# Circadian cortisol and fatigue severity in relapsing-remitting multiple sclerosis

Daniel J.H. Powell [a,b,*], Rona Moss-Morris [b,c], Christina Liossi [b],
Wolff Schlotz [b,d]

[a] Aberdeen Health Psychology Group, Institute of Applied Health Science, University of Aberdeen, AB25 2ZD, UK
[b] Psychology, Faculty of Social and Human Sciences, University of Southampton, SO17 1BJ, UK
[c] Health Psychology Section, Institute of Psychiatry, King's College London, London SE1 9RT, UK
[d] Max Planck Institute of Empirical Aesthetics, 60322 Frankfurt am Main, Germany

**Summary**    Cortisol is a key regulator of the immune system, energy metabolism, and stress, yet its relevance to fatigue experienced by people with relapsing-remitting multiple sclerosis (RRMS) remains uncertain. We examined cortisol secretory activity in RRMS and its association with fatigue severity between-individuals and within-individuals (day-to-day) using a case—control ecological momentary assessment design. While undergoing usual daily routines, 38 people with RRMS and 38 healthy control participants provided saliva samples at strategic time-points over 4 consecutive weekdays to measure the cortisol awakening response (CAR; 0, 30, and 45 min after awakening) and the diurnal cortisol slope (DCS; 6 quasi-random samples provided between 1000 h and 2000 h). Recalled fatigue was measured at baseline, and daily fatigue was measured as the mean average of momentary fatigue ratings provided alongside each DCS sample. Multilevel modeling found CAR output was greater in RRMS than controls, and recalled fatigue in RRMS was associated with both lower waking cortisol level and larger awakening response. Day-to-day, the CAR was not associated with same-day fatigue levels in RRMS. Cortisol appears to have a role in fatigue experienced in RRMS, but whether it is a causal factor remains unclear.
© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

Multiple sclerosis (MS) is a disease characterized by autoimmune-mediated inflammatory demyelination and neurodegeneration (Compston and Coles, 2008). Fatigue is frequently described as one of the most common and disabling symptoms in MS, affecting 60—85% of people with MS

* Corresponding author at: Aberdeen Health Psychology Group, 2nd Floor, Health Sciences Building, Foresterhill Campus, University of Aberdeen, AB25 2ZD, UK. Tel.: +44 1224 438101; fax: +44 1224 438165.
    E-mail address: daniel.powell@abdn.ac.uk (D.J.H. Powell).

(Lerdal et al., 2003; Minden et al., 2006). Current understanding of the etiology of MS fatigue is uncertain, with many disease-mediated mechanisms and secondary factors such as depression and sleep dysfunction potentially implicated (Induruwa et al., 2012).

With no licensed treatment for MS fatigue, robust information about the underlying mechanisms of fatigue experience is required in order to identify potential targets for intervention. There are several mechanisms by which cortisol, the adrenal product of the hypothalamic—pituitary—adrenal (HPA) axis, may be relevant to MS fatigue: (1) cortisol is generally considered the primary endogenous regulator of immune inflammation (Chrousos, 1995); (2) cortisol is an important regulator of energy metabolism via glycogenesis promotion (Sapolsky et al., 2000); and (3) while several reviews highlight a positive association between stressful experience and risk of MS symptom exacerbation (Mohr et al., 2004; Artemiadis et al., 2011), cortisol is fundamental within the stress response (Dickerson and Kemeny, 2004). Hypocortisolism is commonly reported in chronic fatigue syndrome (Papadopoulos and Cleare, 2012) but the importance of HPA axis (dys)function to fatigue experienced by individuals with other chronic conditions has received relatively little attention (Powell et al., 2013). Our understanding of unstimulated cortisol secretory activity in MS and its relevance to MS fatigue is limited, particularly in daily life.

## 1.1. The HPA axis in MS

HPA axis non-suppression by dexamethasone appears most prevalent in disease-active MS, such as in relapse-phase relapsing-remitting MS (RRMS) and progressive MS-types (Ysrraelit et al., 2008). HPA axis hyper-reactivity to CRH stimulation has been reported in MS (e.g., Fassbender et al., 1998) but studies describe both positive and negative associations between Dex/CRH test (Heuser et al., 1994) outcomes and gadolinium-enhancing lesions on MRI (Fassbender et al., 1998; Schumann et al., 2002): an important marker of inflammatory disease activity.

Greater 24h urinary cortisol outputs have been reported in people with MS compared with healthy controls (Ysrraelit et al., 2008) but 24h urinary cortisol observations provide little information regarding cortisol circadian rhythm dynamics. More recently, 2-day salivary cortisol studies have compared facets of the cortisol circadian rhythm in people with MS and healthy controls (Gold et al., 2010; Kern et al., 2011, 2013). Hyper-secretion of cortisol has been reported within the cortisol awakening response (CAR) (Kern et al., 2011, 2013) and in the evening (Gold et al., 2010) in people with RRMS.

Depressive symptomatology may underlie HPA axis hyper-activity in MS, with HPA axis hyperactivity a relatively consistent biological marker in major depressive disorder (Pariante and Lightman, 2008). Several studies have provided empirical support for this hypothesis in MS, but findings remain mixed. Gold et al. (2010) observed that people with RRMS and high levels of depressive symptoms had flatter diurnal cortisol slopes (DCS) and higher evening cortisol levels than people with RRMS and low depressive symptom scores. In a later study, the same research group

reported flatter DCS and greater daily cortisol output in individuals with RRMS and a clinical diagnosis of major depressive disorder compared to individuals with RRMS but without comorbidity (Gold et al., 2011). A further study in RRMS found CARs were larger in a group with more depressive symptoms (Kern et al., 2011). However, most recently, Kern et al. (2013) found no association between depressive symptoms and the CAR in either RRMS or secondary progressive MS.

## 1.2. HPA axis activity and MS fatigue

Current evidence suggests reduced dynamic cortisol variability is more relevant to fatigue experience than lower cortisol outputs, per se (Powell et al., 2013). Gold et al. (2011) remains the only study to examine associations between salivary cortisol and MS fatigue, reporting no association between fatigue and either the CAR or DCS in a RRMS sample where nearly a quarter also had major depressive disorder. Given that fatigue is a common symptom in depression (American Psychiatric Association, 2000), the role of cortisol in the severity of MS-related fatigue remains unclear.

Several qualitative studies have indicated that individuals describe very different levels of symptom intensity from day to day (e.g., Mills and Young, 2008). However, very little research has sought to understand within-person variability in fatigue severity in any population. There is a growing literature proposing that state variation in the CAR is implicated in same-day experience (Powell and Schlotz, 2012; Law et al., 2013). Small yet statistically significant within-person associations have been reported between smaller CARs and greater levels of same-day exhaustion in individuals with burnout (Sonnenschein et al., 2007), while two studies have described within-person associations between low morning cortisol and greater levels of same-day physical symptoms in non-clinical populations (Adam et al., 2006; Gartland et al., 2014). The role of the CAR in daily fatigue experience in MS warrants investigation.

## 1.3. Aims

This daily life study aimed to explore the relationship between cortisol and fatigue severity in RRMS while addressing several limitations of previous studies, which have used relatively short 2-day saliva-sampling protocols with unmonitored compliance, and often included participants with comorbidities. There were two primary objectives: First, to comprehensively explore two facets of the cortisol circadian rhythm (CAR and DCS) in a RRMS group without multi-morbidity; and second, to examine associations between cortisol secretory activity and fatigue severity in RRMS. The following hypotheses were tested in a case—control design: (1) the CAR is larger and DCS is flatter in people with RRMS compared to healthy individuals, independent of depressive symptoms and chronic stress; (2) fatigue severity is associated with an attenuated CAR and a flatter DCS in both groups; (3) within individuals, attenuations in CAR are associated with greater same-day fatigue severity in both groups.

## 2. Materials and methods

Ethical approval was granted by the UK NHS National Research Ethics Service Committee (11/SC/0333) and University of Southampton Psychology Ethics Committee (589). All data were anonymized before analysis, and written informed consent was provided by all participants.

### 2.1. Participants

We recruited 42 people with clinically definite RRMS (Polman et al., 2011), and 40 age- and sex-matched healthy individuals. The RRMS group was recruited between February 2012 and February 2013 from consecutive attendants at neurologist and specialist-nurse clinics at University Hospital Southampton NHS Foundation Trust and Guy's and St Thomas' NHS Foundation Trust, and from UK MS Society postings in Hampshire and Greater London. Of those individuals approached to be part of the RRMS group, 37.1% (76/205) met all eligibility criteria, and 55.3% (42/76) went on to enroll and participate in the study. Control participants were recruited via newspaper and University intranet postings. All participants were of working age (18—65 years), and fluent in the English language.

The RRMS group had several exclusion criteria: a clinical relapse and/or corticosteroid treatment within 3 months; inability to ambulate 300 m without rest with/without use of a walking aid; a diagnosed physical or psychiatric comorbidity; a Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983) depression subscale score $\geq 8$, indicative of moderate depression; current antidepressant medication use; pregnancy; care-giving; and shift-working. Current use of a disease modifying therapy (DMT) was permitted. The control group had the following exclusion criteria: an acute or chronic disease or illness; current use of prescribed medication; pregnancy; care-giving; and shift-working.

Within the RRMS group, one participant withdrew citing incompatibility of the study protocol alongside their job, one withdrew due to non-MS related illness, and two participants' data were lost due to technical faults. Within the control group, two participants were later excluded due to consistent extreme and outlying salivary cortisol levels indicative of endocrine abnormality. This redundancy left 38 well-matched individuals in each group. Participant characteristics are presented in Table 1.

### 2.2. Study procedure

The study implemented an ecological momentary assessment (EMA) design, measuring salivary cortisol and real-time assessments of fatigue severity with high ecological validity and limited recall bias (Stone and Shiffman, 1994; Schlotz, 2012). All participants began by attended one-to-one introductory sessions at the University of Southampton or Institute of Psychiatry, Kings College London. All participants completed baseline questionnaires and were given Daily Life Assessment packs containing 36 pre-labeled synthetic Cortisol Salivettes (*Sarstedt*, Leicester, UK), a pre-programmed electronic handheld device (*Hewlett Packard iPAQ 111 Classic Handheld*, Bracknell, UK) for prompting and guiding the

EMA schedule, and a reference information booklet. Participants were given training in how to provide samples and operate the handheld device, and left once confident in doing so.

### 2.3. Baseline self-report measures

Fatigue severity was measured in both groups by the Fatigue Scale (FS; Chalder et al., 1993). The 11-item FS measures fatigue severity over the last month, with subscales examining physical fatigue (7 items) and mental fatigue (4 items). Response is via 4-point Likert scale. The FS was used with both continuous (range: 0—33) and bimodal (range: 0—11) scoring systems, with higher scores indicating greater fatigue severity. FS subscales showed excellent internal consistencies within the present study (physical fatigue, $\alpha = .93$; mental fatigue, $\alpha = .85$). The bimodal scoring system distinguishes between severe fatigue and normal fatigue by a $\geq$ 4 cut-off (Chalder et al., 1993): a cut-off with sensitivity of 75.5% and specificity of 74.5% based on the fatigue item of the Clinical Interview Schedule (Lewis et al., 1992). We use this cut-off to identify individuals with clinically significant fatigue in secondary analysis. We chose the FS rather than the Fatigue Severity Scale (FSS; Krupp et al., 1989) or Modified Fatigue Impact Scale (MFIS; Multiple Sclerosis Council for Clinical Practice Guidelines, 1998) as our study focussed on fatigue severity, and neither the FSS nor the MFIS measure the concept of severity directly: both measure the impact of fatigue in daily life, while the FSS also contains items assessing determinants of fatigue. The FS has been used in several MS studies (e.g., Skerrett and Moss-Morris, 2006).

Neurological disability was measured in the RRMS group by the self-administered Expanded Disability Status Scale (EDSS-sa; Bowen et al., 2001). Respondents evaluated a series of items regarding functioning and symptoms 'on an average day, at your best'. The EDSS-sa has a bespoke rating system for each item, contributing to an overall score ranging from 0.0 (no neurological impairment) to 10.0 (death from MS). The EDSS-sa is highly correlated ($r = .89$) with the original physician-delivered EDSS (Kurtzke, 1983).

All participants completed the 12-item Chronic Stress Screening Scale (CSSS). The CSSS is a brief summary measure of chronic stress over the prior 3 months derived from the Trier Inventory of Chronic Stress (Schulz et al., 2004). Responses to the CSSS are on a 0—4 rating scale, with higher scores indicating greater experience of chronic stress. The CSSS had excellent internal consistency in the present study ($\alpha = .91$). The CSSS defines an individual as chronically stressed if they are worrying a lot, feeling overextended and overwhelmed, and receiving no recognition for their efforts.

The 14-item Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983) was also completed by all participants to measure anxiety and depression symptoms over the last week on a 0—3 rating scale (maximum score of 21 for each subscale), with higher scores indicating more anxiety and depression symptoms. The internal consistencies for the HADS subscales were excellent for anxiety symptoms ($\alpha = .85$) and adequate for depressive symptoms ($\alpha = .65$). The HADS does not incorporate items regarding somatic symptoms of anxiety or depression, such as fatigue.

**Table 1** Demographic and clinical characteristics of study participants.

|  | RRMS | Control | p |
|---|---|---|---|
| N | 38 | 38 | |
| Age | 41.89 (7.53) [28—54] | 40.34 (8.16) [27—56] | |
| Gender (female/male) | 31/7 | 31/7 | |
| Employment | | | |
| Paid employment | 30 | 33 | |
| Unpaid employment | 3 | 1 | |
| Unemployed | 5 | 4 | |
| EDSS-sa | 4.35 (1.40) [0.00—6.00] | | |
| Years since diagnosis | 6.03 (5.18) [0—20] | | |
| Disease modifying therapy | | | |
| Interferon | 12 | | |
| Glatiramer acetate | 6 | | |
| Natalizumab | 5 | | |
| No DMT | 15 | | |
| Fatigue | | | |
| FS | 17.58 (7.09) [1—32] | 11.55 (2.87) [4—21] | <.001 |
| FS (physical subscale) | 11.18 (4.89) [0—20] | 7.26 (2.34) [2—16] | <.001 |
| FS (mental subscale) | 6.39 (2.66) [1—12] | 4.29 (0.96) [2—7] | <.001 |
| Stress | | | |
| CSSS | 19.82 (9.36) [0—37] | 14.11 (7.93) [1—28] | .006 |
| Anxiety and depression | | | |
| HADS-anxiety | 7.50 (3.90) [2—17] | 4.82 (3.12) [0—12] | .003 |
| HADS-depression | 4.00 (2.29) [0—7] | 2.08 (2.27) [0—7] | <.001 |
| Sleep quality (person-mean) | 6.07 (1.57) [2.75—8.75] | 6.22 (1.97) [2.50—9.25] | .720 |
| Sleeping hours (person-mean) | 7.83 (1.00) [5.82—10.15] | 7.63 (0.89) [4.75—10.24] | .348 |

*Note.* Mean, standard deviation (), and range [] presented for all continuous variables. EDSS-sa indicates self-assessed Expanded Disability Status Scale; DMT, disease modifying therapy; FS, Fatigue Scale; CSSS, Chronic Stress Screening Scale; HADS, Hospital Anxiety and Depression Scale.

## 2.4. Ecological momentary assessment schedule

The EMA schedule was carried out over 4 consecutive weekdays, guided by handheld device auditory prompts, as participants undertook their usual daily routines. The daily EMA schedule was composed of two designs: an event-based fixed occasion design for assessments soon after awakening, and a time-based variable occasion design with stratified random sampling for assessments later in the day (cf. Schlotz, 2012). The event-based design incorporated prompts at awakening (S1 assessment), 30 min after awakening (S2), and 45 min after awakening (S3). S1 assessment time was defined as the time of a pre-set waking alarm that could be no later than 0830 h, or the time of manual engagement with the handheld if waking earlier than expected. The time-based design consisted of six prompts (S4—S9)

distributed between 1000 h and 2000 h by an algorithm that quasi-randomly allocated each prompt within one of the six consecutive 100 min periods, while ensuring no two prompts could be within 30 min. To minimize missed assessments, participants could postpone any S4—S9 assessment for 5, 10, or 15 min.

### 2.4.1. Cortisol measurement
Nine saliva samples were collected per day for the analysis of free cortisol content: samples at S1—S3 measured the CAR; samples at S4—S9 measured the DCS. Upon each prompt, the handheld briefly presented a 3-digit code which participants recorded on the salivette used, with incorrectly coded salivettes later discarded as non-compliant (Stetler et al., 2004). The compliance rate here was 92.0%, with 2518 usable samples from a possible 2736.

During the CAR measurement period (S1–S3), participants were instructed not to eat, drink anything other than water, smoke, brush their teeth, or exercise. Throughout the rest of the day, there were no behavioral instructions prior to saliva sampling but potential confounders (eating, drinking coffee, smoking, physical exertion, sleeping) were monitored by handheld self-reports. We used samples provided at S1–S3 to compute three different facets of the CAR: (1) the area under the curve ground (AUCg); (2) the waking cortisol level (S1); and (3) the area under the curve increase (AUCi); (Pruessner et al., 2003; Fekedulegn et al., 2007). Here, AUCg measured estimated total cortisol output in the 45 min after awakening, S1 cortisol level assessed the end of the pre-awakening cortisol rise, while AUCi measured the dynamic change in cortisol post-awakening. Pre-awakening is thought to be characterized by reduced adrenal sensitivity to ACTH (Born et al., 1999; Hellhammer et al., 2009; Clow et al., 2010), but post-awakening is characterized by heightened adrenal sensitivity to ACTH, mediated by light-sensitivity (Thorn et al., 2004; Clow et al., 2010). A previous systematic review suggested attenuated measures of diurnal cortisol variability, including CAR AUCi, are more relevant to fatigue than measures of output such as CAR AUCg (Powell et al., 2013).

Participants stored used salivettes in their own refrigerators until returning them to the lab at the end of the EMA schedule, where they were frozen at $-20\,°C$ until assay. Samples were sent in one batch to the Biochemical Laboratory at the Division of Theoretical and Clinical Psychobiology, University of Trier, Germany, where analysis for free cortisol content (nmol/L) was by time-resolved immunoassay with fluorescent detection (Dressendörfer et al., 1992). The detection limit for the assay was 0.173 nmol/L. Each sample was measured in duplicate, with an intra-assay coefficient of variance between 4.0% and 6.7%, and inter-assay coefficient of variance between 7.1% and 9.0%.

### 2.4.2. Momentary self-report assessments

Momentary fatigue was measured at each quasi-random assessment (S4–S9) with a single-item developed from the Brief Fatigue Inventory (Mendoza et al., 1999): 'How much fatigue (tiredness, weariness, problems thinking clearly) do you feel right now?' Response was by visual analog scale (VAS) from 0 ('No Fatigue') to 10 ('Extreme Fatigue'). The momentary fatigue item reflected both the physical and mental fatigue components of the generally accepted definition of MS fatigue as ''a subjective lack of physical and/or mental energy...'' (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, p. 2). Convergent and discriminant validity were demonstrated by negative within-person associations with concurrent *Energetic* ($r = -.45$) and *Alert* ($r = -.44$) single-items, and weak associations with both *Anxious* ($r = .09$) and *Distressed* ($r = .13$) single-items. Fatigue reports were provided in 91.1% of quasi-random momentary assessments.

Upon awakening (S1), participants were presented with items regarding sleep quality ('How would you rate your sleep last night?' with VAS response 0 'Very Bad' to 10 'Very Good') and recalled time of sleep ('What time did you go to sleep last night?' with digital clock response). Hours slept was computed as the time elapsed between self-reported time of sleep and time of S1. Individual items were also presented to indicate whether participants had eaten a meal, drank coffee, smoked, exerted physically, or slept in the 30 min prior to the prompt.

## 2.5. Statistical procedures

Statistical analysis was by multilevel modeling (Snijders and Bosker, 2012) using SPSS Version 21. The criterion for statistical significance was $\alpha = .05$. Each multilevel model used to test a hypothesis is specified in the supplementary material (Appendix A) using notation from Snijders and Bosker (2012). For group comparisons using cortisol data, and in addition to multilevel analysis, Cohen's *d* effect sizes were computed from person-means and standard deviations using independent samples *t*-tests to facilitate comparisons with existing research.

Potential covariates were added to each model in a step-wise manner in data-level order: (1) assessment-level (eating, smoking, caffeine, being at work, physical exertion); (2) day level (sleep quality, time of awakening, morning stress); and (3) individual level (age, gender, menstrual phase, contraceptive use, DMT-use). All assessment-level and day-level covariates were centered to the person-mean, unless binary. The criterion for retaining covariates in the final model was $\alpha = .10$. Potential outliers were identified using a mean $\pm 3$ *SD* criterion (Schlotz, 2012) and were excluded in sensitivity analysis. The number of outliers removed in each sensitivity analysis is declared within the Results section.

### 2.5.1. Testing hypothesis 1

Valid CARs were defined as comprising an S1 sample provided within 8 min of the S1 auditory prompt or manual initiation (Smyth et al., 2013), and S2 and S3 samples provided within 37 min and 52 min, respectively, of the S1 auditory prompt (Kudielka et al., 2003). Missing, delayed, or ineligible or incorrectly labeled S1–S3 samples led to the exclusion of that day's CAR. At least two complete CARs from the 4 assessment days were required for a participant's data to be retained.

Two-level models (days nested within individuals) were built for each of the three CAR outcomes: AUCg, AUCi, and S1 cortisol. A natural log-transformation brought the S1 cortisol data closer to a normal distribution; transformation was not required for AUCg or AUCi. The absence of a cortisol level rise post-awakening has been argued to potentially indicate protocol non-adherence (Thorn et al., 2006), so supplementary responder-only CAR analyses are presented. Responders were defined as where S2 or S3 cortisol level was >1.5 nmol/L higher than S1 cortisol level on a given assessment day (Miller et al., 2013). Group differences were determined by a binary fixed effect for group.

The DCS was computed as the linear slope parameter (effect of time) estimated by a 3-level multilevel model (assessments nested within days within individuals) of log-transformed cortisol data collected at S4–S9 assessments. We required cortisol samples with correct codes from at least 50% (3 of 6) of S4–S9 assessments to retain that day's DCS. Group differences in DCS were determined by the group by time interaction effect. HADS-Depression subscale scores

and CSSS scores were entered into each model as fixed effects to adjust the model for depressive symptoms and chronic stress.

### 2.5.2. Testing hypothesis 2

To test the association of fatigue with cortisol secretory activity, we ran the hypothesis 1 models with FS score and group by FS score interaction entered as fixed effects. Where a statistically significant association was found between cortisol (CAR or DCS) and fatigue, sensitivity analysis was conducted whereby models were adjusted for CSSS, HADS-Depression subscale, EDSS-sa, and employment status scores. In supplementary analyses, the RRMS group was divided into those with (RRMS-f, $n = 21$) and without (RRMS-nf, $n = 17$) clinically significant fatigue using the bimodal FS cut-off ($\geq 4$). For group comparisons, specified multilevel models for CAR outcomes and DCS were rerun with dummy variables representing the three groups (RRMS-f, RRMS-nf, control) as binary fixed effects with comparator 0.
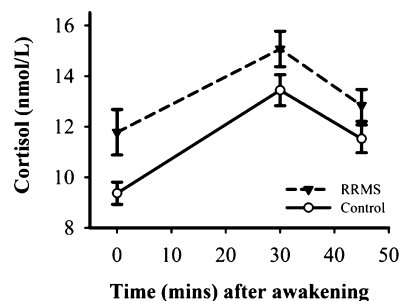
### 2.5.3. Testing hypothesis 3

A 2-level model with daily fatigue severity as outcome was built to test whether the CAR predicted same-day fatigue severity within-individuals. Daily fatigue severity was operationalized as the daily mean of momentary fatigue ratings. CAR measures and group by CAR interaction terms were entered into consecutive models as fixed effects.

## 3. Results

### 3.1. Cortisol awakening response

The CAR AUCg and AUCi analyses were based on 258 assessment days (84.8%) nested within 75 participants (37 RRMS; 38 Control); one participant provided no valid CAR



**Figure 1** Cortisol awakening response represented by the mean of the within-subject means for samples at 0 (S1), 30 (S2), and 45 min (S3) post-awakening. Error bars represent the standard error of the mean.

assessments and was excluded. S1 cortisol analysis was based on 298 assessment days (98.0%) nested within 76 participants. Of the 258 CARs measured, 177 (68.6%) were classified as responder (RRMS, 69.7%; Control, 67.5%).

The CAR profiles for the RRMS and control groups are presented in Fig. 1. As shown in Table 2, a statistically significant group difference was found for CAR AUCg such that, on average, cortisol output in the CAR period was 1.72 nmol/L/min greater in the RRMS group than the control group. Removing an outlying AUCg assessment from the dataset did not substantially affect the result, $p = .044$, 95% CI [0.043, 3.00]. RRMS group CARs typically showed a higher S1 cortisol level, albeit not to statistical significance ($p = .074$), followed by an awakening response (AUCi) that was no different from controls. In responder-only analysis, the group difference for CAR AUCg was diminished, $p = .147$, 95% CI [−0.407, 2.665]. Table 3 presents means and SDs of person-mean CAR data, demonstrating the group difference in AUCg represented a medium effect size.

Although the group difference for CAR AUCg was somewhat diminished in a model adjusted for depressive

**Table 2** Multilevel parameters estimates of group differences in cortisol secretory activity.

| Fixed effect | Coefficient | SE | t | p | 95% CI |
|---|---|---|---|---|---|
| Model for CAR AUCg | | | | | |
| Intercept | 11.831 | 0.543 | 21.772 | <.001 | [10.748, 12.912] |
| Group | 1.715 | 0.767 | 2.236 | .028 | [0.187, 3.242] |
| Model for CAR AUCi | | | | | |
| Intercept | 2.375 | 0.476 | 4.992 | <.001 | [1.431, 3.319] |
| Group | −0.591 | 0.669 | −0.883 | .379 | [−1.919, 0.737] |
| Model for (ln) S1 cortisol | | | | | |
| Intercept | 2.267 | 0.058 | 38.947 | <.001 | [2.151, 2.383] |
| Group | 0.149 | 0.082 | 1.808 | .074 | [−0.015, 0.313] |
| Model for DCS[a] | | | | | |
| Intercept | 1.687 | 0.053 | 32.107 | <.001 | [1.583, 1.792] |
| Time | −0.091 | 0.007 | −12.826 | <.001 | [−0.105, −0.076] |
| Group | 0.109 | 0.074 | 1.469 | .146 | [−0.039, 0.257] |
| Time*Group | −0.005 | 0.010 | −0.512 | .610 | [−0.025, 0.015] |

*Note.* CAR indicates cortisol awakening response; AUCg, area under the curve ground; AUCi, area under the curve increase; S1, cortisol upon awakening; DCS, diurnal cortisol slope. Data presented is from responder and non-responder days.

[a] Fixed effects of significant covariates (recent meal, recent smoking) not presented. Intercepts represent parameters for comparator group (controls). Group fixed effect parameters represent difference between RRMS group and comparator group (i.e., a positive parameter indicates a larger cortisol outcome). For Time*Group, a positive parameter indicates a flatter DCS.

**Table 3** Mean of Person-Means (*SD*) for Cortisol Awakening Response outcomes in each group.

| CAR | RRMS | Control | *t* | *p* | 95% CI | *d* |
|---|---|---|---|---|---|---|
| AUCg (nmol/L/min) | 13.57 (3.77) | 11.78 (2.95) | 2.29 | .03 | [0.23, 3.35] | 0.53 |
| AUCi (nmol/L/min) | 1.88 (3.22) | 2.02 (2.69) | 0.21 | .84 | [−1.51, 1.22] | −0.04 |
| (ln) S1 | 2.41 (0.41) | 2.28 (0.31) | 1.49 | .14 | [−0.04, 0.29] | 0.36 |
| (ln) S2 | 2.71 (0.28) | 2.60 (0.26) | 1.74 | .09 | [−0.02, 0.23] | 0.41 |
| (ln) S3 | 2.56 (0.29) | 2.46 (0.28) | 1.42 | .21 | [−0.04, 0.23] | 0.35 |

*Note.* CAR indicates cortisol awakening response; AUCg, area under the curve ground; AUCi, area under the curve increase; S1, cortisol upon awakening; S2, cortisol 30 min after awakening; S3, cortisol 45 min after awakening. Data presented is from responder and non-responder days. Significance tests use independent samples *t*-test.

symptoms, $\gamma = 2.013$, $SE = 1.306$, $p = .128$, 95% CI [−0.590, 4.616], depressive symptoms were not associated with CAR AUCg in either the RRMS group, $\gamma = 0.033$, $SE = 0.234$, $p = .887$, 95% CI [−0.433, 0.500], or the control group, $\gamma = 0.212$, $SE = 0.244$, $p = .388$, 95% CI [−0.274, 0.698].

### 3.2. Diurnal cortisol slope

The DCS analysis was based on 1637 assessments nested within 296 days within 76 individuals. Group comparisons are presented within Table 2 (fitted linear regression lines for each participant are presented in Fig. B.1 in Appendix B). As expected, there was a statistically significant decreasing trend in cortisol levels over time in both groups, but the DCS did not differ between groups.

### 3.3. Cortisol and fatigue

Parameters from models testing associations between FS score and different facets of cortisol secretion in the two groups are presented in Table 4. In the RRMS group, CAR AUCg was not associated with FS score. However, S1 cortisol was negatively associated with FS score in the RRMS group, such that lower cortisol levels were associated with greater fatigue (see Fig. B.2A in Appendix B); an association that was reflected in both the physical and mental FS subscales. In addition, a larger CAR AUCi was associated with higher FS score in the RRMS group, such that those with greater fatigue had larger rises in cortisol post-awakening (see Fig. B.2B in Appendix B). Similar associations with CAR AUCi were found for both FS subscales. No CAR measure was associated with FS score in the control group. The DCS was not associated with FS score in either group.

Across all participants, FS score was associated with HADS-Depression score ($r = .335$, $p = .003$) and CSSS score ($r = .228$, $p = .047$). However, cortisol-fatigue associations in the RRMS group remained after controlling for depressive symptoms (HADS Depression score), chronic stress (CSSS score), neurological disability (EDSS score), and employment hours. For both S1 and CAR AUCi, excluding cortisol outliers (2 S1 outliers; 4 AUCi outliers) and an individual who reported FS = 1 did not substantially affect any association with FS score.

#### 3.3.1. Clinically significant fatigue
The RRMS group was divided into fatigued (RRMS-f, $n = 21$) and non-fatigued (RRMS-nf, $n = 16$) subgroups

based on the standard cut-off on the FS. There were no statistically significant subgroup differences for age, gender, paid employment, or awakening time ($ps > .270$). Self-reported levels of sleep quality were similar in the RRMS-f and RRMS-nf subgroups ($M_{RRMS-f} = 5.937$, $SD = 1.812$; $M_{RRMS-nf} = 6.245$, $SD = 1.232$, $U = 168.500$, $p = .769$), as were HADS-Depression scores ($M_{RRMS-f} = 4.428$, $SD = 2.158$; $M_{RRMS-nf} = 3.471$, $SD = 2.401$, $U = 136.500$, $p = .213$) and CSSS scores ($M_{RRMS-f} = 20.952$, $SD = 9.573$; $M_{RRMS-nf} = 18.412$, $SD = 9.172$, $t = −0.829$, $p = .413$). However, the RRMS-f subgroup was somewhat more disabled than the RRMS-nf subgroup ($M_{RRMS-f} = 4.691$, $SD = 0.887$; $M_{RRMS-nf} = 3.794$, $SD = 1.705$, $U = 114.500$, $Z = 1.912$, $p = .056$).

Compared to the control group, CAR AUCg was elevated only in the RRMS-nf subgroup, $\gamma = 2.170$, $SE = 0.951$, $t = 2.281$, $p = .025$, 95% CI [0.275, 4.064], and not the RRMS-f subgroup, $\gamma = 1.032$, $SE = 0.864$, $t = 1.193$, $p = .237$, 95% CI [−0.691, 2.754]. There was no statistically significant difference between RRMS subgroups for CAR AUCg, $\gamma = 1.138$, $SE = 1.053$, $t = 1.081$, $p = .283$, 95% CI [−0.960, 3.237]. S1 cortisol levels were higher in the RRMS-nf subgroup compared to both the control group, $\gamma = 0.274$, $SE = 0.102$, $t = 2.696$, $p = .009$, 95% CI [0.072, 0.478], and the RRMS-f subgroup, $\gamma = 0.228$, $SE = 0.114$, $t = 2.003$, $p = .049$, 95% CI [0.001, 0.456]. The difference in CAR AUCi between RRMS-f and RRMS-nf subgroups did not quite reach statistical significance, $\gamma = 1.686$, $SE = 0.854$, $t = 1.973$, $p = .052$, 95% CI [−0.012, 3.384], while we detected no difference in CAR AUCi between RRMS-f and controls, $\gamma = 0.574$, $t = 0.822$, $SE = 0.698$, $p = .413$, 95% CI [−0.814, 1.961], or RRMS-nf and controls, $\gamma = −1.112$, $SE = 0.776$, $t = −1.432$, $p = .156$, 95% CI [−2.655, 0.431]. Mean CAR profiles for each subgroup are presented in Fig. 2.

There was no difference in DCS between subgroups, but cortisol levels were elevated in the RRMS-nf group at 1000 h compared to the control group, $\gamma = 0.192$, $SE = 0.094$, $t = 2.047$, $p = .044$, 95% CI [0.005, 0.378]. RRMS-f vs Control and RRMS-f vs RRMS-nf subgroup comparisons at 1000 h were not statistically significant. Therefore, despite cortisol levels being similar at S3 for all RRMS-f and RRMS-nf subgroups, cortisol level decreased more slowly between 45 min after awakening and 1000 h in the RRMS-nf subgroup. This effect remained after controlling for awakening time.
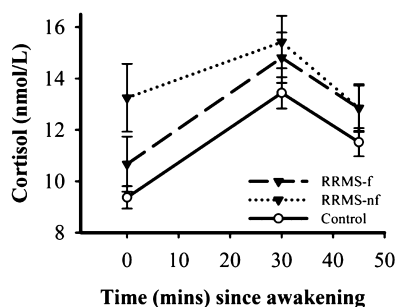
#### 3.3.2. Within-person analysis
No associations between facets of the CAR and same-day fatigue severity were statistically significant in either group.

**Table 4** Multilevel parameter estimates of associations between Fatigue Scale score and cortisol secretory activity in the RRMS group and control group.

| Fixed effect | RRMS | | | | | Control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coef. | SE | t | p | 95% CI | Coef. | SE | t | p | 95% CI |
| Model for CAR AUCg | | | | | | | | | | |
|   FS Score | −0.089 | 0.076 | −1.168 | .246 | [−0.241, 0.063] | 0.017 | 0.189 | 0.090 | .928 | [−0.360, 0.394] |
| Models for CAR AUCi | | | | | | | | | | |
|   FS Score | 0.157 | 0.064 | 2.442 | .017 | [0.029, 0.285] | 0.087 | 0.160 | 0.543 | .588 | [−0.231, 0.405] |
|   FS Physical | 0.216 | 0.093 | 2.316 | .023 | [0.031, 0.400] | 0.094 | 0.194 | 0.482 | .631 | [−0.292, 0.480] |
|   FS Mental | 0.391 | 0.175 | 2.235 | .028 | [0.044, 0.739] | 0.219 | 0.502 | 0.324 | .663 | [−0.776, 1.214] |
| Models for (ln) S1 cortisol | | | | | | | | | | |
|   FS Score | −0.022 | 0.008 | −2.781 | .007 | [−0.038, −0.006] | −0.008 | 0.019 | −0.387 | .700 | [−0.046, 0.031] |
|   FS Physical | −0.028 | 0.012 | −2.454 | .016 | [−0.051, −0.005] | −0.009 | 0.024 | −0.393 | .695 | [−0.058, 0.039] |
|   FS Mental | −0.060 | 0.021 | −2.865 | .005 | [−0.102, −0.018] | −0.011 | 0.059 | −0.191 | .849 | [−0.129, 0.106] |
| Model for DCS | | | | | | | | | | |
|   Time*FS Score | 0.0010 | 0.0010 | 1.038 | .302 | [−0.0009, 0.0030] | 0.0045 | 0.0024 | 1.847 | .069 | [−0.0004, 0.0093] |

*Note.* CAR indicates cortisol awakening response; AUCg, area under the curve ground; AUCi, area under the curve increase; S1, cortisol upon awakening; DCS, diurnal cortisol slope. Data presented is from responder and non-responder days.



**Figure 2** Cortisol awakening response represented by the mean of the within-subject means for samples at 0 (S1), 30 (S2), and 45 min (S3) post-awakening. Error bars represent the standard error of the mean.

In the RRMS group, CAR AUCg, *p* = .814, 95% CI [−0.062, 0.079], S1 cortisol, *p* = .625 95% CI [−0.776, 0.467], and CAR AUCi, *p* = .911, 95% CI [−0.067, 0.075], were not associated with same-day fatigue severity. In the control group, an association between greater CAR AUCg and higher same-day fatigue was close to statistical significance, $\gamma = 0.081$, *SE* = 0.042, *t* = 1.942, *p* = .054, 95% CI [−0.001, 0.164], but neither S1 cortisol, *p* = .496, 95% CI [−0.460, 0.946] nor CAR AUCi, *p* = .895, 95% CI [−0.078, 0.068] was associated with fatigue within individuals.

## 4. Discussion

The present study provided some support for our first hypothesis that the CAR is larger in people with RRMS than healthy individuals, although this difference was only apparent for cortisol output (CAR AUCg) and not the post-awakening cortisol increase (CAR AUCi). Diurnal cortisol slopes were similar across groups. Group differences remained after adjusting for severity of neurological and depressive symptoms, and chronic stress. Although total cortisol output was associated with RRMS, this did not appear to

explain the fatigue experienced by many with RRMS. RRMS fatigue was associated with a low waking (S1) cortisol level and greater post-awakening cortisol increases (AUCi), rather than cortisol output (AUCg). There was no support for the final hypothesis in either group: daily CARs did not predict same-day fatigue ratings within individuals.

Our data showing that cortisol output in the CAR is elevated in RRMS compared to healthy controls supports previous case—control studies with CAR comparisons (Kern et al., 2011, 2013). Our findings add to other studies using a variety of methods that have reported hyperactivity in some facet of basal cortisol secretion in RRMS (Ysrraelit et al., 2008; Gold et al., 2010; Kern et al., 2011, 2013; Melief et al., 2013). However, our data contests the hypothesis that comorbid depression drives cortisol hyper-secretion in RRMS (Gold et al., 2010, 2011; Kern et al., 2011); we demonstrated heightened CAR output in individuals with RRMS who did not have comorbid major depressive disorder and had relatively mild levels of depressive symptoms. In other studies, depression in MS was not associated with 24 h urinary cortisol (Ysrraelit et al., 2008), cerebrospinal fluid cortisol (Melief et al., 2013), or salivary cortisol in a 2-day study (Kern et al., 2013). Of course, results we present do not rule out major depressive disorder further exaggerating HPA axis hyperactivity in RRMS beyond that driven by RRMS-specific disease processes. Like Kern et al. (2013), we found no evidence that neurological disability was implicated in salivary cortisol outcomes and agree that this likely points to the present disease state, rather than accumulated deficits, being responsible for changes in HPA axis activity.

Our data suggest greater cortisol output within the CAR in RRMS is characterized by a larger pre-awakening rise in cortisol levels followed by a post-awakening rise that is broadly similar to that of healthy individuals. The CAR profiles presented by Kern et al. (2013) seem to follow a similar pattern, with cortisol levels already higher in the RRMS group upon awakening followed by a post-awakening rise similar in size to controls.

Pre-awakening HPA axis activity is thought to consist of a period of dissociation between ACTH and cortisol secretion (Clow et al., 2010) evidenced by ACTH levels increasing more quickly than cortisol pre-awakening (Wilhelm et al., 2007). This pre-awakening dissociation may be attributable to enhanced inhibitory actions by the hippocampus while rapid eye movement sleep is dominant and hippocampal activity is increased (Clow et al., 2010). Several studies have reported that smaller hippocampal volume or hippocampal damage is associated with higher S1 cortisol levels (Frodl and O'Keane, 2013) and studies have demonstrated smaller hippocampal regions in RRMS (Sicotte et al., 2008; Gold et al., 2010). We speculate that the heightened CAR output in RRMS reported in the present study could be attributed to a reduction in the expected ACTH-cortisol dissociation prior to awakening due to impaired hippocampal functioning in RRMS. Flatter DCS has previously been associated with a smaller CA23DG hippocampal region volume in RRMS (Gold et al., 2010).

Bearing in mind average CAR output (AUCg) and fatigue were greater in the RRMS group when compared to controls, and the post-awakening rise (AUCi) was broadly similar across group, it may seem surprising to find no association between RRMS fatigue severity and CAR output, and yet a positive association with fatigue and the post-awakening rise. The lack of a relationship between fatigue and overall cortisol output is in line with a systematic review we previously conducted on cortisol secretory activity and fatigue in physical health conditions (Powell et al., 2013). However, the systematic review found attenuated diurnal cortisol variability (e.g., reduced post-awakening increase) was most relevant to fatigue across populations; we have found a contrary positive association in the RRMS group in the present study. Of note, the only study of a clinical population included in the systematic review that reported a positive association between the post-awakening cortisol rise and fatigue severity was in rheumatoid arthritis (Dekkers et al., 2000). With rheumatoid arthritis being, like MS, an autoimmune disease characterized by high levels of inflammation, there may be different mechanisms of fatigue in inflammatory autoimmune disease vs fatigue in other clinical groups.

Cortisol is known to be an important endogenous regulator of inflammation (Chrousos, 1995), and studies have shown concurrently high levels of cortisol and pro-inflammatory cytokines in MS (e.g., Ysrraelit et al., 2008). Speculatively, MS-related fatigue may manifest in the absence of sufficient down-regulation of inflammation by cortisol, particularly in the mornings. This suggestion is supported by our finding that average cortisol levels were still elevated at 1000 h in the RRMS not-fatigued subgroup. Future research into the mechanisms underlying associations between cortisol dysregulation and fatigue in MS should seek to simultaneously examine both cortisol and inflammatory markers and their association with MS-related fatigue in a longitudinal design.

Within individuals, we found no association between day-to-day changes in the CAR and same-day fatigue severity in RRMS. This finding differs from negative associations reported between the CAR and same-day symptoms in clinical burnout (Sonnenschein et al., 2007) and same-day physical symptoms in general population samples (Adam et al., 2006; Gartland et al., 2014). The fact we found no association between the CAR and same-day fatigue in RRMS might suggest that day-to-day variability in MS fatigue is related to psychosocial and contextual factors in daily life, which may include perceived demand and sleep quality. A broad range of psychological factors have previously been shown to correlate with recalled fatigue in MS (Bol et al., 2009), while interventions based on a cognitive behavioral model of MS fatigue (van Kessel and Moss-Morris, 2006) have been shown to successfully reduce fatigue in MS (van Kessel et al., 2008). The role of psychosocial factors in accounting for fatigue variability within people with MS warrants further investigation, particularly as these factors may be amenable to change.

Importantly, MS fatigue appears a highly complex phenomenon of multifactorial origin with variability not only between individuals but also within individuals. Previous studies have indicated positive associations between MS fatigue and pro-inflammatory cytokines (Flachenecker et al., 2004; Heesen et al., 2006), brain lesion load (Colombo et al., 2000; Tedeschi et al., 2007), psychosocial stress (Trojan et al., 2007), and various associations with cognitive and behavioral factors (Skerrett and Moss-Morris, 2006). However, evidence is frequently mixed; for example, MS fatigue has been associated with smaller lesion loads in another study (Codella et al., 2002). Several reviews have now been written describing current understanding of the multifactorial origins of MS fatigue (see Kos et al., 2008; Krupp et al., 2010; Induruwa et al., 2012).

The present study has a number of methodological strengths to highlight. First, all participants were clinically stable (remission-phase) with sufficient recovery time (>3 months) from relapse and associated corticosteroid treatments, and had no other comorbidity. These eligibility criteria contributed toward a relatively homogeneous RRMS sample, which increased our confidence in attributing findings to RRMS and to MS-related fatigue rather than an extraneous factor. We acknowledge this necessarily limits the generalizability of the findings.

The cortisol sampling protocol was the most comprehensive to date in an MS population: nine saliva samples over 4 weekdays provided greater measurement reliability and power to detect effects than has been achieved in previous 2-day protocols (Gold et al., 2010, 2011; Kern et al., 2011, 2013). Sampling over consecutive days limited the opportunity to self-select assessment days. Despite these methodological improvements, it should be noted that it has been suggested that 6 days' and 10 days' sampling may be required to adequately examine between-person associations with the CAR AUCi and DCS, respectively (Hellhammer et al., 2007; Segerstrom et al., 2014). Many steps were taken to maximize compliance with the sampling protocol in daily life, including using audible prompts and random code generation. We report excellent rates of compliance. However, future studies may also use mobile polysomnography or actigraphy to objectively validate time of awakening and promptness of S1 saliva. Although all participants were briefed to engage with their handheld upon awakening and to pre-set the S1 waking alarm, it was impossible to be definitively certain this occurred for all sampling days.

There are other limitations to acknowledge. Firstly, we did not directly assess MS patients for sleep disorders.

Although we excluded participants with multimorbidity during recruitment, sleep disorders are underdiagnosed in MS (Brass et al., 2014). Sleep disorders, such as restless leg syndrome, periodic leg movement disorder, and sleep apnea are all relatively common in MS (Marrie et al., 2014) and are associated with increased fatigue (Veauthier and Paul, 2014). However, we report similar ratings of sleep quality across groups, so believe it is unlikely that sleep disorders have had a biasing effect on our results. Secondly, although we did investigate DMT-use as a potential covariate in analyses, we could not include specific DMTs as covariates due to limited numbers. Interferon has an influenza-like side effect in around half of MS patients (Filippini et al., 2003) which may have affected fatigue symptoms, but we found similar FS scores for those taking interferon and those who were not ($p = .832$). In addition, a large cross-sectional study found no differences in fatigue ratings between MS patients taking immunosuppressant or immunomodulatory medications and MS patients who were treatment-free (Putzki et al., 2008).

In summary, we have demonstrated greater CAR output in RRMS, independent of depression. We found associations of low waking cortisol (pre-awakening rise) and greater post-awakening cortisol rises with higher ratings of recalled fatigue severity in RRMS. These associations were independent of depressive symptoms and stress, both factors that are related to fatigue in MS (Trojan et al., 2007). In people with RRMS, there was no within-person association between the CAR and same-day fatigue. Cortisol secretory activity appears to be implicated in fatigue experience in RRMS, but whether it is a causal factor remains unclear.

## Role of the funding sources

## Conflict of interest

None declared.

## Acknowledgments

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.03.010.

## References

Adam, E.K., Hawkley, L.C., Kudielka, B.M., Cacioppo, J.T., 2006. Day-to-day dynamics of experience cortisol associations in a population-based sample of older adults. Proc. Natl. Acad. Sci. U. S. A. 103, 17058–17063.

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). American Psychiatric Association, Washington, DC.

Artemiadis, A.K., Anagnostouli, M.C., Alexopoulos, E.C., 2011. Stress as a risk factor for multiple sclerosis onset or relapse: a systematic review. Neuroepidemiology 36, 109–120.

Bol, Y., Duits, A.A., Hupperts, R.M.M., Vlaeyen, J.W.S., Verhey, F.R.J., 2009. The psychology of fatigue in patients with multiple sclerosis: a review. J. Psychosom. Res. 66, 3–11.

Born, J., Hansen, K., Marshall, L., Molle, M., Fehm, H.L., 1999. Timing the end of nocturnal sleep. Nature 397, 29–30.

Bowen, J., Gibbons, L., Gianas, A., Kraft, G.H., 2001. Self-administered Expanded Disability Status Scale with functional system scores correlates well with a physician-administered test. Mult. Scler. 7, 201–206.

Brass, S.D., Li, C.-S., Auerbach, S., 2014. The underdiagnosis of sleep disorders in patients with multiple sclerosis. J. Clin. Sleep Med. 10, 1025–1031.

Chalder, T., Berelowitz, G., Pawlikowska, T., Watts, L., Wessely, S., Wright, D., Wallace, E.P., 1993. Development of a fatigue scale. J. Psychosom. Res. 37, 147–153.

Chrousos, G.P., 1995. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. N. Engl. J. Med. 332, 1351–1363.

Clow, A., Hucklebridge, F., Stalder, T., Evans, P., Thorn, L., 2010. The cortisol awakening response: more than a measure of HPA axis function. Neurosci. Biobehav. Rev. 35, 97–103.

Codella, M., Rocca, M.A., Colombo, B., Rossi, P., Comi, G., Filippi, M., 2002. A preliminary study of magnetization transfer and diffusion tensor MRI of multiple sclerosis patients with fatigue. J. Neurol. 249, 535–537.

Colombo, B., Boneschi, F.M., Rossi, P., Rovaris, M., Maderna, L., Filippi, M., Comi, G., 2000. MRI and motor evoked potential findings in nondisabled multiple sclerosis patients with and without symptoms of fatigue. J. Neurol. 247, 506–509.

Compston, A., Coles, A., 2008. Multiple sclerosis. Lancet 372, 1502–1517.

Dekkers, J.C., Geenen, R., Godaert, G.L.R., van Doornen, L.J.P., Bijlsma, J.W.J., 2000. Diurnal courses of cortisol, pain, fatigue, negative mood, and stiffness in patients with recently diagnosed rheumatoid arthritis. Int. J. Behav. Med. 7, 353–371.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.

Dressendörfer, R.A., Kirschbaum, C., Rohde, W., Stahl, F., Strasburger, C.J., 1992. Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. J. Steroid Biochem. Mol. Biol. 43, 683–692.

Fassbender, K., Schmidt, R., Mössner, R., Kischka, U., Kühnen, J., Schwartz, A., Hennerici, M., 1998. Mood disorders and dysfunction of the hypothalamic–pituitary–adrenal axis in multiple sclerosis: association with cerebral inflammation. Arch. Neurol. 55, 66–72.

Fekedulegn, D.B., Andrew, M.E., Burchfiel, C.M., Violanti, J.M., Hartley, T.A., Charles, L.E., Miller, D.B., 2007. Area under the curve and other summary indicators of repeated waking cortisol measurements. Psychosom. Med. 69, 651—659.

Filippini, G., Munari, L., Incorvaia, B., Ebers, G.C., Polman, C., D'Amico, R., Rice, G.P.A., 2003. Interferons in relapsing remitting multiple sclerosis: a systematic review. Lancet 361, 545—552.

Flachenecker, P., Bihler, I., Weber, F., Gottschalk, M., Toyka, K.V., Rieckmann, P., 2004. Cytokine mRNA expression in patients with multiple sclerosis and fatigue. Mult. Scler. 10, 165—169.

Frodl, T., O'Keane, V., 2013. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol. Dis. 52, 24—37.

Gartland, N., O'Connor, D.B., Lawton, R., Bristow, M., 2014. Exploring day-to-day dynamics of daily stressor appraisals, physical symptoms and the cortisol awakening response. Psychoneuroendocrinology 50, 130—138.

Gold, S.M., Kern, K.C., O'Connor, M.-F., Montag, M.J., Kim, A., Yoo, Y.S., Giesser, B.S., Sicotte, N.L., 2010. Smaller cornu ammonis 2—3/dentate gyrus volumes and elevated cortisol in multiple sclerosis patients with depressive symptoms. Biol. Psychiatry 68, 553—559.

Gold, S.M., Kruger, S., Ziegler, K.J., Krieger, T., Schulz, K.H., Otte, C., Heesen, C., 2011. Endocrine and immune substrates of depressive symptoms and fatigue in multiple sclerosis patients with comorbid major depression. J. Neurol. Neurosurg. Psychiatry 82 (7), 814—818.

Heesen, C., Nawrath, L., Reich, C., Bauer, N., Schulz, K., Gold, S.M., 2006. Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? J. Neurol. Neurosurg. Psychiatry 77, 34—39.

Hellhammer, D.H., Wüst, S., Kudielka, B.M., 2009. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 34, 163—171.

Hellhammer, J., Fries, E., Schweisthal, O.W., Schlotz, W., Stone, A.A., Hagemann, D., 2007. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology 32, 80—86.

Heuser, I., Yassouridis, A., Holsboer, F., 1994. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. J. Psychiatr. Res. 28, 341—356.

Induruwa, I., Constantinescu, C.S., Gran, B., 2012. Fatigue in multiple sclerosis—a brief review. J. Neurol. Sci. 323, 9—15.

Kern, S., Krause, I., Horntrich, A., Thomas, K., Aderhold, J., Ziemssen, T., 2013. Cortisol awakening response is linked to disease course and progression in multiple sclerosis. PLOS ONE 8, e60647.

Kern, S., Schultheiß, T., Schneider, H., Schrempf, W., Reichmann, H., Ziemssen, T., 2011. Circadian cortisol, depressive symptoms and neurological impairment in early multiple sclerosis. Psychoneuroendocrinology 36, 1505—1512.

Kos, D., Kerckhofs, E., Nagels, G., D'hooghe, M.B., Ilsbroukx, S., 2008. Origin of fatigue in multiple sclerosis: review of the literature. Neurorehabil. Neural Repair 22, 91—100.

Krupp, L.B., LaRocca, N.G., Muir-Nash, J., Steinberg, A.D., 1989. The Fatigue Severity Scale: application to patients with multiple sclerosis and systemic lupus erythematosus. Arch. Neurol. 46, 1121—1123.

Krupp, L.B., Serafin, D.J., Christodoulou, C., 2010. Multiple sclerosis-associated fatigue. Expert Rev. Neurother. 10, 1437—1447.

Kudielka, B.M., Broderick, J.E., Kirschbaum, C., 2003. Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. Psychosom. Med. 65, 313—319.

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis. Neurology 33, 1444—1452.

Law, R., Hucklebridge, F., Thorn, L., Evans, P., Clow, A., 2013. State variation in the cortisol awakening response. Stress 16, 483—492.

Lerdal, A., Celius, E.G., Moum, T., 2003. Fatigue and its association with sociodemographic variables among multiple sclerosis patients. Mult. Scler. 9, 509—514.

Lewis, G., Pelosi, A.J., Araya, R., Dunn, G., 1992. Measuring psychiatric disorder in the community: a standardized assessment for use by lay interviewers. Psychol. Med. 22, 465—486.

Marrie, R.A., Reider, N., Cohen, J., Trojano, M., Sorensen, P.S., Cutter, G., Reingold, S., Stuve, O., 2014. A systematic review of the incidence and prevalence of sleep disorders and seizure disorders in multiple sclerosis. Mult. Scler., http://dx.doi.org/10.1177/1352458514564486.

Melief, J., Wit, S., Eden, C., Teunissen, C., Hamann, J., Uitdehaag, B., Swaab, D., Huitinga, I., 2013. HPA axis activity in multiple sclerosis correlates with disease severity, lesion type and gene expression in normal-appearing white matter. Acta Neuropathol. (Berl.), 1—13.

Mendoza, T.R., Wang, X.S., Cleeland, C.S., Morrissey, M., Johnson, B.A., Wendt, J.K., Huber, S.L., 1999. The rapid assessment of fatigue severity in cancer patients. Cancer 85, 1186—1196.

Miller, R., Plessow, F., Kirschbaum, C., Stalder, T., 2013. Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: evaluation of salivary cortisol pulse detection in panel designs. Psychosom. Med. 75, 832—840.

Mills, R.J., Young, C.A., 2008. A medical definition of fatigue in multiple sclerosis. QJM 101, 49—60.

Minden, S.L., Frankel, D., Hadden, L., Perloffp, J., Srinath, K.P., Hoaglin, D.C., 2006. The Sonya Slifka Longitudinal Multiple Sclerosis Study: methods and sample characteristics. Mult. Scler. 12, 24—38.

Mohr, D.C., Hart, S.L., Julian, L., Cox, D., Pelletier, D., 2004. Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. BMJ 328, 731—733.

Multiple Sclerosis Council for Clinical Practice Guidelines, 1998. Multiple Sclerosis Council for Clinical Practice Guidelines: Fatigue and Multiple Sclerosis. Paralyzed Veterans of America, Washington, DC.

Papadopoulos, A., Cleare, A.J., 2012. Hypothalamic—pituitary—adrenal axis dysfunction in chronic fatigue syndrome. Nat. Rev. Endocrinol. 8, 22—32.

Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31, 464—468.

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B., Wolinsky, J.S., 2011. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann. Neurol. 69, 292—302.

Powell, D.J., Schlotz, W., 2012. Daily life stress and the cortisol awakening response: testing the anticipation hypothesis. PLoS ONE 7, e52067.

Powell, D.J.H., Liossi, C., Moss-Morris, R., Schlotz, W., 2013. Unstimulated cortisol secretory activity in everyday life and its relationship with fatigue and chronic fatigue syndrome: a systematic review and subset meta-analysis. Psychoneuroendocrinology 38, 2405—2422.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916—931.

Putzki, N., Katsarava, Z., Vago, S., Diener, H.C., Limmroth, V., 2008. Prevalence and severity of multiple-sclerosis-associated fatigue in treated and untreated patients. Eur. Neurol. 59, 136—142.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55—89.

Schlotz, W., 2012. Ambulatory psychoneuroendocrinology: assessing salivary cortisol and other hormones in daily life. In: Mehl, M.R., Conner, T.S. (Eds.), Handbook of Research Methods for Studying Daily Life. Guilford Press, New York, NY, pp. 193—209.

Schulz, P., Schlotz, W., Becker, P., 2004. Trierer Inventar zum Chronischen Stress (TICS) [Trier Inventory for Chronic Stress (TICS)]. Hogrefe Verlag, Gottingen.

Schumann, E.M., Kümpfel, T., Then Bergh, F., Trenkwalder, C., Holsboer, F., Auer, D.P., 2002. Activity of the hypothalamic—pituitary—adrenal axis in multiple sclerosis: correlations with gadolinium-enhancing lesions and ventricular volume. Ann. Neurol. 51, 763—767.

Segerstrom, S.C., Boggero, I.A., Smith, G.T., Sephton, S.E., 2014. Variability and reliability of diurnal cortisol in younger and older adults: implications for design decisions. Psychoneuroendocrinology 49, 299—309.

Sicotte, N.L., Kern, K.C., Giesser, B.S., Arshanapalli, A., Schultz, A., Montag, M., Wang, H., Bookheimer, S.Y., 2008. Regional hippocampal atrophy in multiple sclerosis. Brain 131, 1134—1141.

Skerrett, T.N., Moss-Morris, R., 2006. Fatigue and social impairment in multiple sclerosis: the role of patients' cognitive and behavioral responses to their symptoms. J. Psychosom. Res. 61, 587—593.

Smyth, N., Clow, A., Thorn, L., Hucklebridge, F., Evans, P., 2013. Delays of 5—15 min between awakening and the start of saliva sampling matter in assessment of the cortisol awakening response. Psychoneuroendocrinology 38, 1476—1483.

Snijders, T.A.B., Bosker, R.J., 2012. Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling, 2nd edition. SAGE Publications, London, UK.

Sonnenschein, M., Mommersteeg, P.M.C., Houtveen, J.H., Sorbi, M.J., Schaufeli, W.B., van Doornen, L.J.P., 2007. Exhaustion and endocrine functioning in clinical burnout: an in-depth study using the experience sampling method. Biol. Psychol. 75, 176—184.

Stetler, C., Dickerson, S.S., Miller, G.E., 2004. Uncoupling of social zeitgebers and diurnal cortisol secretion in clinical depression. Psychoneuroendocrinology 29, 1250—1259.

Stone, A.A., Shiffman, S., 1994. Ecological momentary assessment (EMA) in behavioral medicine. Ann. Behav. Med. 16, 199—202.

Tedeschi, G., Dinacci, D., Lavorgna, L., Prinster, A., Savettieri, G., Quattrone, A., Livrea, P., Messina, C., Reggio, A., Servillo, G., Bresciamorra, V., Orefice, G., Paciello, M., Brunetti, A., Paolillo, A., Coniglio, G., Bonavita, S., Di Costanzo, A., Bellacosa, A., Valentino, P., Quarantelli, M., Patti, F., Salemi, G., Cammarata, E., Simone, I., Salvatore, M., Bonavita, V., Alfano, B., 2007. Correlation between fatigue and brain atrophy and lesion load in multiple sclerosis patients independent of disability. J. Neurol. Sci. 263, 15—19.

Thorn, L., Hucklebridge, F., Esgate, A., Evans, P., Clow, A., 2004. The effect of dawn simulation on the cortisol response to awakening in healthy participants. Psychoneuroendocrinology 29, 925—930.

Thorn, L., Hucklebridge, F., Evans, P., Clow, A., 2006. Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. Psychoneuroendocrinology 31, 1009—1018.

Trojan, D.A., Arnold, D., Collet, J.-P., Shapiro, S., Bar-Or, A., Robinson, A., Le Cruguel, J.-P., Ducruet, T., Narayanan, S., Arcelin, K., Wong, A.N., Tartaglia, M.C., Lapierre, Y., Caramanos, Z., Da Costa, D., 2007. Fatigue in multiple sclerosis: association with disease-related, behavioural and psychosocial factors. Mult. Scler. 13, 985—995.

van Kessel, K., Moss-Morris, R., 2006. Understanding multiple sclerosis fatigue: a synthesis of biological and psychological factors. J. Psychosom. Res. 61, 583—585.

van Kessel, K., Moss-Morris, R., Willoughby, E., Chalder, T., Johnson, M.H., Robinson, E., 2008. A randomized controlled trial of cognitive behavior therapy for multiple sclerosis fatigue. Psychosom. Med. 70, 205—213.

Veauthier, C., Paul, F., 2014. Sleep disorders in multiple sclerosis and their relationship to fatigue. Sleep Med. 15, 5—14.

Wilhelm, I., Born, J., Kudielka, B.M., Schlotz, W., Wüst, S., 2007. Is the cortisol awakening rise a response to awakening? Psychoneuroendocrinology 32, 358—366.

Ysrraelit, M.C., Gaitán, M.I., Lopez, A.S., Correale, J., 2008. Impaired hypothalamic—pituitary—adrenal axis activity in patients with multiple sclerosis. Neurology 71, 1948—1954.

Zigmond, A.S., Snaith, R.P., 1983. The Hospital Anxiety and Depression Scale. Acta Psychiatr. Scand. 67, 361—370.