Sleep deficiency and evening cortisol levels in a heterogeneous patient population on long-term sick leave

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Abstract

Objectives Sleep deficiency is implicated in long-term sick leave and is found to frequently co-occur and interact with both mental disorders and physical illness. Higher prevalence of sleep deficiency in clinical populations compared to the general population indicates a complex relationship between sleep and disease. A dysfunctional HPA-axis has been associated to conditions like depression, anxiety and chronic fatigue syndrome. This study used elevated evening cortisol levels as a predictor for sleep deficiency.

Methods Saliva cortisol measures were assessed in a heterogeneous patient population on long-term sick leave (*N* = 44). The participants were diagnosed with depression, anxiety and/or chronic fatigue syndrome. Saliva cortisol was collected on two consecutive days at awakening, +30 min (CAR) and at 22:00. Multiple logistic regression were used to measure potential risk factors for sleep deficiency.

Results Mental health (anxiety and depression) was a strong risk factor for sleep deficiency. The study did not find a significant relationship between elevated evening cortisol levels and sleep deficiency. CAR-to-evening cortisol slope was not significantly different in patients diagnosed with depression, anxiety or chronic fatigue.

Conclusion This study replicates previous studies demonstrating that anxiety and depression, both comorbid and independently, are significant risk factors for sleep deficiency. No relationship was found between elevated evening cortisol levels and sleep deficiency.

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Contents

1. Introduction

Sleep deficiency is common in Norway with 11.7 % of the population meeting the clinical criteria for an insomnia diagnosis [1]. Insomnia is one of the most common sleep disorders [2] and is defined as difficulties initiating or maintaining sleep, experiencing early morning awakenings and decreased daytime functioning for no less than 1 month [3–6]. Sleep problems are a major health complain and a common reason for medical consultations in Norway [7]. According to the 2011 update of the National Institutes of Health (NIH) Sleep Research Plan [8], the term sleep deficiency is characterized as an inadequate quantity or quality of sleep obtained for optimal wellbeing, performance and health [8,9]. Sleep deficiency may occur from sleep deprivation, sleep disorders, insufficient sleep duration or poor sleep quality [10].

Norway has the highest rate of sickness absence in the OECD [11] and with around 90 recognized sleep disorders, serious health effects may arise as consequences of inadequate sleep [12,13]. Sleep deficiency have been implicated in long term sick leave [14] and is linked to stress [15], psychological distress [16] and somatic disease [17]. Research show that sleep problems frequently co-occur and interact with both mental disorders and physical illness [3,5,18]. Furthermore, higher prevalence of sleep deficiency in clinical populations compared to the general population indicates a complex relationship between sleep and disease [19].

Homeostatic imbalance could potentially lead to sleep deficiency [20]. Furthermore, sleep problems like insomnia symptoms have been found to predict sick leave [21], and is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis [13]. The relationship between sleep and stress is critical in insomnia [3]. Prior research have found that insomniacs have higher evening cortisol levels after stress exposure than healthy controls [22]. In addition, sleep deprivation is strongly linked to elevated evening cortisol levels [23–25]. Elevated evening cortisol levels have been shown to indicate sleep deficiency [26] and alterations of the HPA-axis [27].

1.1. Stress - allostasis and allostatic load

Homeostasis, the process of maintaining a complex dynamic equilibrium, is constantly challenged by internal and external stressors [28]. Thus, stress can be defined as a state of threatened homeostasis where adaptive responses are needed in order to re-achieve a balanced state [29]. Because we often encounter environmental conditions requiring our urgent attention and reactions, it has been theorized that the burden of chronic stress could enhance adaptive responses in the body called allostasis [23]. Allostasis provide compensatory feedback during stress in order to re-achieve the state of homeostasis [30,31]. Disrupted sleep is known to enhance physiological stress [32] which is something that has been seen in individuals with depression and/or anxiety [33].

Chronic stressors could activate pathological mechanisms in the human body referred to as allostatic load [34,35]. It is also known that chronic activation of the HPA-axis may contribute to allostatic load by abnormal activation levels, further targeting brain structures like the amygdala in which behavioral changes may enhance fear and anxiety [36,37]. Studies on chronic stress in animal models have demonstrated neuronal hypertrophy (dendritic growth) in the amygdala which is believed to affect fear and anxiety [23,37].

The mechanisms of allostatic load describe how long-term exposure to chronic stress (seen as fluctuations or heightened neural and neuroendocrine responses), can lead to disease by an inefficient management of the structures promoting adaptation or by stress itself [35,38].

1.2. Mechanisms of stress – the HPA-axis

The HPA-axis can be described as a regulatory system connecting the central nervous system (CNS) with the endocrine system [39]. The hypothalamus, the pituitary gland and the adrenal cortex are the main parts of the HPA-axis. Corticotropin releasing hormone (CRH) is released from the hypothalamus and transported via the portal vessels to the anterior pituitary. Vasopressin (AVP) is found in some CRH-cells in the hypothalamic paraventricular nucleus (PVN) [40]. This nucleus is also the location for CRH production [40]. AVP act in combination with CRH to potentiate adrenocorticotropic hormone (ACTH) secretion into the bloodstream [42,43]. The main target of ACTH is the adrenal cortex, a site that regulates cortisol release by the adrenal zona fasciculata [44]. The HPA-axis itself is adjusted by a

negative feedback regulation of cortisol on the secretion of CRH and ACTH [45]. This regulation is known to reduce the prolongation of cortisol release as it helps maintain a pulsatile and circadian secretion [44,46]. A weakened negative feedback regulation could lead to an inadequate suppression of CRH and AVP, and possibly result in elevated cortisol levels [47]. HPA-axis hyperactivity is strongly associated with sleep deficiency [48], and a vast majority of research on both humans and rodents indicate that a dysfunctional HPA-axis could increase sleep disruptions [22,49].

The HPA-axis is a central component in an individual's stress response. In rats, CRH has been found to influence HPA-activity [50]. Timpl et al. (1998) [51] found a reduction in the release of ACTH and corticosterone in mice lacking a specific type of CRH receptor (Crhr 1).

A crucial aspect of the stress response is that it is only intended to operate for a limited period of time. Chronic stressors could accelerate an enhanced system-activation, something that is considered to be a fundamental concept in the generation of stress-enhanced pathology [29]. It is known that hypo- or hyperactivity of the pituitary- adrenal interaction could lead to alterations of the nervous system [52]. Increased sympathetic nervous system activity could have an effect on ACTH and on the adrenal glands which could further enhance the elevation of cortisol secretion [53]. Hyperactivity of the HPA-axis is associated with psychiatric disorders and clinical syndromes such as depression and insomnia, respectively [54,55].

Increased cortisol secretion in humans, and corticosterone in rats, have been associated with behavioral deficits like depression [56–58] and anxiety [59–62]. Depressed patients are also at increased risk for hypercortisolism compared to the general population [63]. Intravenous injections of CRH have an effect in healthy individuals that is similar to the pattern observed in hypercortisolemic depressive patients [64]. The overactive state of hypercortisolism could be attributable to a defective feedback regulation [65,66]. According to Carroll et al. [67] depressive hypercortisolism could emerge from adrenal cortex dysfunction in which cortisol secretion acts independently from ACTH resulting in irregular cortisol secretion.

Another condition associated with alteration of the HPA-axis is chronic fatigue syndrome (CFS). This condition is characterized by severe exhaustion and a combination of symptoms, particularly somnolence and both physical and mental fatigue [68,69]. It is estimated that 20-25 % of individuals diagnosed with stress-related disorders, such as CFS,

experience hypocortisolism [70]. Individuals diagnosed with CFS could be characterized as having a heightened negative feedback regulation [71] and a hyporesponsiveness on the various levels of the HPA-axis [70]. In addition, they commonly display a flattened circadian cortisol cycle [71–74]. A dysfunctional cortisol signaling, such as found in CFS, could be a result of a declined hormone bioavailability and/or decreased hormone sensitivity [75].

1.3. The relationship between stress and sleep

The stress response is adjusted by the central nervous system (CNS) and other peripheral organs. Two essential components of the response are CRH and the locus coeruleus-norepinephrine systems [76,77] , both contributing with notable physiological responses to stress [78]. As the main regulator of the HPA-axis, CRH could influence stress-induced changes in sleep by increasing insomnolence [79]. This could be a possible reasons to why CRH is so strongly associated with sleep deficiency [80,81]. Cortisol is suggested to have a modulatory effect on sleep [82] A distinct increase of CRH in the limbic system and pituitary of sleep deprived rats could influence behavior alterations and sleep-regulation [83,84]. Rats limited to 4 hours of sleep for an 8-day period showed increased ACTH and glucocorticoids (corticosterone) concentrations [85]. Other studies have also reported similar findings, indicating sleep loss to be a likely cause behind increased ACTH and cortisol levels [80,86,87].

It is important to focus on the complex reciprocal relationship between stress and sleep, considering that one third of the population will experience sleep problems at some time in their life [88]. A dysfunctional HPA-axis is strongly associated with sleep deficiency [89,90] and studies have indicated a positive correlation between poor sleep and HPA-axis activity in chronic insomniacs [48,91].

1.4. The link between sleep deficiency and evening cortisol levels

Cortisol secretion follows a pulsatile pattern, characterized by a relatively high awakening level that continues to rise until it peaks at about 30 minutes after awakening. This peak is called the cortisol awakening response (CAR) and is followed by a decline throughout the day, before reaching nadir (i.e. lowest level) [92] at around midnight [93,94]. The diurnal cortisol pattern is relatively independent from sleep and environmental inputs [80],

and is regulated by the hypothalamic suprachiasmatic nuclei (SCN) [95], a structure enhancing wakefulness by generation of daytime alertness [96]. The state of sleep has an inhibitory effect on cortisol secretion [54] however increased nocturnal HPA activity (possibly from nocturnal awakenings), contribute to sleep deficiency and is strongly linked to elevated evening cortisol levels in both healthy individuals and insomniacs [26,97]. Prior research are not conclusive, however, one study did not find elevated evening cortisol levels in individuals reporting sleep problems during the previous four weeks [98].

1.4.1. Evening cortisol levels in individuals with depression, anxiety and CFS

It has been shown that depressed individuals have higher cortisol concentrations particularly around nadir compared to age-matched controls [55,99,100]. A study on the relationship between depression and cortisol levels revealed that participants with severe depressive symptoms had higher evening cortisol levels than those with mild or low depressive symptoms [101]. Similar findings were reported in a study where elevated evening cortisol levels were more common in patients with severe psychiatric disorders than in those with less severe disorders [102]. Grynderup et al. (2013) [103] conducted a study on depression and cortisol levels and found that a smaller difference between morning and evening cortisol levels could be a risk factor for depression.

A positive relationship between anxiety and evening cortisol levels has been indicated in several studies [104–106]. However, Vreeburg et al. (2010) [107] found no such associations. In CFS, evening cortisol levels are typically reduced compared to healthy controls [74,108]. It is common for individuals with CFS comorbid with depression to display a pattern of evening hypocortisolism [74].

1.5. Sleep, stress and sick leave

Studies have shown that comorbid mental disorders are significant risk factors for sleep deficiency [109–111]. Furthermore, sleep deficiency has been identified as a serious risk factor for long-term sick leave [21,112–114] and to have a major impact on stress [115,116]. Prior research have identified sleep disturbances as common manifestations of both depression and anxiety [117]. Sleep disturbance is defined as chronic sleep disruption, in which the latter

have been characterized as dysregulation of sleep homeostasis and sleep deficiency [8]. Mood disorders are found in one-third up to one-half of all patients with sleep deficiency [118], and depression, more than any other psychiatric disorder, is strongly linked to sleep problems [119]. Insomnia, and sleep problems in general, are therefore often found to co-occur and interact with depression [112,120]. Because of such a strong interaction, the DSM-IV lists sleep problems as part of the clinical criteria for the diagnosis of depression [121].

Knudsen et al. (2013) [122] found that comorbid anxiety and depression were significant risk factors for sick leave. Another study showed that anxiety, more than depression, was identified as a crucial risk factor for sick leave [123].

Individuals with CFS on long-term sick leave report poor and altered sleep [124,125]. Even though sleep alterations could influence the etiology of CFS, they are not believed to be the common reason behind daytime fatigue [126]. A dysfunctional stress response is often common in CFS where symptoms of emotional distress are typically manifested somatically instead of emotionally [127]. The main purpose of sick leave is to contribute economically to those affected by illness or disease. Sick leave is meant to promote recovery and for people to regain their work capacity [113]. Mental health problems constitute considerable health problems in the Norwegian workforce [128]

Two common consequences from being on sick leave is experiencing depression and increased stress [129]. Moreover, a study conducted on patients' daily life whilst on sick leave, revealed poor sleep to be a common consequence, mainly due to the situation of being absent from work [113]. The negative effects of long-term sick leave is therefore strongly linked to both sleep and psychological well-being [113]. This correlates with a recent study showing that return to work was associated with improved sleep [113]. By improving sleep one does not only increase the chances of returning to work, but improvement could also be crucial in the rehabilitation and prevention of sick leave due to other stress-related disorders [130]. The risk of having comorbid conditions (where insomnia co-occur with psychiatric and medical disorders) are significantly reduced by sleep improvement [111].

A cross-sectional study demonstrated that those who reported troubles initiating sleep, maintaining sleep, or experienced early morning awakenings were more likely to have two or more health problems than those without these symptoms [111,131]. Undiagnosed comorbid conditions in patients on long-term sick leave could predict their work ability and extent of sick leave [132]. It has been suggested that insomnia, either as a primary diagnosis or

secondary to other illnesses, should receive a greater emphasis for clinical intervention in order to lessen the economic burden accompanying sick leave [21].

1.6. Aim

The aim of the present study was to examine the effects of sleep deficiency, measured on Insomnia Severity Index (ISI) [133] in a heterogeneous patient population. We expect that patients with elevated evening cortisol levels would report higher levels of sleep deficiency regardless of any diagnosis.

2. Methods

2.1. Sampling procedure

From January to June 2012, 112 patients between 18-59 years of age were referred to Hysnes Vocational Rehabilitation Center by their general practitioners (GP) for a 3.5-week in-patient intervention. The patients had been on sick leave longer than 8 weeks due to musculoskeletal pain, fatigue and/or common mental disorders. The participants were asked upon admission if they would be interested in participating in a study while receiving the intervention. Participants were excluded if they did not define return to work as a personal goal, had a severe mental disorder (acute psychosis, an ongoing manic episode or suicidal ideation), were not able to communicate in Norwegian, or were pregnant. Participants met with a physician, a psychologist and a physical therapist at a designated out-patient clinic to be examined before participating in the intervention. Prior to this examination the participants filled out an extensive web-based survey provided by CheckWare™. This survey included measures of sleep problems, mental distress, fear-avoidance measures, fatigue and socio-demographics.

A total of 44 participants, 9 men and 33 women (2 missing), between 22 to 60 years of age (M = 39.95, SD = 10.35) were included in the study. Demographic information including *age*, *sex* and *BMI* (weight and height) was assessed by questionnaire and at out-patient clinic.

2.2. Measures

2.2.1. Depression and anxiety

The Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) [134] is a semi-structured interview used to diagnose major mental disorders according to DSM-IV-TR [135]. The SCID-I include obligatory questions where symptoms are rated in a categorical system before an algorithm is used in order to provide a final diagnosis [134]. The SCID-I was performed by a clinical psychologist at the out-patient clinic to assess the presence of individuals with a diagnosis of *depression* (*n*=9) and *anxiety* (*n*=5).

2.2.2. Psychological and somatic measures

Sleep deficiency was measured by the Insomnia Severity Index (ISI) [133]. The ISI comprises seven items evaluating perceived insomnia severity in the previous two weeks [136]. The questionnaire assess sleep-onset, sleep maintenance, early and nocturnal awakenings, satisfaction with current sleep pattern, interference with daily functioning, recognizable impairments attributed to sleep problem, and level of concern caused by the sleep problem [133,137]. Each question is scored on a 5-point Likert scale (0-4) with a total range of 0-28 [133]. Scores 15-21 indicate moderate, clinically significant insomnia, and scores 22-28 indicate severe, clinically significant insomnia [138]. This study uses a cut-off score of >14 which has shown to have optimal specificity (94%) and sensitivity (94%) [137]. Different versions of the ISI are available; patient, clinician, and significant others [139]. This study uses the patient (self-administered) version only.

Chronic fatigue was measured by the Chalder Fatigue Questionnaire [140]. The questionnaire assesses symptoms of cognitive difficulties, sleepiness, tiredness, loss of interest, lack of energy, lack of muscle-strength and lack of endurance [140,141]. Each question is scored on a 4-point Likert scale (0-3) in which the response alternatives are scored bimodally, 0-0-1-1, with a total range of 0-11 [141]. In this study, the 11 item questionnaire, which has an internal consistency, measured by Chronbach's alpha, of 0.89 was used [142]. The questionnaire was later revised to include two questions on duration and extent [143]. A cut-off indicating fatigue was a score of ≥5, with a duration of more than 6 months. This cut-off has been validated for a Norwegian population [143]

2.3. Cortisol sampling

Saliva was collected at awakening, 30 minutes after awakening (CAR), and at 22:00 hours. In order to control for cortisol variability, which are known to depend on day of the week[144], collection was conducted on two consecutive days; Tuesdays and Wednesdays or Wednesdays and Thursdays. A former study found no differences in cortisol values between Tuesdays-Thursdays [145]. All participants were told to avoid touching the cotton swab with their fingers when removing or inserting it into the sampling tube (SARSTEDT AG & Co, Salivette® Cortisol). They were further instructed to avoid caffeinated drinks, smoking,

physical activity and eating 1 hour prior to sampling. Written instructions were also provided along with the sampling tubes. All participants were told to keep the swab in the mouth for 1 minute and to note the time of sampling on the instruction sheet. The samples were domestically stored in a refrigerated state during the collection period before they were further stored at minus 20 degrees C. At this temperature cortisol samples remain stable for 9 months [146].

2.4. Cortisol analysis

The cortisol samples in the current study were analyzed within 7 months at the Department of Medical Biochemistry at St. Olavs Hospital, Trondheim. The assay used for determination of cortisol in saliva was Roche Modular E with an analytic variability of 7.9 % at 12 nmol/L.

2.5. Statistical analysis

All statistical analyses were conducted using R statistical software (R Development Core Team 2012) for Windows and IBM SPSS statistics 21 (SPSS Inc., Chicago, IL USA) for Windows.

Participants with one or more missing CAR or evening measures were excluded from further data analysis. In addition, descriptive statistics were used to characterize basic features for each variable of interest. Mean awakening, CAR and evening values were cleaned in order to remove outliers (values > 3 standard deviations from the mean) [147]. Pearson product-moment correlation coefficients were used to measure the relationship between the two days of saliva collection, and we used the mean over the two collection days for further analyses [148]. Pearson product-moment correlation coefficients were also computed to assess the relationship between depression, anxiety and CFS.

The diurnal cortisol rhythm was measured by the slope, as an indication of the rate of decline. We only included participants with valid CAR and evening samples for both days of saliva collection. The slope was calculated as the difference between CAR and evening cortisol levels in the valid saliva samples, and elevated evening cortisol levels (i.e. flat CAR-

to-evening slope) was by determined by values >1 standard deviation above the mean cortisol slope.

The associations between baseline characteristics and sleep deficiency (yes or no) were analyzed using students t-test for continuous variables: cortisol slope, evening cortisol levels, age, and BMI, or Fischer's exact test for categorical variables: sex, depression, anxiety, and CFS. All statistical analyses in the current study operated with a *p*-value of <.05 to be of statistical significance.

2.5.1. Regression analysis

Logistic regression analysis was used to examine the relationship between sleep deficiency and elevated evening cortisol levels (i.e. flat CAR-to-evening slope). The following covariates (determined a priori based on known influence on saliva cortisol measures) were included in the analyses: age (continuous), sex (male, female), BMI (continuous), depression (yes, no), anxiety (yes, no) and CFS (yes, no).

The main predictor variable of interest was elevated evening cortisol levels (a flat cortisol slope), while depression, anxiety and CFS together with age, sex and BMI were other covariates included in the analysis. The dependent variable, *sleep deficiency*, was dichotomized and results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). In addition, the probability values (*p*-values) and the coefficient for the constants (Bs) together with the standard errors (SEs) are given.

2.6. Ethics

Participation was voluntary and all participants provided written informed consent. The study was approved by the Regional Committees for Medical and Health Research Ethics (REC) and conducted in accordance with the Declaration of Helsinki.

3. Results

3.1. Descriptive statistics

From a total of 44 participants, 19 (43 %) reported sleep deficiency, 6 (16 %) had elevated evening cortisol levels, 9 (21 %) were diagnosed with depression, 5 (12 %) were diagnosed with anxiety and 31 (74 %) were diagnosed with CFS. Table 1 provides an overview of the participant characteristics listed by the presence or absence of sleep deficiency.

Table 1 Participant characteristics and their association between the presence or absence of sleep deficiency. The level (SD), frequency (%) or mean (SD) are listed together with the *p*-value for the association.

	Sleep deficiency		
Characteristic	Yes (*n*=19)	No (*n*=25)	*p*-value
Cortisol slope CAR-to-evening, nmol/L, mean (SD)			
1. Flat	-8.33 (.58)	-8.33 (1.15)	.54
2. Not flat	-18.57 (4.85)	-18.77 (7.31)	.93
Evening cortisol levels, nmol/L, mean (SD)	7.08 (2.27)	6.91 (2.22)	.81
Age in years, mean (SD)	39.1 (11.8)	40,5 (9,2)	.66
Sex			
1. Male	3 (33.3 %)	9 (66.7 %)	.47
2. Female	16 (48.5 %)	17 (51.5 %)	
BMI, kg/m², mean (SD)	26,9 (4,2)	27,0 (5,4)	.96
Depression			
1. Yes	**8 (88.9 %)**	**1 (11.1 %)**	**.00**
2. No	**11 (31.4 %)**	**24 (68.6 %)**	
Anxiety*			
1. Yes	**5 (100 %)**	**0 (0 %)**	**.01**
2. No	**14 (36.8 %)**	**24 (63.2 %)**	
CFS**			
1. Yes	15 (48.4 %)	16 (51.6 %)	.73
2. No	4 (36.4 %)	7 (63.6 %)	

Significant P-values are bolded (p<.05); *Anxiety data missing on one participant; **Chronic fatigue data missing on two participants.

Descriptive statistics revealed that 3 participants were diagnosed with all three conditions; 3 participants were diagnosed with depression and anxiety; 3 participants were diagnosed with CFS and depression, and 4 participants were diagnosed with CFS and anxiety. Correlation coefficient revealed a significant relationship between depression and anxiety (r=.35, p<.05).

Sleep deficiency was not associated with mean evening cortisol levels or CAR-to-evening slope. Looking at the covariates, neither age, sex nor BMI were associated with sleep deficiency.

Sleep deficiency was, however, significantly associated with both depression and anxiety. These findings indicate that depression and anxiety are independently associated with a greater prevalence of sleep deficiency (p<.05). CFS, however was not associated with sleep deficiency. The CAR-to-evening cortisol slope in CFS was not significantly different from the slope in the other two conditions.

3.2. Cortisol values

From the cortisol samples returned, 14 samples were not taken by the participants leaving a total of 249 individual samples. In order to examine for normality and control for measurement inaccuracies, all cortisol values were checked for outliers. After removal of 5 outliers the cortisol data showed a normal distribution so there was no need for transformations.

Correlations coefficients between the two consecutive days of saliva collection indicated positive correlations between the two awakening (r=.45, p=<.01), and CAR (r=.75, p=<.01) measures, but not between the two evening (r=.23, p=.14) measures. Average cortisol values for the two consecutive days of saliva collection are shown for participants with sleep deficiency (Figure 1) and without sleep deficiency (Figure 2).

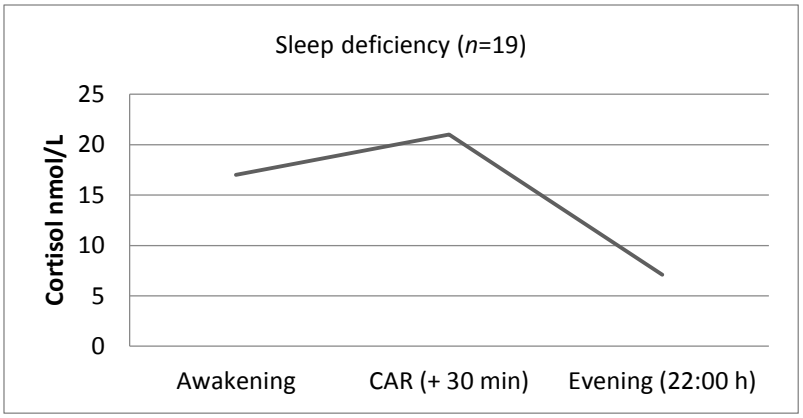

Figure 1 Average cortisol values for participants with sleep deficiency. Saliva concentration at awakening, CAR (+30 min) and evening (22:00 h) are shown.

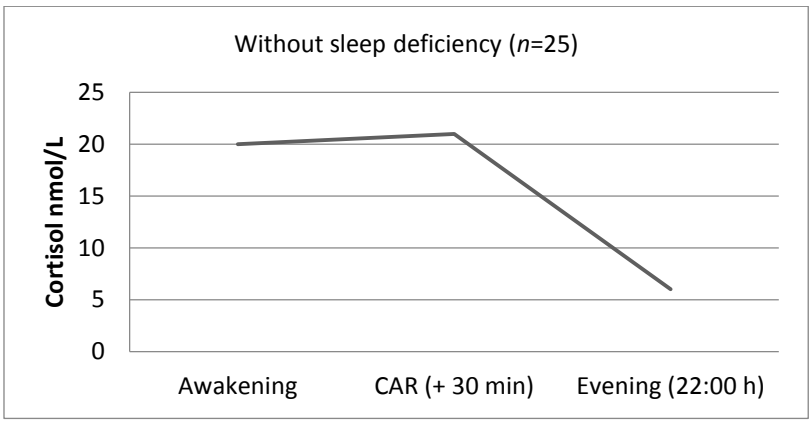

Figure 2 Average cortisol values for participants without sleep deficiency. Saliva concentration at awakening, CAR (+30 min) and evening (22:00 h) are shown.

3.3. Cortisol slope

In order to identify participants with elevated evening cortisol levels we calculated the slope from CAR to evening. Mean CAR cortisol value was 21.8 nmol/L based on 79 individual values, and mean evening cortisol value was 6.9 nmol/L based on 84 individual values. The average CAR-to-evening cortisol slope was -16.5 indicating a decline throughout the day.

3.4. Regression analysis

Multiple regression analyses were conducted to examine the relationship between sleep deficiency and various potential predictors. The analyses revealed that mental health (depression and anxiety) was the strongest predictor of sleep deficiency, whereas evening cortisol levels, age, sex, BMI and CFS showed more inconsistent relationships.

The results did not reveal a significant main effect of having a flat CAR-to-evening slope on sleep deficiency. Specifically, a flat CAR-to-evening slope (i.e. elevated evening cortisol levels) was not associated sleep deficiency (OR 1.07, 95% CI: .19-6.13). In addition, a non-flat CAR-to-evening slope (i.e. not having elevated evening cortisol levels) was not associated with sleep deficiency (OR .94, 95% CI: .16-5.39; data not shown). Table 3 summarizes the results.

Table 3. Summary of multiple regression analysis for the potential predictors of sleep deficiency.

				95 % CI for OR	
Variable	B (SE)	P-value	OR	Lower	Upper
Flat CAR-to-evening slope	.06 (.89)	.94	1.07	.19	6.13
Age	-.01 (.03)	.65	.99	.93	1.05
Sex	.63 (.79)	.42	1.88	.40	8.82
BMI	-.01 (.06)	.92	.99	.88	1.12
Depression	**2.86 (1.12)**	**.01**	**17.45**	**1.94**	**157.20**
Anxiety*	**∞ (∞)**	**.01**	**∞**	**1.32**	**∞**
CFS	.49 (.72)	.49	1.64	.40	6.76

*Fischer's exact test used instead of logistic regression due to small sample size. All participants with anxiety (*n*=5) reported sleep deficiency.

The results indicate significant main effects of depression and anxiety on sleep deficiency. Specifically, they were both independently associated with a flat CAR-to-evening slope. However, because all participants diagnosed with anxiety reported sleep deficiency, accurate calculation in the regression model was not performed. Instead, Fischer's exact test was used with results indicating an OR of infinity (Table 3).

4. Discussion

The goal of the current study was to examine the effects of sleep deficiency, measured on Insomnia Severity Index (ISI) [133], in a heterogeneous patient population. We expected that patients with elevated evening cortisol levels would report higher levels of sleep deficiency regardless of any diagnosis.

This study did not find that self-reported sleep deficiency was associated with elevated evening cortisol levels in participants diagnosed with depression, anxiety or CFS on long-term sick leave. Thus, the hypothesis that patients with elevated evening cortisol levels would report higher levels of sleep deficiency, regardless of diagnosis, was not supported.

The results from the current study contrast prior research on the effects of sleep on evening cortisol levels [27,97,149]. For example, Rodenbeck et al. (2002) [97] found alterations in HPA-activity in participants with sleep problems. Moreover, insomnia and sleep deprivation have been linked to hyperactivity of the HPA-axis [89], and increased evening cortisol levels, respectively [23].

Because an essential aim of sleep is to restore depleted energy levels [12], and because sleep is such a fundamental component of human homeostasis [150] we expected participants reporting sleep deficiency to display an abnormal HPA-activation. Our study did not support HPA-axis dysfunction to be associated with sleep deficiency [89,90].

It is known that glucocorticoids could influence stress and brain functioning, and that a dysfunctional HPA-axis could lead to increased saliva cortisol concentration [151]. The latter has been found to indicate depression [152], however no such association was found in the current study. Consistent with prior research on comorbidity and sleep, our results indicate that comorbid mental disorders were significant risk factors for sleep deficiency [109–111]. Breslau et al. (1996) [153] found a strong association between depression and sleep disturbances, and that sleep disturbance per se, could signal an increased risk for the onset of depression.

A prior study conducted by Grynderup et al. (2013) [103] found that a small difference between cortisol levels in the morning and evening could be a potential risk factor for depression. Even though depression was significantly related to sleep deficiency in our study, it was not related to elevated evening cortisol levels.

The current study did not reveal a significant relationship between CFS and sleep deficiency, nor did it indicate hypocortisolemia in CFS [74]. Interestingly, the majority of participants with CFS in our study reported having sleep deficiency, but the relationship with elevated cortisol levels were not statistically significant. Nater et al. (2008)[154] found that CFS is associated with a weak reduction in cortisol concentration across the day, and that this decreased diurnal cortisol variation is significantly different from the pattern found in healthy controls [72]. This pattern could also be indicative of a flattened diurnal cortisol rhythm, but our results found no significant difference between the cortisol slope in CFS and the other two conditions.

Neuroendocrine dysfunction, often reported in CFS, could occur as consequences of sleep disruptions [155]. CFS is typically associated with low evening cortisol levels [108] and many individuals with the condition display a flattened circadian cortisol cycle [154]. Our study did not find a flattened CAR-to-evening slope in patients diagnosed with CFS. We speculate whether comorbidity and/or light symptomatology could be potential reasons as to why the cortisol slope was similar in CFS and the other conditions.

Hansen et al. (2012) [98] did not find any relationship between sleep problems and morning-to-evening cortisol slope in CFS, but at follow up three months later, those with sleep problems displayed a flattened cortisol profile [98]. This suggests that a follow up could have been used in the current study to capture potential changes in cortisol secretion.

Even though the results were not significant, the OR for age, sex and BMI revealed that only sex had an effect on sleep deficiency indicating that being female was associated with an increased risk for sleep deficiency.

A major strength in this study is the use of cortisol secretion measured in saliva [156]. Many studies have used saliva sampling [157–160] because it is a non-invasive, reliable technique, used for determining adrenal cortical activity [94]. Another major strength was the implementation of SCID-1 as a diagnostic tool to diagnose individuals with depression and anxiety.

4.1. Limitations

The analyses in the current study were not adjusted for comorbidities other than simple descriptive statistics between the conditions of interest. Nor did the study control for symptom severity in depression, anxiety or CFS and could potentially have missed significant results in those with more severe symptomatology.

The study did not quire about participants' stress level during the days of saliva collection. Because insomniacs are known to have higher evening cortisol levels after stress exposure than healthy controls [22] a diary study of self-reported stress during the collection days could potentially control for external influences. The logistic regression model was unstable due to a predictor variable with a distribution of zero counts in one cell.

Even though the relationship between depression and anxiety was strongly significant one could argue that the correlation is weak considering only 5 participants were diagnosed with anxiety. The study population was not homogenous and a relatively high comorbidity rate was observed in the anxiety condition were 4 out of 5 of participants diagnosed with anxiety also reported CFS. Any inferences made must be interpreted with caution due to a small sample size. Future studies could include larger samples, particularly for a potential anxiety population.

In general, the coefficients should be no less than twice the size of its standard error to be of statistical significance [161]. Looking at the coefficient for the constants, only depression and anxiety revealed coefficients of significance, the latter being reported as infinity. Another limitation is the large confidence interval obtained for depression and anxiety. Larger studies have the ability to potentially offer more precise estimates of effect, and the ability to obtain narrower confidence intervals [162].

Multilevel modelling could have been used in order to examine possible relationships between the variables of interest and cortisol levels. Hruschka et al. (2005) [163] describe how multilevel modelling have the ability to model variation between- and within individuals.

No significant relationship was found between the two evening measures of the two consecutive days of two days of saliva collection.

To conclude, the current study did not support the hypothesis that elevated evening cortisol levels were a risk factor for sleep deficiency. Mental health (anxiety and depression) was a strong risk factor for sleep deficiency. The study did not find a significant relationship

between elevated evening cortisol levels and sleep deficiency. CAR-to-evening cortisol slope was not significantly different in patients diagnosed with depression, anxiety or chronic fatigue.

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