean cortisol levels of all participants at the five individual measuring points (0, 15, 30, 60 min after awakening) were calculated. Second, the degree of increase (percentage) in cortisol level between two measuring points (e.g. between 0 and 15 min after awakening, etc.) was calculated. This percentage of increase was then applied to the individual participant to calculate the missing value. This method
was used to take into account the inter-individual differences in cortisol output.

### 2.5. CFS recovery definition

Recovery from CFS was defined in accordance with the FITNET trial as a combination of fatigue scores (CIS-20 fatigue scale < 40), physical functioning (CHQ physical functioning scale ≥ 85%), school attendance within normal limits (> 90%), and whether the patient rates him- or herself as having recovered (SRI: “I have completely recovered” or “I feel much better”) (Nijhof et al., 2011a,b, 2012).

### 2.6. Statistical analyses

First, we analysed differences in relevant characteristics between adolescents with and without CFS. Depending on distributions of continuous parameters, (non)parametric statistics were applied; tests for proportional data were applied where appropriate.

Second, we analysed (change of) cortisol response as a determinant of recovery. To that end, we first evaluated possible confounders of that relationship, tabulating pre-treatment characteristics by the two categories of the determinant (above and below mean AUCG). Subsequently, we have analysed cortisol response as a determinant of recovery using logistic regression with recovery (yes/no) as dependent variable and cortisol response as independent variable. Both change in AUCG (delta AUCG post-treatment—pre-treatment) and pre-treatment AUCG were entered as independent variables. Data were expressed as absolute measures in nmol/L/h and z-scores. The same model was used for the adjustment with possible confounders. Differences within groups at different points of measurement were tested using a paired-samples t-test.

The significance level for all group comparisons and regression modelling was set at p < 0.05. Statistical analyses were performed using IBM SPSS Statistics 20.

### 3. Results

Our first research aim was to assess whether cortisol awakening responses (CAR) were different between CFS patients and healthy controls. CFS-patients reported significantly (p < 0.01) more fatigue (51.4 ± 4.3 vs. 20.5 ± 10.2), physical disabilities (59.0% ± 16.7 vs. 96.0% ± 6.9), and school absence (56.7% ± 31.1 vs. 1.2% ± 3.8) than healthy controls. Table 1 shows pre-treatment characteristics of CFS patients and healthy controls. More CFS patients used medication, particularly contraceptives. Of CFS-patients, 30 used oral contraceptives, 3 non-systemic corticosteroids (inhalation or cream), and 26 used other medication, such as paracetamol or melatonin (n = 2). Among healthy controls, one adolescent used medication (paracetamol), eight others used contraceptives. Fig. 1a shows that CFS-patients had significantly lower cortisol levels than controls at 0, 15 and 30 min after awakening, resulting in a significantly lower AUCG (AUCG CFS: 756.7 ± 200.4; AUCG controls: 912.0 ± 241.9, mean difference

### Table 1 Baseline (pre-treatment) characteristics of CFS patients and healthy controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy controls (n = 39)</th>
<th>CFS-patients (n = 118)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol levels (nmol/L/h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>912.0 (241.9)</td>
<td>756.7 (200.4)</td>
<td>0.001&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.2 (0.5)</td>
<td>15.8 (1.4)</td>
<td>0.005&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Girls&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.1</td>
<td>79.7</td>
<td>0.055&lt;sup&gt;∗∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.3 (2.9)</td>
<td>21.0 (2.8)</td>
<td>0.246&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Illness duration (months)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>16.5 (11.0)</td>
<td>—</td>
</tr>
<tr>
<td>Psychopathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1 (5.5)</td>
<td>11.3 (5.3)</td>
<td>&lt;0.001&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Activity and sleep characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.4 (18.9)</td>
<td>62.7 (16.7)</td>
<td>&lt;0.001&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sleep during 2 weeks&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8 h48 (0 h33)</td>
<td>9 h48 (1 h14)</td>
<td>0.001&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sleep duration before CAR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8 h46 (1 h33)</td>
<td>9 h26 (1 h45)</td>
<td>0.036&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Awakening time&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8 h43 (1 h20)</td>
<td>8 h43 (1 h25)</td>
<td>0.970&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bed time&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22 h53 (0 h46)</td>
<td>22 h15 (1 h33)</td>
<td>0.001&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sample characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication use&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.6</td>
<td>50.4</td>
<td>0.002&lt;sup&gt;∗∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oral contraceptives (% of girls)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.0</td>
<td>40.9</td>
<td>0.345&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smoking&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8</td>
<td>2.6</td>
<td>1.000&lt;sup&gt;∗∗&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Depression score as measured with CDI score, physical activity as measured with an actometer in number of accelerations per 5-min period, sleep during 2 weeks and before CAR in hours. BMI is the Body Mass Index.

<sup>a</sup> Mean (SD).

<sup>b</sup> Percentage.

<sup>c</sup> Median (IQR).

<sup>∗</sup> Independent samples student t-test/Mann–Whitney U-test.

<sup>∗∗</sup> χ²-test/Fisher’s Exact test.
Cortisol Awakening Response (CAR)

A. pre treatment

B. post treatment

![Graph showing cortisol levels before and after treatment for CFS patients and healthy controls.](Image)

**Fig. 1** Cortisol Awakening Response in CFS patients and healthy controls at baseline (pre-treatment), as well as in recovered and non-recovered patients post-treatment. Adjusted for gender, age, BMI, depression score, sleep duration and physical activity (actometer).

(95% CI): $-155.3$ ($-242.9$, $-67.7$), $p = 0.001$). Additional adjustment for the possible confounders, age, gender, BMI, depression score, sleep duration, and activity level (actometer), had no effect on the results (Fig. 1a).

Our second research aim was to assess whether pre-treatment (or change of AUCG) of CFS patients is associated with recovery. Overall, 43 of CFS patients were recovered post-treatment; 5 (9%) assigned to usual care and 38 (62%) assigned to FitNet. Pre-treatment, there were no significant differences between recovered and non-recovered patients (AUCG: $749.3 \pm 174.3$ vs. $761.9 \pm 218.0$, mean difference (95% CI): $-12.6$ ($-89.0$, $63.8$), $p = 0.744$).

Table 2 shows characteristics of CFS patients dichotomized by below and above mean AUCG levels. Only sleep duration before CAR was clearly significantly different and therefore a possible confounder for this relationship. Next, we analysed whether absolute changes of salivary cortisol response between pre- and post-treatment were associated with recovery. Table 3 shows a statistically significant positive relation between change of AUCG and recovery. Pre-treatment AUCG was not a significant predictor of recovery. Adjustment for possible confounders, age, gender, BMI, depression score, sleep duration, and activity level, had no effect on the results. Indeed, with recovery, post-treatment AUCG had significantly increased compared to pre-treatment levels (mean difference in AUCG (95% CI): 175.0 (98.1–251.8), $p < 0.001$) and more so as compared with non-recovery (AUCG: post-treatment mean difference AUCG recovered–non-recovered (95% CI): 98.3 (6.3–190.3), $p = 0.037$), resulting in an improvement of the AUCG of recovered patients to ‘normal’ levels (Fig. 1b). In contrast, salivary cortisol levels of non-recovered patients post-treatment improved slightly but not significantly; AUCG: MD (95% CI): 47.2 ($-13.6$–108.0), $p = 0.123$.

4. Discussion

This study is the first in which HPA-axis functioning was assessed in a large group of adolescents with CFS, compared to healthy peers, with a 6-month follow-up. The data support our hypothesis that an association exists between recovery from CFS and a change in cortisol levels after treatment. We identified a mild hypofunction of the HPA-axis at CFS diagnosis compared to healthy peers, manifesting itself in a significantly impaired salivary cortisol awakening response. This initial hypocortisolism was significantly reversed after recovery from CFS, whereas non-recovered adolescents had a persistent hypocortisolism. Hypocortisolism may thus represent an aspect of the illness, disappearing with its recovery. Pre-treatment cortisol levels did not predict recovery. Although the amount of sleep was significantly associated with hypocortisolism, we were not able to identify ‘disease-behaviour’ or pre-treatment characteristics as potential
Table 2 Pre-treatment characteristics of CFS-patients dichotomized by above or below the mean AUCG of salivary cortisol.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>&lt;756.7 nmol/L (n = 61)</th>
<th>&gt;756.7 nmol/L (n = 47)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td>15.7 (1.3)</td>
<td>15.9 (1.4)</td>
<td>0.665*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>77.0</td>
<td>80.9</td>
<td>0.813†</td>
</tr>
<tr>
<td>Illness duration (months)</td>
<td>21.0 (2.6)</td>
<td>20.8 (2.8)</td>
<td>0.815†</td>
</tr>
<tr>
<td>Randomization to FitNet at T0</td>
<td>17.0 (10.0)</td>
<td>16.0 (11.0)</td>
<td>0.872‡</td>
</tr>
<tr>
<td>Depression</td>
<td>11.0 (5.2)</td>
<td>11.8 (5.6)</td>
<td>0.488*</td>
</tr>
<tr>
<td>Physical activity</td>
<td>62.3 (15.3)</td>
<td>63.5 (18.5)</td>
<td>0.718*</td>
</tr>
<tr>
<td>Sleep during 2 weeks</td>
<td>9 h54 (h10)</td>
<td>9 h37 (h19)</td>
<td>0.221†</td>
</tr>
<tr>
<td>Sleep duration before CAR</td>
<td>9 h55 (1h38)</td>
<td>8 h52 (1h1)</td>
<td>0.002‡</td>
</tr>
<tr>
<td>Medication use</td>
<td>44.3</td>
<td>56.5</td>
<td>0.244*</td>
</tr>
<tr>
<td>Oral contraceptives (% of girls)</td>
<td>36.1</td>
<td>43.6</td>
<td>0.638**</td>
</tr>
</tbody>
</table>

Depression score as measured with CDI score, physical activity as measured with an actometer in number of accelerations per 5-min period, sleep during 2 weeks and before CAR in hours. BMI is the Body Mass Index.

* Mean (SD).
† Percentage.
‡ Median (IQR).
§ Independent samples student t-test/Mann–Whitney U-test.
** χ²-test/Fisher’s Exact test.

Our study has limitations. The HPA-axis does not function in isolation, but interacts with other systems in the body, such as other parts of the central nervous system (Chrousos et al., 2009; Cleare, 2004). Because we studied cortisol output in response to awakening, we examined one part of the HPA-axis and should take this into account when interpreting the results of this study. Assessing awakening cortisol levels however has been accepted as a good indication of the functioning of the HPA-axis (Jessop and Turner-Cobb, 2008; Wust et al., 2000). The fact that we only sampled on one day, instead of two or more consecutive days, might be considered a limitation (Papadopoulos et al., 2009). However, it has been shown that the cortisol awakening response is stable over time, on both intra- and inter-individual levels (ter Wolbeek et al., 2007; Wust et al., 2000).

A strength of this study is the sample size of CFS-patients, which is large, relative to other studies (Tak et al., 2011).

Table 3 Relation of AUCG to recovery.

<table>
<thead>
<tr>
<th>AUCG</th>
<th>Non-adjusted OR (95% CI)</th>
<th>p-Value</th>
<th>Adjusted OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔAUCG</td>
<td>1.81 (1.13–2.90)</td>
<td>0.014</td>
<td>1.93 (1.18–3.17)</td>
<td>0.009</td>
</tr>
<tr>
<td>Pre-treatment AUCG</td>
<td>1.00 (0.99–1.00)</td>
<td>0.753</td>
<td>1.00 (0.99–1.00)</td>
<td>0.988</td>
</tr>
</tbody>
</table>

OR is the odds ratio, Δ: difference post-treatment–pre-treatment.

Adjusted for age, gender, BMI, pre-treatment disease duration, activity level, and hours of sleep before CAR.

Data of AUCG (absolute measures in nmol/L/h) were expressed as z-scores.

In our cohort study we have assessed all possible confounders simultaneously. The meta-analysis by Tak et al. (2011) suggests a multifactorial model of HPA axis dysfunction in CFS in which levels of physical activity, presence of depression and use of medication did moderate the findings of low cortisol. Nevertheless, most studies have not assessed all these factors simultaneously; consequently, the degree to which factors affect the HPA axis remains unclear (Tak et al., 2011). Other advantages of our study include a non-stressful method of assessing a biologically active hormone, and because patients acted as their own controls before and after treatment we excluded intra-individual factors that might complicate comparisons of HPA-axis assessments.

Our results concerning salivary hypocortisolism are in line with studies in adult CFS where hypocortisolism has consistently been demonstrated (Heim et al., 2000; Tak et al., 2011). Our finding that a lower AUCG was significantly associated with more hours of sleep in the night prior to sampling, is in agreement with prior studies, except one (Federenko et al., 2004) showing that cortisol increases when the amount of sleep decreases (Cleare, 2003; Kudielka and Kirschbaum, 2003; Wust et al., 2000). Also, the reversion of initial hypocortisolism, which could be attributed to recovery from CFS, is in concordance with a normalization in cortisol levels that
has been shown after CBT in one study with adult CFS-
patients (Roberts et al., 2009).

We could not confirm that CFS-patients with a more
dysregulated pre-treatment HPA-axis, e.g. with lower levels
of salivary cortisol, responded less to treatment, as the adult
study of Roberts et al. (2010) suggested.

Instead, our data shows that normalization of cortisol
levels is associated with treatment success. This suggests
that hypofunction of the HPA-axis in patients with CFS might
be part of the symptom complex.

In our study, the association between the difference
between pre- and post-treatment salivary cortisol and recov-
er of CFS is not explained by behavioural symptoms such as
disturbed sleep pattern and physical activity. HPA-axis hypo-
function solely as a secondary response to the illness and its
associated behaviour is therefore unlikely. Alternatively, low
salivary cortisol could represent an epiphenomenon of the
illness, or a nonspecific response to a chronic illness (Fries
et al., 2005; Van Houdenhove et al., 2009).

Regardless of whether disruption of the HPA axis is primary
or secondary, a greater understanding of the complexities of
CFS is gained from understanding the mechanisms by which
HPA-axis changes occur. This knowledge could improve the
treatment of CFS. CBT is the current mainstay of treatment of
adolescent CFS, but not all patients recover. Identifying along
treatment the individuals with a lower chance of ultimate
treatment success is important, since a delay in recovery has
a severe impact on social and educational development.
Assessing pre-treatment hypocortisolism did not identify
these patients. More importantly, the change in hypocorti-
solism seemed to be associated with recovery. We advocate
that given a difference post-treatment and no difference
pre-treatment, that difference must have originated some-
where in between that time span, and that may be of high
clinical interest in the future. If the conversion moment were
to be known it may be possible to use that as a predictor of
recovery earlier during treatment. This hypothesis empha-
sizes that assessing HPA-axis functioning during treatment
alongside with the outcome parameters could earlier identify
those likely not to recover. Cortisol might serve as a process
‘biomarker’ for treatment success.

It has previously been demonstrated that cortisol-replace-
ment therapy can lead to a significant, temporary, improve-
ment of fatigue, disability and other features of CFS in adults
(Cleare et al., 1999; McKenzie et al., 1998). However, it is not
recommended as a treatment of choice in CFS, due to the
observation that only a minority of patients gain benefit, and
the long-term effects of cortisol-replacement are unknown. It
is possible that only a subgroup of patients benefit.

Future research could be aimed at assessing HPA-axis
function repeatedly during therapy, and the subsequent
role of additional low-dose hydrocortisone to enhance
CBT effectiveness.

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The sponsor of the study had no role in study design, data
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Conflict of interest

None declared.

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