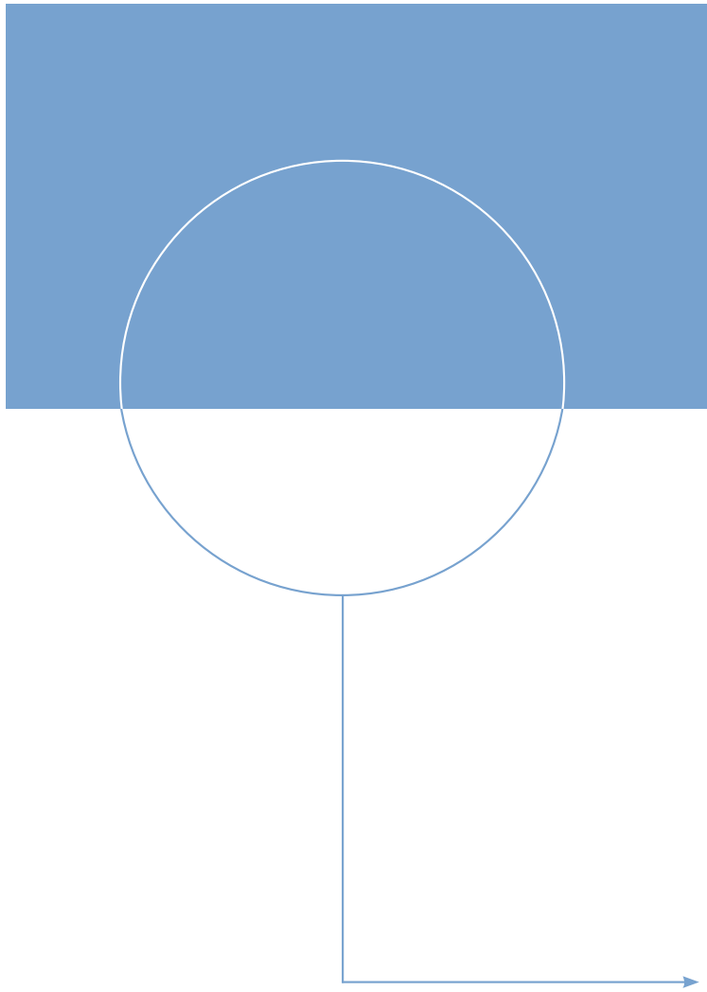

Master's thesis

2019

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Differences in diurnal saliva cortisol variation between patients with Chronic Fatigue Syndrome and patients with Fibromyalgia, and the role of Insomnia Severity as a predictor.

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Master's Thesis in Neuroscience
Submission date: June 2019
Supervisor: Tore C. Stiles

Norwegian University of Science and Technology
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Abstract

Objective: Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a severe chronic disease, that severely impacts the quality of life. The underlying pathophysiology of CFS/ME is still unknown, but a substantial body of research has focused on the potential role of the hypothalamic-pituitary-adrenal (HPA) axis. The overarching aim of this study was to further examine the differences in HPA axis functioning in CFS/ME and Fibromyalgia patient groups. This was done by examining diurnal cortisol variance, and evening cortisol levels using saliva samples.

Method: Female patients with CFS/ME (n = 18) and Fibromyalgia (n = 15) were recruited from a 10-week specialized multidisciplinary rehabilitation program in Trondheim, Norway. Six saliva cortisol samples were collected from each participant at fixed time points throughout the day. Participants also completed self-report questionnaires assessing their level of insomnia severity, fatigue, anxiety, depression, and pain intensity.

Results: CFS/ME patients exhibited significantly less cortisol variation within a day, compared to fibromyalgia patients. Insomnia severity was not a significant predictor of cortisol variation within a day. However, higher levels of insomnia were significantly associated with higher levels of cortisol in the evening irrespectively of primary diagnosis.

Conclusion: CFS/ME patients had significantly less cortisol variance than fibromyalgia patients, still they do not significantly differ in levels of symptoms such as fatigue, pain intensity, insomnia severity, anxiety, and depression. This may indicate that significantly lower cortisol variance is maintained by other mechanisms in CFS/ME patients compared to Fibromyalgia patients, further suggesting different pathophysiology between the two disorders.

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Finally, I would like to thank my friends and family for all the encouragement and support.

Abbreviations

CFS	chronic fatigue syndrome
ME	myalgic encephalomyelitis
FM	fibromyalgia
HPA	hypothalamic-pituitary-adrenal
CNS	central nervous system
PVN	paraventricular nucleus
CRH	corticotropin releasing hormone
AVP	arginine vasopressin
ACTH	adrenocorticotropic hormone
CAR	cortisol awakening response
AUC	area under the curve
IL-6	interleukin-6
TNF	tumor necrosis factors
HADS	hospital anxiety and depression scale
M.I.N.I.	mini-international neuropsychiatric interview
HPLC	high-performance liquid chromatography
ISI	insomnia severity index
IIS	insomnia interview schedule
REK	regional committee for medical and health research ethics
BMI	body mass index
CBT	cognitive behavioral therapy

Contents

1 Introduction

1.1 Chronic Fatigue Syndrome

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is an illness characterized by persistent, extensive and unexplainable fatigue that does not improve from rest (Fukuda et al., 1994). According to the Fukuda criteria, which is the main criteria for CFS/ME used within research, patients also experience other symptoms in addition to the fatigue. Additional symptoms include substantial impairment in short-term memory and/or concentration, sore throat, tender lymph nodes, multi-joint pain, newly occurring headaches, and unrefreshing sleep (Fukuda et al., 1994). For a diagnosis to be made, the patient has to experience at least four of these symptoms in addition to the fatigue that has been present for at least six months (Fukuda et al., 1994). There is no biological marker for CFS/ME. Thus, clinicians have to ensure that no other medical or severe psychological condition, previous or present, can explain the fatigue. CFS/ME is a disorder that severely impairs the daily functioning of the individual, and often for a period of several years (Brown, Brown, & Jason, 2011; De Gucht, Garcia, den Engelsman, & Maes, 2017).

Due to different criteria, diagnostic methods, as well as different target populations, the prevalence rates of CFS/ME vary substantially among studies. The estimated prevalence is 0.4-2.4% in the general population (Johnston, Brenu, Staines, & Marshall-Gradisnik, 2013). While based on the national guidelines from the Norwegian Directorate of Health (2015) the prevalence is 0.2% when applying the Canada criteria, and 0.4% when applying the Fukuda criteria. Patients diagnosed using the Canada criteria tend to have less comorbidity with psychiatric conditions, be more physically impaired, and experience more fatigue/weakness and neurological symptoms compared to patients diagnosed using the Fukuda criteria (Jason, Torres-Harding, Jurgens, & Helgerson, 2011). CFS/ME is also more frequently seen in females (Faro et al., 2016).

Little is known about the cause of the disorder, but various possibilities have so far been investigated. Some of the current areas of focus are immunology, metabolic factors, and neurology (Scheibenbogen et al., 2017). With little information about what causes the disorder, there is also little information on how to treat it.

1.2 Fibromyalgia

A patient group that often experience similar symptoms to CFS/ME, is fibromyalgia patients. Based on the 2016 Fibromyalgia criteria, Fibromyalgia (FM) can be characterized as diffuse, widespread, and generalized chronic pain that has been present for at least 3 months at the current level (Wolfe et al., 2016). Pain has evolutionary worked as a warning system and a strong motivational cue for avoidance. For chronic pain patients, this pain can persist beyond the time of healing, or it can be sustained for more than three months. In addition to the pain, FM patients typically also experience cognitive symptoms, unrefreshing sleep, and fatigue (Wolfe et al., 2016). Studies have shown a prevalence of FM from 0.2% to 5% (Heidari, Afshari, & Moosazadeh, 2017). A large German population study found a prevalence of 2.1% in the general population (Wolfe, Brahler, Hinz, & Hauser, 2013), similar prevalence rates have also been found in other population studies (Cabo-Meseguer, Cerdá-Olmedo, & Trillo-Mata, 2017; Park & Gilmour, 2017). Similar to the CFS/ME patient group, there is also a greater prevalence of FM among women (Park & Gilmour, 2017; Wolfe et al., 2013). To this day, the pathophysiology underlying FM is still not fully understood (Clauw et al., 2018). There is however evidence suggesting that FM is a disorder of the central nervous system (CNS), mainly seen through central sensitization, leading to increase in pain response to a normally non-painful stimulus (Clauw, 2015). It has also been theorized that changes in the CNS lead to the development of CNS-mediated somatic symptoms like fatigue, memory difficulties and mood disorders (Sluka & Clauw, 2016). Neuroimaging studies suggest that "central sensitization" is not the cause of syndromes like fibromyalgia, but that "central sensory augmentation" may be a predisposing factor (Jensen et al., 2012).

Previously, it has been hypothesized that CFS/ME and fibromyalgia are versions of the same disease, a theory called The Single Syndrome Hypothesis (Ciccone & Natelson, 2003; Wessely, Nimnuan, & Sharpe, 1999). This was mainly due to the ambiguity, and the complex, chronic and unknown etiology of both disorders. Patients in both groups share symptoms of pain, fatigue and sleep disturbance. The patients also often have a variety of coexisting medical and/or psychiatric conditions that complicate diagnosis (Landis, 2011). Still, there appears to be different underlying pathophysiology behind CFS/ME and FM, suggesting that they are in fact separate illnesses (Abbi & Natelson, 2013).

1.3 Sleep disturbances in CFS/ME and Fibromyalgia

Both CFS/ME and FM patients have a higher prevalence of sleep disturbances than the general population (Theadom & Cropley, 2010). For CFS/ME the most common issue with sleep is non-restorative sleep. As many as 87-95% of CFS/ME patients self-report having problems with non-restorative sleep (Mariman et al., 2013). Non-restorative sleep is characterized by not feeling rested, even with an adequate amount of sleep. Other common sleep complaints are; excessive daytime sleepiness, difficulty initiating sleep, and sleep fragmentation (Mariman, Vogelaers, Hanoulle, Delesie, & Pevernagie, 2012). A review calculated the average prevalence from different studies and estimated a 68-86% prevalence of self-reported insomnia among CFS/ME patients (Ferre, 2016). Evidence of sleep disturbances in fibromyalgia patients was found already in 1975 (Moldofsky, Scarisbrick, England, & Smythe, 1975). These disturbances included difficulty with sleep onset/maintenance and/or persistent nonrestorative sleep (Moldofsky et al., 1975). It has been estimated that 70-80% of FM patients suffer from sleep disturbances, and 23% of FM patients report having insomnia (Landis, 2011). It has been theorized that sleep difficulties might play a role in maintaining FM-related fatigue and discomfort/pain (Nicassio, Moxham, Schuman, & Gevirtz, 2002). Lack of restorative sleep has also been found to cause hyperalgesia, increased pain sensation (Sutton & Opp, 2014). Based on these findings, the severity of symptoms experienced by FM, and also CFS/ME, patients may be modulated by the interaction of sleep disturbance and daytime pain/distress (Edinger, 2005). Thus, it is believed that therapy designed to improve sleep may interrupt the sleep-pain/distress cycle, and lead to overall improvements in fatigue and pain symptoms (Edinger, 2005)

Insomnia is a diagnosis that often goes hand-in-hand with psychiatric conditions. It has been estimated that 50-80% of patients with a psychological disorder, most commonly anxiety and depression, also have insomnia (Morin & Ware, 1996). When this is the case, insomnia is often viewed as the secondary problem, and often a symptom of the primary problem. This has led to a lack of focus on insomnia in the mental health sector, leading to many patients not receiving adequate treatment (Harvey, 2001). There is a higher prevalence of insomnia, anxiety, and depression among CFS/ME patients, compared to the general population (Reeves et al., 2006; Taylor, Loades, Brigden, Collin, & Crawley, 2017). Research has shown that using insomnia as a therapeutic target, can be useful in the treatment of other comorbid disorders, like for instance depression (Bei et al., 2018; Manber et al., 2008). Thus, insomnia might be a useful therapeutic target, for treatment of CFS/ME and FM (Kallestad, Jacobsen, Landro, Borchgrevink, & Stiles, 2015).

1.4 The Stress Response

Sleep plays a critical role in our health. It is important for our metabolic rate, immune system, memory consolidation, and it is believed to have a stress-buffering effect. This stress-buffering effect is associated with having a healthy variance of cortisol during the day and parasympathetic tone (Palesh et al., 2008). The fluctuation of cortisol, which is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, is a vital part of the stress response. The HPA axis consists of multiple structures. These influence each other directly and through feedback interactions. First, we have the paraventricular nucleus (PVN) of the hypothalamus, which releases corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) (Tomas, Newton, & Watson, 2013). These hormones cause the pituitary to secrete adrenocorticotropic hormone (ACTH) into the systemic circulation (Tomas et al., 2013). The ACTH then bind at the adrenal gland, which stimulates the synthesis and secretion of cortisol. Cortisol is then released in a pulsatile fashion, and it plays an important role in ensuring strict regulation of both feedforward and feedback loops in the HPA axis (Tomas et al., 2013). Circulating cortisol will activate mineralocorticoid and glucocorticoid receptors in the PVN, which then will decrease the secretion of CRH, AVP, and ACTH (Tomas et al., 2013).

Cortisol

The secretion of cortisol from the adrenal glands in response to stress has various effects on the body. Cortisol is mostly known as a stress hormone, making the body able to deal with stressors. Cortisol is also involved in regulating various processes in the body and brain including arousal, energy and metabolic processes, mood, the functioning of immune and inflammatory systems, vasoconstriction, and maintaining homeostasis (Sapolsky, Romero, & Munck, 2000).

The secretion of cortisol follows a diurnal rhythm. A peak in cortisol values occurs typically 30-40 minutes after awakening. The level of secretion then decreases until around midnight (Stone et al., 2001). Within large epidemiological studies, the three main parameters used for measuring the diurnal rhythm of cortisol are cortisol awakening response (CAR), the area under the curve (AUC), and diurnal cortisol slope (Adam & Kumari, 2009). These methods have also been robustly linked to psychosocial processes and health outcomes, implying clinical relevance (Adam & Kumari, 2009). The different parameters are believed to reflect different aspects of HPA axis functioning (Ryan, Booth, Spathis, Mollart, & Clow, 2016). The CAR, which is the rise in cortisol 30-40 minutes after awakening, is important for mobilizing the bodies energy reserves when we wake-up (Pruessner et al., 1997). It is also

believed to be important for switching the activity of the immune system in the morning (Hucklebridge, Clow, Abeyguneratne, Huezo-Diaz, & Evans, 1999), and anticipation of the upcoming day´s events (Fries, Dettenborn, & Kirschbaum, 2009). Findings suggest that the CAR might be mediated by an extra-pituitary pathway to the adrenal from the suprachiasmatic nucleus (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). The AUC is typically used to estimate the overall secretion of cortisol within a day (Pruessner et al., 1997), but it will provide no indication of diurnal change (Adam & Kumari, 2009). The diurnal slope is the rate of decline in cortisol levels from the morning to the evening, here a steeper decline has been correlated to better psychosocial and physical health (Adam & Kumari, 2009). The diurnal slope is believed to be an indication of the function of the HPA axis negative feedback loop, which is strongly linked to the stress response (Dmitrieva, Almeida, Dmitrieva, Loken, & Pieper, 2013). Stress will normally cause elevations in diurnal cortisol, facilitating recruitment of energy and coping resources. A theory is that repeated HPA axis activation, or overactivation, alters the relationship between chronic stress and cortisol secretion (Milrad et al., 2017). Repeated or sustained cortisol elevations may lead to a breakdown in the negative feedback system of cortisol secretion, resulting in hypofunction in the HPA axis seen through a flattened diurnal slope (Dmitrieva et al., 2013). A flattened diurnal slope is believed to indicate an inability to mobilize energy and resources when facing a stressor. A flattened curve has also been correlated with more negative clinical and health outcomes (Milrad et al., 2017). It has been hypothesized that the pathophysiologic basis of CFS/ME, might be a breakdown in this negative feedback system, from a mode of overactivation, to a mode of underactivation (Van Houdenhove, Kempke, & Luyten, 2010).

Evening rates of cortisol has been shown to be useful in linking both sleep, cortisol and proinflammatory cytokines. High levels of evening cortisol have previously been seen as an indicator of insomnia (Leproult, Copinschi, Buxton, & Van Cauter, 1997; Spiegel, Leproult, & Van Cauter, 1999). Thus, evening measures of cortisol might be an indicator of the biological severity of insomnia (Basta, Chrousos, Vela-Bueno, & Vgontzas, 2007).
The quantity and quality of our sleep have also been known to negatively influence pro-inflammatory cytokines, which are fatigue-inducing. The focus has especially been put on Interleukin-6 (IL-6) which is involved in peripheral pain sensory neurons, and Tumor Necrosis Factors (TNF) which is involved in central sensitization of pain (Basta et al., 2007). Greater evening cortisol has also been linked to greater levels of circulating pro-inflammatory cytokines (Lattie et al., 2012). Findings have been mixed in relation to pro-inflammatory cytokines in CFS/ME patients (Groven, Fors, Iversen, White, & Reitan, 2018; Vollmer-Conna

et al., 2007), but findings indicate a trend towards higher levels of pro-inflammatory cytokines among CFS/ME patients (Groven et al., 2018). Increased levels of pro-inflammatory cytokines have also been found in fibromyalgia patients (Rodriguez-Pinto, Agmon-Levin, Howard, & Shoenfeld, 2014; Wang, Moser, Schiltenwolf, & Buchner, 2008).

HPA axis function in CFS/ME and Fibromyalgia patients

The first publication to identify dysfunction in the HPA axis among CFS/ME patients was published already in 1991 (Demitrack et al.). Here the focus was on the similarities between the symptoms experienced by CFS/ME patients, and the symptoms seen with Addison's disease (Demitrack et al., 1991). To this date, there is substantial evidence of hypocortisolism in CFS/ME (Papadopoulos & Cleare, 2011). With hypocortisolism, we typically see a shortening of the diurnal cortisol slope. In addition, we also see dysfunction in the resilience of the HPA axis, and an increase in fatigue, pain, and stress sensitivity (Fries, Hesse, Hellhammer, & Hellhammer, 2005). This can develop in multiple ways, including reduced synthesis of hormones from the HPA axis, down-regulation of receptors, and/or increased sensitivity of the negative feedback sensitivity of the HPA axis (Fries et al., 2005). Recent studies have found a decreased cortisol awakening response (CAR) among CFS/ME patients, but no significant difference in the total cortisol output during the day (Roerink et al., 2018). In addition, there has been found an increase in evening cortisol among CFS/ME patients (Nater et al., 2008a). Lowered levels at awakening, less negative diurnal slope throughout the day, and relative high bedtime levels lead to a blunted diurnal profile of cortisol (Dmitrieva et al., 2013). This can be caused by a failure to activate the HPA axis in the morning, and/or a failure to deactivate it in the evening (Fries et al., 2005; Heim, Newport, et al., 2000). The blunted diurnal cortisol curve has been interpreted as a physiological expression of vital exhaustion, which leads to a mental state where the ability to adapt to stress is disrupted (B.M. Kudielka, Bellingrath, & Hellhammer, 2006). If this is the case, a blunted diurnal cortisol curve might be a biological factor contributing to the maintenance of CFS/ME (Kallestad et al., 2015).

The activity of the HPA-axis in Fibromyalgia patients is more poorly understood (Turner-Cobb, Osborn, da Silva, Keogh, & Jessop, 2010). Studies have found lower cortisol levels in FM patients, than in the general public (Gur, Cevik, Nas, Colpan, & Sarac, 2004; Riva, Mork, Westgaard, & Lundberg, 2012). There have also been studies finding contradictory results (Geiss, Rohleder, & Anton, 2012). Some researchers believe these inconsistencies may be due to only subgroups of FM patients experiencing HPA axis alterations. One theory is that

the changes in cortisol after awakening can be linked to the level of fatigue (Doerr, Fischer, Nater, & Strahler, 2017). If this is the case only those FM patients with a higher degree of fatigue would experience lower increases in cortisol after awakening (Doerr et al., 2017). Another study suggested that reduced levels of cortisol secretion during a day in FM patients was linked to comorbid affective disturbances (Wingenfeld, Nutzinger, Kauth, Hellhammer, & Lautenbacher, 2010).

For this thesis, it was hypothesized that patients with a primary diagnosis of CFS/ME would have less diurnal variation in cortisol than FM patients. This is mainly based on two points. Firstly, greater fatigue has been related to a smaller diurnal cortisol slope (Doerr et al., 2017). Both patient groups typically experience fatigue, making it likely that both patient groups will have a smaller diurnal slope than non-fatigued individuals. However, since CFS/ME patients typically have fatigue as their primary symptoms, and generally experience more of it than FM patients, it is hypothesized that their slope will be smaller. The second point is that smaller diurnal cortisol slope is based on less good health outcomes (Dmitrieva et al., 2013). In general, both disorders tend to impact the individual in several aspects of daily life, but when examining results from the Short Form 36 Health Survey (J. Ware & Sherbourne, 1992) the CFS/ME patients show worse health outcomes than FM patients, on all eight health concepts [physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional wellbeing, social functioning, energy/fatigue, and general health perception] (Baraniuk et al., 2013; Hoffman & Dukes, 2008; Nacul et al., 2011). This is an indicator of worse quality of life and health outcomes among CFS/ME patients compared to FM patients. Thus, it would be expected for CFS/ME to experience a more blunted diurnal cortisol curve, reflecting less variance in cortisol within a day. In this thesis, we chose to use a measure of cortisol variance calculated as the range of the highest and the lowest samples of the five samples taken within a day. By using this measure we are able to capture the variance to a larger degree, than if the variance was only measured from morning and evening samples, which is a more common approach. Variance in cortisol has also been found to be more linked to the level of functioning throughout the day, and a more long-term measure of health outcome, than for instance CAR.

1.5 Purpose and aim

The dysfunction of the HPA axis in CFS/ME has been explored frequently in the last years, but the focus has mainly been on the cortisol awakening response, and less focus has been put on variability in cortisol during the day. In Fibromyalgia there has been little conclusive research in the field, and there appears to be few studies that have looked at CFS/ME and FM separately, but under the same conditions. This thesis contributes to creating a better understanding of the pathological factors separating CFS/ME and FM, which can help further improve the management of patients within these groups.

The overarching aim of the study is to further examine the differences in HPA axis functioning in CFS/ME and FM patient groups through examining diurnal cortisol variance. The hypothesis are that 1) Patients with CFS/ME will exhibit significant less saliva cortisol variation during a day compared to patients with fibromyalgia. 2) Higher levels of insomnia severity will be significantly associated with lower cortisol variation during a day, and higher levels of saliva cortisol at the evening irrespective of diagnostic status.

2 Methods

2.1 Sample

The full sample included 58 participants. Patients were recruited from a 10-week specialized multidisciplinary rehabilitation program in Trondheim, Norway. All patients starting rehabilitation between 19.11.18 and 11.02.19 were offered to participate in the study, but participation was voluntary. All participants were referred to the rehabilitation program by their general practitioner. In order to be referred they needed to have chronic pain or CFS/ME diagnosis. The chronic pain disorders seen included Fibromyalgia, arthritis, generalized pain and/or shoulder pain, myalgia, and persistent somatoform pain disorder. From this sample, 18 participants were excluded because they did not have a primary diagnosis of CFS/ME or Fibromyalgia. Eight male participants were also excluded to avoid gender as a confounding variable. Lastly, two participants were excluded because their cortisol measures were recorded as severe outliers. One patient did not want to participate, and four patients expressed interest but did not return the samples. After this, we were left with 33 patients. The participants were between the age of 20 and 66 (M = 35). For the diagnosis of CFS/ME both the Fukuda and the Canada criteria were used, and for fibromyalgia the 2016 Fibromyalgia criteria were used. All participants were informed about the process and the purpose of the study. None of the participants had major ongoing psychological conditions including psychosis, bipolar disorder, and substance abuse. None of the participants were pregnant.

2.2 Assessments

Psychological and Medical Examination

Before starting the rehabilitation program, all participants went through a psychological exam by a licensed psychologist. Here comorbid mental disorders were assessed using Mini-International Neuropsychiatric Interview (M.I.N.I) (Sheehan et al., 1998). A physician also performed individual medical examinations, including blood samples from each participant. Blood samples were used to ensure that a correct diagnosis had been made. During these exams it was also examined if FM patients would fit the diagnostic criteria for CFS/ME.

In addition to this, semi-structured diagnostic interviews were performed using an adapted version of the Insomnia Interview Schedule (Morin, 1993) to assess the sleeping pattern of all participants.

Cortisol

The sampling procedure was designed to be as user-friendly and stress-free as possible to get the highest possible participation rate. Participants were asked to measure their level of free cortisol in saliva at three different time points during their treatment period. Participants were all informed on the purpose and process of the study. Because of low participation rates analysis were based on only the first round of sampling. For the sampling, purpose-designed polyester Salivette produced by Sarstedt INC, Rommelsdorf, Germany, were used to collect saliva. All participants were instructed on how to accurately take the samples. Participants were instructed to keep the swab in their mouth for approximately 60 seconds, or until it felt soaked. They were encouraged to avoid touching the cotton swab with their fingers or lips when removing it or inserting it into the sampling tube. To avoid contamination of the samples participants were asked to avoid eating, smoking and other nicotine products, drinking caffeinated drinks, working out, and brushing their teeth in the hour before sampling, but ideally to avoid drinking coffee or smoking two hours before taking the sample. In addition to the oral instructions a handout was given with detailed instructions. Participants were also asked to fill in a form stating the time of each sample, medication taken, and an area to note down any abnormalities in their health (i.e. fever, sore throat). The participants were asked to do the cortisol sampling at five different times of the day, 08:00, 10:00, 14:00, 18:00, and 22:00. This was done to get a measure of their diurnal cortisol rhythm, and to capture as much variation as possible. Participants were asked to take the samples on days that best represented their average days, and try to go about their day as normal. Participants were encouraged to write down the accurate time of sampling and to note down any deviations. After sampling, the participants were instructed to store the samples in their refrigerators before returning the samples to the clinic the next day when they attended the rehabilitation program.

The samples were then sent to the Department of Medical Biochemistry at St. Olav's Hospital, Trondheim, for analysis. The analyze tool used was Agilent 1290 high-performance liquid chromatography (HPLC) with Agilent 6410 Triple Quad LC/MS-MS detector. This has an analytic variability of 5.5 % at 2 nmol/L.

The researcher was visible and available to answer questions from the participants on the days when they had rehabilitation. The researcher also provided the participants with individual feedback on the results of their tests, and provide answers to any questions.
When handing in the cortisol samples the participants were also asked to fill in forms assessing their level of insomnia, fatigue, anxiety, depression, and pain in the last week.

Insomnia

The insomnia severity index (ISI) was used to register the levels of insomnia symptoms. The ISI consists of seven items that measure the nature, severity, and impact of insomnia symptoms. The items are as follows: difficulty falling asleep, difficulty staying asleep, problems with waking up too early, current sleep pattern, noticeability to others, worried about sleep, and interference with daily functioning. A five-point Likert scale was used to indicate the level of severity on each item, giving a maximum score of 28. A value of 14 or higher is viewed as an indication of clinical insomnia. A value of eight or lower is seen as an indicator of normal sleep. Reliability and validity have been established for the ISI (Bastien, Vallières, & Morin, 2001; Morin, Belleville, Bélanger, & Ivers, 2011). ISI is also the recommended outcome measure for insomnia severity in clinical trials (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006).

Fatigue

The Chalder Fatigue Scale (Chalder et al., 1993) was used to assess the level of fatigue. The Chalder Fatigue Scale is a questionnaire consisting of 11 items, that are meant to measure both physical and mental fatigue. For each item there are four possible responses, these are scored bimodally 0-0-1-1 (less than normal = 0, like normal = 0, more than normal = 1, a lot more than normal = 1). A score of five or higher is viewed as an indication of chronic fatigue. The Chalder Fatigue Scale has shown high reliability and validity scores (Morriss, Wearden, & Mullis, 1998).

Pain

To assess the level of pain one item was used from the Short Form-8 (SF-8) (J. C. Ware, Kosinski, & Keller, 1995). In this item the participants were asked to indicate on a Likert scale, from 0 = no pain, to 6 = very strong pain, the level of pain experienced in the last week. A large Norwegian study found that this item was valid as a self-reported measure of pain (Landmark, Romundstad, Borchgrevink, Kaasa, & Dale, 2013).

Depression and anxiety

The Hospital Anxiety and Depression Scale (HADS) was used to determine the participants levels of anxiety and depression (Zigmond & Snaith, 1983). This scale consists of 14 items. From these half form an anxiety subscale, while the second half form the depression subscale. The validity of the HADS for use with CFS/ME patients has been documented (McCue, Buchanan, & Martin, 2006)

2.3 Statistical analysis

All statistical analyses were conducted using IBM SPSS statistical 21 software (SPSS Inc., Chicago, IL USA) for Windows. Patients were divided into groups based on their primary diagnosis, CFS/ME (n = 18) and Fibromyalgia (n = 15). Descriptive statistics were calculated to analyze the basic features of each subgroup. Normal distribution was evaluated for each symptom measure using Kolmogorov-Smirnov test. Group differences in normally distributed measures were assessed using independent t-tests, while group differences in skewed distributions were assessed using Mann Whitney U tests. Normally distributed data are presented with mean and standard deviation, while skewed data are presented with medians and standard errors. Pearson product-moment correlations were also calculated to access the relationship between the different symptom measures.

Z-scores for each subgroup were calculated to examine the dataset for outliers. Scores labeled "Probable outlier" ($z > 2.58$) or "Extreme outliers" ($z > 3.29$) were excluded, or if the participant had taken another round of samples, these were used instead. Two participants were excluded because of outliers, and no other measures available.

The cortisol parameters used were values for each fixed-time-slot, and a cortisol variance measure to examine the diurnal variation in the data. The cortisol variance measure was calculated as the highest value measured in a day, minus the lowest value measured in a day. All cortisol values are provided in nmol/L. A Kolmogorov-Smirnov test was performed on the cortisol measures to check for normal distribution. Following standard practices, all cortisol values were log-transformed to correct for non-normality (Stone et al., 2001). Independent t-tests were calculated to look for differences in the means between the two subgroups.

To test the first hypothesis, that CFS/ME patients will exhibit significant less saliva cortisol variation during a day compared to FM patients, a hierarchical multiple regression analysis was conducted. The dependent value was range of cortisol values. In step one the age variable was entered, and in step two the use of oral contraceptives (no = 0, yes = 1) was entered to statistically control for their potential confounding effects. In step three primary diagnosis (FM = 0, CFS/ME = 1) was entered.

To test the second hypothesis whether higher levels of insomnia severity were significantly associated with lower cortisol variation during daytime (08 am-10 pm) and higher levels of saliva cortisol at nighttime (10 pm), irrespective of primary diagnosis, two hierarchical multiple regression analysis were conducted. The factors entered were the same in both analysis, but the dependent variable was different. For the first regression analysis the dependent variable was cortisol variance during the daytime, while in the second analysis the dependent variable was cortisol level at 10 pm. In step one age was entered, in step two the use of oral contraceptives was entered, in step three primary diagnosis was entered, and in step four scores of insomnia severity was entered. All statistical analyses in the current study operated with a p-value of $< .05$ to be of statistical significance.

2.4 Ethics

Participation in the project was voluntary. The project has been approved by the Regional Committee for Medical and Health Research Ethics (REK).

3 Results

3.1 Descriptive statistics

Kolmogorov-Smirnov tests were calculated to control for normal distribution in all symptom variables, with a significant test indicating a non-normal distribution. The age, insomnia, anxiety, depression and pain variables showed normal distribution. Levels of fatigue were not normally distributed. Age and various symptoms between groups are described in Table 1, as well as the results of the statistical analyses examining group differences. Due to the lack of normal distribution, the fatigue variable was described in median values, and standard errors, and group differences are analyzed using a Mann Whitney U test. The two groups neither differed significantly in age, insomnia severity, fatigue, anxiety, depression nor pain intensity. Differences in sleep are further displayed in Table 2. This displays that there was no large difference between the groups in terms of sleep, but the fibromyalgia group had a larger percentage of normal sleepers than the CFS/ME group, but the difference is small.

Table 1

Group differences in age and various symptoms, including results from independent t-test and Mann Whitney U test for the fatigue variable.

	CFS/ME *(n = 18)*				Fibromyalgia (*n = 15)*						
	M	*Median*	*SD*	*SE*	*M*	*Median*	*SD*	*SE*	*t*	z	*p-value*
Fatigue *		9.00		0.70		13.84		0.27		1.81	.086
Age	31.67		12.49		38.00		9.41		1.62		.116
Insomnia	12.33		5.49		12.87		6.48		0.26		.800
Anxiety	6.11		3.31		8.67		5.25		1.64		.116
Depression	7.50		3.17		8.53		5.04		0.69		.498
Pain	3.11		1.18		3.73		0.80		1.73		.093

Fatigue = sum score on the Chalder Fatigue Scale.
Insomnia = sum score on the Insomnia Severity Index.
Anxiety and depression = sum score on the Hospital Anxiety and Depression Scale.
Pain = score on the level of somatic pain from the Short-Form 8.

* Variable did not have normal distribution according to Kolmogorov-Smirnov test.

Table 2

Group differences in sleep, showing percentage from each subgroup.

	CFS/ME	Fibromyalgia
ISI		
(≤ 8)	22%	27%
(9 – 13)	39%	33%
(≥ 14)	39%	40%
Sleep apnea	5%	7%
Circadian Rhythm Disorder	11%	-

ISI = score on Insomnia Severity Index.

A Pearson product-moment correlation coefficient was computed to assess correlations between the different symptoms. The results are presented in Table 3. Insomnia severity was significantly correlated with levels of depression and pain intensity, but neither with levels of fatigue nor anxiety. Levels of anxiety and depression also correlated significantly. Level of fatigue did not correlate significantly with any of the other symptoms.

Table 3

Pearson correlations between the different symptoms

	Fatigue	Anxiety	Depression	Pain
Insomnia	.01	.34	.49 *	.51**
Fatigue	--	-.28	-.10	-.15
Anxiety		--	.64 **	.30
Depression			--	.21
Pain				--

Insomnia = sum score on the Insomnia Severity Index.
Fatigue = sum score on the Chalder Fatigue Scale .
Anxiety and depression = sum score on the Hospital Anxiety and Depression Scale.
Pain = score on level of somatic pain from the Short-Form
* p = < 0.05.
** p = < 0.01.

3.2 Cortisol values

The Kolmogorov-Smirnov tests indicated a lack of normal distribution in the morning and evening measure of cortisol. Therefore all cortisol values were logarithmically transformed. After this the data showed normal distribution. Table 4 displays the logarithmically transformed means and standard deviations for the two groups as well as the results of the independent t-test. The only significant group difference was in the cortisol sample taken at 08:00. A correlation analysis was also done to assess the correlation between the different symptoms and the variance measure and evening measure of cortisol. The Pearson product-moment correlation coefficient showed that both insomnia severity (r = .40, p = .023) and pain intensity (r = .46,
p = .008) were significantly correlated with evening cortisol. Anxiety (r = .19, p = .269), depression (r = .20, p = .27), and fatigue (r = -.07, p = .69) were not significantly correlated with evening cortisol. None of the symptom variables were significantly correlated with the measure of cortisol variance.

Table 4

Means (M), standard deviations (SD) for the various cortisol measures, and results of the independent t-test to examine group differences. Cortisol values have been logarithmically transformed, and are given in nmol/L

	CFS/ME *(n = 18)*		Fibromyalgia *(n = 15)*			
	M	SD	M	SD	*t*	*p-value*
Variance	0.72	0.18	0.85	0.24	1.75	.090
08:00	**0.59**	**0.38**	**0.84**	**0.24**	**2.17**	**.038**
10:00	0.60	0.31	0.63	0.20	0.75	.461
14:00	0.37	0.27	0.31	0.16	-0.83	.414
18:00	0.19	0.29	0.09	0.16	-1.18	.246
22:00	-0.05	0.25	-0.20	0.24	-1.59	.122

Variance = Highest cortisol value minus lowest value.

3.3 Differences in cortisol variance between CFS/ME and Fibromyalgia

To test if the diagnostic group was significantly associated with cortisol variance a hierarchical multiple regression analysis was performed. The dependent variable was logarithmically transformed cortisol variance. Age was entered in the first step, and use of oral contraceptives in the second step to statistically control for their potential confounding effects. In the third step diagnostic group (CFS/ME vs Fibromyalgia) was entered. The results are summarized in Table 5. The regression model explained 17% of the variance in the cortisol variance variable. Neither age nor use of oral contraceptives was significantly associated with cortisol variance. However, as predicted, diagnostic status was significantly associated with cortisol variance indicating significantly lower cortisol variance in the CFS/ME group compared to the Fibromyalgia group.

Table 5

Summary of the hierarchical multiple regression analysis with cortisol variance as the dependent variable.

Variable	β	*t*	*p*	*F*	df	*p*	R^2
Overall model 1				0.02	1,32	.905	.000
Age	0.00	0.12	.905				
Overall model 2				0.74	2,32	.487	.047
Age	0.00	0.59	.558				
Oral Contraceptives	0.13	1.21	.236				
Overall model 3				1.99	3,32	.138	.170
Age	0.00	0.21	.834				
Oral Contraceptives	0.17	1.63	.113				
Diagnostic group	**- 0.16**	**- 2.08**	**.047**				

Oral contraceptives = no oral contraceptives (0), use of oral contraceptives (1).

Diagnostic group = Fibromyalgia (0), CFS/ME (1)

3.4 The role of insomnia severity

In order to test the hypothesis that higher levels of insomnia severity are significantly associated with cortisol variance, a multiple regression analysis was performed. The dependent variable was log transformed cortisol variance. Age, use of oral contraceptives or not, and diagnostic status were entered simultaneously with insomnia severity to statistically control for their potential confounding effects. The result of step four of the regression analysis is summarized in Table 6. Levels of insomnia severity were not significantly associated with cortisol variance when age and use of contraceptives and diagnostic status were statistically controlled for.

Table 6

Summary of multiple regression analysis of insomnia severity as a predictor of cortisol variance.

Variable	β	*t*	*p*	*F*	df	*p*	R^2
Overall model 1				1.45	4,32	.244	.172
Age	0.00	0.24	.812				
Oral Contraceptives	0.18	1.62	.117				
Diagnostic group	- 0.16	- 2.03	.052				
Insomnia	0.00	0.20	.840				

Oral contraceptives = No contraceptives (0), use of oral contraceptives (1).
Diagnostic group = Fibromyalgia (0), CFS/ME (1)
Insomnia = sum score on the Insomnia Severity Index.

In order to test the hypothesis that higher levels of insomnia severity are significantly associated with evening cortisol a hierarchical multiple regression analysis was performed. The dependent variable was log transformed evening cortisol. In the first step age was entered. In the second step use of oral contraceptives or not was entered, while in step three diagnostic status was entered. In the final step insomnia severity was entered. The results are summarized in Table 7. Age was significantly associated with evening cortisol, with older females having significantly higher evening cortisol values. Neither use of contraceptives nor diagnostic status were significantly associated with evening cortisol. However, as predicted, insomnia severity was significantly associated with evening cortisol. Higher levels of insomnia severity were significantly associated with higher evening cortisol levels.

Table 7

Summary of the hierarchical multiple regression analysis examining the association between insomnia severity and evening cortisol.

Variable	β	t	p	F	df	p	R^2
Overall model 1				6.63	1,32	.015	.176
Age	**-0.01**	**-2.57**	**.015**				
Overall model 2				4.25	2,32	.024	.221
Age	**-0.01**	**-2.91**	**.007**				
Oral contraceptives	-0.15	-1.31	.200				
Overall model 3				3.45	3,32	.029	.263
Age	**-0.01**	**-2.64**	**.013**				
Oral contraceptives	-0.17	-1.54	.134				
Diagnostic group	0.11	1.30	.206				
Overall model 4				4.18	4,32	.009	.374
Age	**-0.01**	**-2.38**	**.025**				
Oral contraceptives	-0.14	-1.32	.196				
Diagnostic group	0.12	1.51	.143				
Insomnia	**0.01**	**2.22**	**.034**			.	

Oral contraceptives = No oral contraceptives (0), use of oral contraceptives (1).
Diagnostic group = Fibromyalgia (0), CFS/ME (1)
Insomnia = sum score on the Insomnia Severity Index.

3.5 Association between cortisol variance and evening cortisol

Cortisol variance and evening cortisol were not significantly correlated (r = -.138, p = .43).

3.6 Comparison to other studies

Since this study lacks a healthy control group, and no national or international norms exist, it is difficult to decide to what extent the cortisol values found in the present study differ from values from a representative healthy sample. In order to compensate for this lack of information we have chosen to compare our results with data from two international studies of high quality that have collected similar saliva cortisol measures from healthy controls. The median values from the two studies are presented in Table 8 together with the median values from the two subgroups in the present study. In order to be able to compare our results with these two studies, we used the median values before the scores were log transformed.

As can be seen from Table 8, our CFS/ME group have lower median cortisol variance compared to the healthy groups from both Miller et al. (2016) and Mikkelsen et al. (2017). Median evening cortisol values appear to be within a normal range. The fibromyalgia group also have lower median variance scores, then both healthy groups, but higher median variance than the CFS/ME patient group. Median evening cortisol values from the fibromyalgia group is lower than both healthy groups. These comparisons must be treated with caution since the studies have assessed saliva cortisol somewhat differently in different laboratories.

Table 8

For comparison, median levels of cortisol are presented from two different healthy populations. Cortisol values are given in nmol/L.

	CFS/ME*	FM*	Miller et al., 2016	Mikkelsen et al., 2017	
	(n=18)	*(n=15)*	*(n= 7292)*	2007 *(N=3616)*	2009 *(N=2819)*
Variance	4.24	5.76	6.06	9.8	12.2
Morning	4.92	6.26	6.68	11.2	13.6
10:00	4.54	3.87	3.75	-	-
14:00	2.45	1.65	2.13	-	-
18:00	1.53	1.22	0.7	-	-
Evening	0.68	0.5	0.6	1.4	1.4

Variance = Highest cortisol value minus the lowest value.
Miller = Morning sample 1h after awakening, evening 16h after awakening. Only scores from females included.
Mikkelsen = morning sample 30 min after awakening, evening sample at 20:00, sample also includes 22% males.
* Data from the present study

4. Discussion

As predicted, CFS/ME patients exhibited significantly less saliva cortisol variation within a day, compared to fibromyalgia patients when statistically controlling for age and use of oral contraceptives. When comparing the median cortisol variance found in CFS/ME patients in this study, to the median cortisol variances found in studies on healthy populations by Miller et al (2016), and Mikkelsen et al (2017), we see that the CFS/ME patients also have less cortisol variation than a healthy population. Moreover, higher levels of insomnia severity were also significantly associated with higher levels of saliva cortisol in the evening irrespectively of primary diagnosis. However, higher levels of insomnia severity were not significantly associated with cortisol variation within a day.

4.1 Findings: Differences in diurnal cortisol variance

Our findings are further supported by other studies that have found alterations in HPA axis activity in CFS/ME patients, seen through decreased diurnal slopes of cortisol (Jerjes, Cleare, Wessely, Wood, & Taylor, 2005; Nater et al., 2008b; Papadopoulos & Cleare, 2011). Research has also found decreased diurnal slopes in fibromyalgia patients (Doerr et al., 2017; Riva et al., 2012), but results here have been more mixed (L. J. Crofford et al., 1994; McCain & Tilbe, 1989). Our findings are supportive of a more blunted diurnal cortisol curve being present in CFS/ME patients, than in fibromyalgia patients. Our findings can be closely linked to research by Tak (2011). Here statistical significant basal hypocortisolism was found in CFS/ME patients, but not in fibromyalgia patients. However, studies that only included females had a trend towards lower levels of cortisol. These results indicate that hypocortisolism was present in CFS/ME patients, and possibly also present in female fibromyalgia patients, but to a lesser degree (Tak et al., 2011).

It is noteworthy that CFS/ME patients have significantly lower saliva cortisol variance compared to Fibromyalgia patients although they do not significantly differ in levels of symptoms such as fatigue, pain intensity, insomnia severity, anxiety, and depression. This may indicate that significantly lower cortisol variance are maintained by other mechanisms in CFS/ME patients compared to Fibromyalgia patients. Although the present study lacks results from a healthy control group, comparing our results with healthy controls from other studies suggest that the CFS/ME patients also have lower cortisol variance than healthy subjects. If

CFS/ME patients have lower cortisol variance compared to fibromyalgia patients and healthy controls, but similar levels of psychiatric symptoms such as anxiety and depression as fibromyalgia patients, this suggests that the low cortisol variance in CFS/ME is less likely to be purely maintained by psychiatric disorders. Thus, it might be more relevant to examine biological factors which may create, or maintain, low cortisol variance.

A possible explanation of the blunted diurnal cortisol curve in CFS/ME is through the concept of allostatic load. Allostatic load refers to an imbalance in the systems that are involved in adaptation to environmental challenges (McEwen, 2006). This imbalance can be due to chronic stress, or it can be due to an imbalance in the adaptive systems, leading to a failure to shut off the system, or a failure to activate the system to the needed level (McEwen, 2006). Prior to the onset of CFS/ME patients mostly experience a combination of psychosocial and/or physical stressors (Van Houdenhove et al., 2010). A recent study found that 64% of CFS/ME patients have an infection-related episode prior to the onset of the disease (Chu, Valencia, Garvert, & Montoya, 2019). In addition to this, 39 % experience stress or a major life event prior to the onset of the disease (Chu et al., 2019). It has been hypothesized that these stressors create an allostatic load, through a failure to deactivate the system, which then eventually causes the system to switch to a mode of underactivation (Van Houdenhove et al., 2010). The underactivation of the system will then cause an elevation in inflammatory cytokines. These cytokines are able to influence the brain into causing flu-like physiological symptoms such as light fever, and characteristics fatigue/pain symptoms (Van Houdenhove et al., 2010). This can lead to a loss in the ability to mobilize resources that are needed in order to adapt to environmental challenges. An addition theory holds that the symptoms and sensations that come with the allostatic load might become an additional stressor. This will then further increase the allostatic load and eventually maintain the allostatic overload on its own (Arroll, 2013). This could explain why the CFS/ME symptoms are maintained after the initial stressor has passed (Arroll, 2013). All-in-all, these systematic changes may be the cause, or at least a maintaining factor, for several of the symptoms experienced by CFS/ME patients including the blunted cortisol response.

Another possible explanation of the blunted diurnal cortisol curve is that some subgroups of CFS/ME patients may have a risk factors, in terms of genetics and/or of early development origin, affecting the systems that promote adaptation to environmental challenges (Niitsu et al., 2019; Wu et al., 2013). This then leads to a weakening of their ability to respond

adaptively to chronic stressors or traumatic events later on in life (Niitsu et al., 2019; Wu et al., 2013). Both CFS/ME and FM are believed to have a heritable component, and research within CFS/ME have found that first-degree relatives have a significantly greater risk for CFS/ME (Albright, Light, Light, Bateman, & Cannon-Albright, 2011; Markkula et al., 2009).

Patients with CFS/ME and fibromyalgia share several of their core symptoms including pain, sleep disturbances, fatigue, and cognitive deficits. In addition to the overlap in symptoms, there is also an overlap in the comorbid disorders seen in both patient groups, including anxiety, depression, chronic tension-type-headaches, and irritable bowel syndrome (Aaron, Burke, & Buchwald, 2000). There is also a high degree of comorbidity between the two disorders. It has been estimations that 20-70% of patients with fibromyalgia, also meet the criteria for comorbid CFS/ME and that 35-70% of those with CFS/ME also having comorbid fibromyalgia (Aaron et al., 2000). However, the degree of overlap will also vary based on which diagnostic criteria has been used for diagnosing both CFS/ME and fibromyalgia. The reality is that the two diseases have very similar symptomatology, but our study shows that when one is able to separate out those with primary CFS/ME and primary fibromyalgia there is, in fact, a difference between the two. The diagnosis is then primarily based on the patient's own perception of his/her fatigue. An interesting finding was that there was no significant difference in scores on Chalders Fatigue Scale. However, a trend was present, and it is possible that the difference would have been significant with a larger sample size. On the other hand, chronic fatigue is largely based on the extent to which the fatigue reduces daily functioning, more than each individual´s subjective experience of the intensity of the fatigue. This can also be a sign of the fatigue being more biologically conditioned rather than psychological.

When our stress response is functioning as it should, cortisol normally has a protective effect when the body is exposed to stressors. This happens through inducing gluconeogenesis, causing metabolic effects, which in turn cause an increase in the organism´s energy supplies (Kaplan, 1988). In addition to this, glucocorticoids have an immunosuppressive effect on several immune functions. Included in this is inhibiting the secretion of cytokines, which are important immune and inflammatory mediators (Bateman, Singh, Kral, & Solomon, 1989). Due to this, a long-lasting decrease in cortisol variance might promote disinhibition of immune functions, resulting in increased vulnerability for the development of autoimmune

disorders, inflammation, chronic pain syndromes, allergies and asthma (Heim, Ehlert, & Hellhammer, 2000).

Alterations in HPA axis functioning has typically been seen to develop after the onset of CFS/ME, and then it is believed to play an important role in both maintaining symptoms and the further development of the disease (Papadopoulos & Cleare, 2011). Thus, restoring a normal cortisol variance might be a possible therapeutic target for CFS/ME. One clinical trial found increased cortisol levels in CFS/ME patients after six months of Cognitive Behavioral Therapy (CBT), suggesting that CBT might be efficient in restoring normal HPA axis functioning (Roberts, Papadopoulos, Wessely, Chalder, & Cleare, 2009). The improvements seen by CBT is believed to be caused by reversing some of the effects that have occurred due to a low level of activity, depression, and stress in early life (Papadopoulos & Cleare, 2011).

Immunosuppressive treatments have also been found to be efficient for alleviating symptoms for some subgroups (Blomberg, Gottfries, Elfaitouri, Rizwan, & Rosen, 2018). This, in addition to the prevalence of CFS/ME being higher among women, that it runs in families, the alterations in cytokine profiles, metabolic alterations, and that it can be triggered by infections is suggestive of CFS/ME having an autoimmune etiology (Sotzny et al., 2018). Research in the field is currently focusing on finding autoantibodies, which can potentially be biomarkers for CFS/ME, and potential targets for the development of future treatments (Sotzny et al., 2018).

4.2 Findings: The role of insomnia severity

Unexpectedly, insomnia severity could not predict levels of cortisol variation within a day. This is against studies that have found correlations between insomnia severity and diurnal cortisol slopes in the general populations (Abell, Shipley, Ferrie, Kivimaki, & Kumari, 2016; Castro-Diehl et al., 2015). On the other hand, the results are in support of other studies with CFS/ME patients that have found no significant correlation between sleep and diurnal cortisol slopes (Strickland, 1997; Torres-Harding et al., 2008). Our findings indicated that variation in cortisol within a day, was not dependent on the severity of insomnia.

For evening levels of cortisol, regression analysis showed that insomnia severity was a good predictor, independently of primary diagnosis, when statistically controlling for age and use of oral contraceptives. This indicates that sleep disturbances have the same effect on the level

of evening cortisol independently of the primary diagnosis. Several studies have supported elevated evening cortisol in relation to insomnia in the general population (Hirotsu, Tufik, & Andersen, 2015; Rodenbeck, Huether, Rüther, & Hajak, 2002). No study was found that examined naturally occurring evening cortisol in relation to insomnia severity in a CFS/ME patient group. Strickland (1997) tried to examine this but had too small variance in the evening cortisol to examine any associations with symptom variables. Another, more recent study, focused on evening cortisol in CFS/ME patients, but in relation to depression and immunological factors (Milrad et al., 2018).

Greater insomnia severity has been found to be associated with greater inflammation in CFS/ME patients (Milrad et al., 2017). Greater inflammation cause greater levels of inflammatory cytokines, that like mentioned earlier, can cause fatigue/pain symptoms and in addition also cause symptoms of depression (Dantzer, O'Connor, Lawson, & Kelley, 2011). In this way, insomnia can also affect proinflammatory cytokines that can explain several of the symptoms that CFS/ME patients experience, and thus treating insomnia might be relevant for treating CFS/ME. This has been supported by studies that have found that improving insomnia severity, also improves fatigue levels (Kallestad et al., 2015). An interesting finding in relation to this was that there was no correlation between evening cortisol and the cortisol variance. Suggesting that the two measures capture two different phenomena. This means that treatment of insomnia might be a possible target for lowering the cortisol response in the evening. However, due to the lack of correlation between the cortisol variance measure and the evening measure of cortisol, it is unlikely that improving evening cortisol through improving insomnia severity will have a significant effect on daily cortisol variance. Based on findings from this study it appears that treatment of insomnia should not be the main treatment target. Lowering evening cortisol might improve sleep, but it is not likely to have a significant effect on the bigger symptom picture of CFS/ME.

4.3 Strengths of the study

One of the main strengths of this study was the consistency in the data collection. Recruitment, informing, instructing, and frequent follow-up of participants were all conducted by the same researcher, securing standardized procedures. The instruments used to measure symptoms were also standardized and validated, and thus easy to replicate, adding additional strength to the study.

Another strength was the diagnostic precision used on diagnosing all the participants, and the uniformity of it. All participants were diagnostically evaluated by physicians, psychologists, and physiotherapists that are experienced with working with these patient groups.

An additional strength is that the confounding variables age and use of oral contraceptives were controlled for. Gender is also another known confounding variable within cortisol research, thus the analysis are only based on female participants.

An additional strength is that there are few other studies that have separated fibromyalgia and CFS/ME patients and examined them as different groups but using the same study design and conditions (L. J. Crofford et al., 2004). Thus, this thesis is contributing with new knowledge to the field.

This thesis contributes to creating a better understanding of the pathological factors separating CFS/ME and FM, which can help further improve clinical management and diagnostic precision in both the field of CFS/ME and fibromyalgia.

4.4 Limitations

A large limitation of our study was the small sample size, leading to the statistical tests having low statistical power. Thus, the chance of finding results that are genuinely true is low. It also reduces the likelihood that statistically significant results reflect true effects (Button et al., 2013). For some of the multiple regression analysis more factors were added, then our sample size allowed, which weakens their reliability. Still, we do think that findings are interesting, and should therefore be further explored with a larger sample size.

We also chose to exclude male participants which reduced the representativeness of our sample. However, it ensured that our results were not attributable to the confounding effects of sex. There was also a lack of key information for examining the role of other possible confounding variables. For instance, the study did not examine the effect of the score on the Body Mass Index (BMI), which is usually included as a covariate in endocrinological and immunological research (O'Connor et al., 2009).

Another limitation was that we did not know the degree of compliance with the guidelines when participants collected saliva at home. It has been found that saliva sampling conducted

by participants themselves at home can be prone to measurement error due to a lack of compliance to the given sampling times (Brigitte M. Kudielka, Broderick, & Kirschbaum, 2003). Our design was less prone to this, due to the fact that we did not measure the cortisol awakening response (CAR) directly, but measuring the variance within a day instead. Still, if participants collected saliva at different times than assigned this could have affected the cortisol variance recorded.

In relation to this, another limitation was that cortisol samples were not taken in relation to the time of awakening. The participants should at least have been asked to record their time of awakening. Current recommendations suggest that they should have taken their samples not at given clock hours, but in relation to hours after awakening (Adam & Kumari, 2009), but there have not been any studies that have examined the implications of choosing one approach over the other (Adam et al., 2017). Without knowing the time of awakening we cannot say whether CAR variance was included in our measure of variance or not. This is of relevance because the CAR has been viewed as a different index of cortisol regulation (Clow et al., 2010).

For this study both the Canada and the Fukuda criteria were used for CFS/ME patients, and the 2016 Fibromyalgia criteria were used for fibromyalgia patients (Wolfe et al., 2016). There are several diagnostic criteria for both CFS/ME and fibromyalgia, an additional limitation of this study was therefore that our findings might not have been the same if different diagnostic criteria were used.

Cortisol secretion tend to follow a certain pattern, but the amount secreted tend to vary between individuals, but it can also variate within individuals. Therefore the use of two consecutive days is viewed as a standard and valid methodology for assessing diurnal cortisol slopes (Castro-Diehl et al., 2015). On the other hand, Adam (2018) found that studies analyzing data from one day of data collection did not show a weaker association between health outcomes, and the diurnal cortisol slopes than studies using two or more days. Therefore, a possible weakness in our analysis was that we only used data from one day of sampling, but the extent of this possible weakness is unknown. The levels of all symptoms were also measured at one-time point, thus we cannot know with certainty that they give a representative picture of the participants´ symptom intensity overall, or just for a small given period of time.

Sleep has been analyzed based on the scores on the Insomnia Severity Index (ISI). A possible limitation with using this measurement tool with these patient groups was that ISI does not have a factor measuring non-restorative sleep. Since this is one of the main sleep complaints among CFS/ME patients, it is likely that the ISI will underscore the level of sleep disturbances. In addition, in cross-sectional studies like this, the direction of cause and effect may be difficult to assess. Therefore, we are not able to rule out if salivary cortisol affects sleep, or if sleep affects salivary cortisol.

In this thesis, fibromyalgia patients were used as a control group for CFS/ME patients. A greater value would have been added to the thesis if an additional control group with healthy matched controls were included. This would have given more insights to say whether the findings deviates from normal functioning or not. This was extra relevant since the method of using and analyzing cortisol variance is so different from study to study, making it hard to compare our patient groups to population groups from other studies.

Another limitation of the study was that we only measured naturally occurring cortisol. Thereby we do not know how the systems would respond to an actual stressor. If we would have used challenge tests, like the Trier Stress Test, we would have been more able to measure more of the cortisol systems optimal flexibility and might have been able to capture more subtle alterations in cortisol variation. This would then be a measure of the participants ability to mobilize resources when faced by a stressor, while our natural measures of cortisol were more of an indication of baseline function, and what happens between the stressors (Tak et al., 2011).

The final limitation is that this was not a prospective study, making it still unknown if a blunted diurnal saliva cortisol slope plays a pathophysiological role, or if it is a consequence of CFS/ME.

Based on these limitations the results must be treated with caution, and replication is called for by future studies.

4. Conclusion

In this study, significantly lower variation in saliva cortisol was found in CFS/ME patients compared to fibromyalgia patients. This differences in cortisol variation could not be related to any of the measured symptoms including fatigue, pain intensity, insomnia severity, anxiety and depression. This may indicate that significantly lower cortisol variance is maintained by other mechanisms in CFS/ME patients than in Fibromyalgia patients. These mechanisms appear to be not only maintained by psychiatric disorders, but also by biological factors. This study also found that higher levels of insomnia severity predicted higher levels of evening cortisol irrespectively of primary diagnosis. However, insomnia severity was not a significant predictor of cortisol variance within a day. In addition, there was also found no correlation between the cortisol variance measure and the measure of evening cortisol. Based on this it is unlikely that improving evening cortisol through improving insomnia severity will have a significant effect on daily cortisol variance, thus insomnia should not be the main treatment target for CFS/ME. However, the study has several limitations, thus all results must be treated with caution, and needs further replications.

5. References

Abbi, B., & Natelson, B. H. (2013). Is chronic fatigue syndrome the same illness as fibromyalgia: evaluating the 'single syndrome' hypothesis. *QJM, 106*(1), 3-9. doi:10.1093/qjmed/hcs156

Abell, J. G., Shipley, M. J., Ferrie, J. E., Kivimaki, M., & Kumari, M. (2016). Recurrent short sleep, chronic insomnia symptoms and salivary cortisol: A 10-year follow-up in the Whitehall II study. *Psychoneuroendocrinology, 68*, 91-99. doi:10.1016/j.psyneuen.2016.02.021

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology, 34*(10), 1423-1436. doi:10.1016/j.psyneuen.2009.06.011

Adam, E. K., Quinn, M. E., Tavernier, R., McQuillan, M. T., Dahlke, K. A., & Gilbert, K. E. (2017). Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. *Psychoneuroendocrinology, 83*, 25-41. doi:10.1016/j.psyneuen.2017.05.018

Albright, F., Light, K., Light, A., Bateman, L., & Cannon-Albright, L. A. (2011). Evidence for a heritable predisposition to Chronic Fatigue Syndrome. *BMC Neurol, 11*, 62. doi:10.1186/1471-2377-11-62

Arroll, M. A. (2013). Allostatic overload in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Med Hypotheses, 81*(3), 506-508. doi:10.1016/j.mehy.2013.06.023

Baraniuk, J. N., Adewuyi, O., Merck, S. J., Ali, M., Ravindran, M. K., Timbol, C. R., . . . Petrie, K. N. (2013). A Chronic Fatigue Syndrome (CFS) severity score based on case designation criteria. *Am J Transl Res, 5*, 53-68.

Basta, M., Chrousos, G. P., Vela-Bueno, A., & Vgontzas, A. N. (2007). Chronic Insomnia and stress system *Sleep Med Clin, 2*, 279-291.

Bastien, C., Vallières, A., & Morin, C. M. (2001). Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Medicine, 2*(4), 297-307. doi:10.1016/s1389-9457(00)00065-4

Bateman, A., Singh, A., Kral, T., & Solomon, S. (1989). The Immune-Hypothalamic-Pituitary-Adrenal Axis*. *Endocrine Reviews, 10*(1), 92-112. doi:10.1210/edrv-10-1-92

Bei, B., Asarnow, L. D., Krystal, A., Edinger, J. D., Buysse, D. J., & Manber, R. (2018). Treating insomnia in depression: Insomnia related factors predict long-term depression trajectories. *J Consult Clin Psychol, 86*(3), 282-293. doi:10.1037/ccp0000282

Blomberg, J., Gottfries, C. G., Elfaitouri, A., Rizwan, M., & Rosen, A. (2018). Infection Elicited Autoimmunity and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: An Explanatory Model. *Front Immunol, 9*, 229. doi:10.3389/fimmu.2018.00229

Brown, M., Brown, A. A., & Jason, L. (2011). Illness duration and coping style in chronic fatigue syndrome. *Psychol Rep, 106*(2), 383-393.

Button, K. S., Ioannidis, J. P., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S., & Munafo, M. R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci, 14*(5), 365-376. doi:10.1038/nrn3475

Buysse, D. J., Ancoli-Israel, S., Edinger, J. D., Lichstein, K. L., & Morin, C. M. (2006). Recommendations for a Standard Research Assesment of Insomnia *Sleep, 29*(9), 1155-1173.

Cabo-Meseguer, A., Cerdá-Olmedo, G., & Trillo-Mata, J. L. (2017). Fibromyalgia: Prevalence, epidemiologic profiles and economic costs. *Medicina Clínica (English Edition), 149*(10), 441-448. doi:10.1016/j.medcle.2017.10.011

Castro-Diehl, C., Roux, A. V. D., Redline, S., Seeman, T., Shrager, S. E., & Shea, S. (2015). Association of Sleep Duration and Quality With Alterations in the Hypothalamic-Pituitary Adrenocortical Axis: The Multi-Ethnic Study of Atherosclerosis *J Clin Endocrinol Metab, 100*(8), 3149-3158.

Chalder, T., Berelowitz, G., Pawlikowska, T., Watts, L., Wessely, S., Wright, D., & Wallace, E. P. (1993). Development of a Fatigue Scale *Journal of Psychosomatic Research, 37*(2), 147-153.

Chu, L., Valencia, I. J., Garvert, D. W., & Montoya, J. G. (2019). Onset Patterns and Course of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Front Pediatr, 7*, 12. doi:10.3389/fped.2019.00012

Ciccone, D. S., & Natelson, B. H. (2003). Comorbid illness in women with Chronic Fatigue Syndrome: A test of the Single Syndrome Hypothesis. *Psychosom Med, 65*, 268-275
Clauw, D. J. (2015). Fibromyalgia and related conditions. *Mayo Clin Proc, 90*(5), 680-692. doi:10.1016/j.mayocp.2015.03.014
Clauw, D. J., D'Arcy, Y., Gebke, K., Semel, D., Pauer, L., & Jones, K. D. (2018). Normalizing fibromyalgia as a chronic illness. *Postgrad Med, 130*(1), 9-18. doi:10.1080/00325481.2018.1411743
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*(1), 97-103. doi:10.1016/j.neubiorev.2009.12.011
Crofford, L. J., Pillemer, S. R., Kalogeras, K. T., Cash, J. M., Michelson, D., Kling, M. A., . . . Wilder, R. L. (1994). Hypothalamic-Pituitary-Adrenal Axis Perturbations in Patients with Fibromyalgia. *Arthritis & Rheumatism, 37*(11), 1583-1592.
Crofford, L. J., Young, E. A., Engleberg, N. C., Korszun, A., Brucksch, C. B., McClure, L. A., . . . Demitrack, M. A. (2004). Basal circadian and pulsatile ACTH and cortisol secretion in patients with fibromyalgia and/or chronic fatigue syndrome. *Brain Behav Immun, 18*(4), 314-325. doi:10.1016/j.bbi.2003.12.011
Dantzer, R., O'Connor, J. C., Lawson, M. A., & Kelley, K. W. (2011). Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology, 36*(3), 426-436. doi:10.1016/j.psyneuen.2010.09.012
De Gucht, V., Garcia, F. K., den Engelsman, M., & Maes, S. (2017). Do changes in illness perceptions, physical activity, and behavioural regulation influence fatigue severity and health-related outcomes in CFS patients? *J Psychosom Res, 95*, 55-61. doi:10.1016/j.jpsychores.2017.02.009
Demitrack, M. A., Dale, J. K., Straus, S. E., Laue, L., Listwak, S. J., Kruesi, M. J., . . . Gold, P. W. (1991). Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *J Clin Endocrinol Metab, 73*(6), 1224-1234. doi:10.1210/jcem-73-6-1224
Dmitrieva, N. O., Almeida, D. M., Dmitrieva, J., Loken, E., & Pieper, C. F. (2013). A day-centered approach to modeling cortisol: diurnal cortisol profiles and their associations among U.S. adults. *Psychoneuroendocrinology, 38*(10), 2354-2365. doi:10.1016/j.psyneuen.2013.05.003
Doerr, J. M., Fischer, S., Nater, U. M., & Strahler, J. (2017). Influence of stress systems and physical activity on different dimensions of fatigue in female fibromyalgia patients. *J Psychosom Res, 93*, 55-61. doi:10.1016/j.jpsychores.2016.12.005
Edinger, J. D. (2005). Behavioral Insomnia Therapy for Fibromyalgia Patients. *ARCH INTERN MED, 165*.
Faro, M., Saez-Francas, N., Castro Marrero, J., Aliste, L., Fernandez de Sevilla, T., & Alegre, J. (2016). Gender Differences in Chronic Fatigue Syndrome. *Reumatol Clin., 12*(2), 72-77.
Ferre, A. (2016). Chronic fatigue syndrome and sleep disorders: Clinical associations and diagnostic difficulties. *Neurología (English Edition), 33*(6), 385-394.
Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *Int J Psychophysiol, 72*(1), 67-73. doi:10.1016/j.ijpsycho.2008.03.014
Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology, 30*(10), 1010-1016. doi:10.1016/j.psyneuen.2005.04.006
Fukuda, K., Straus, S. E., Hickie, I., Sharpe, M. C., Dobbins, J. G., Komaroff, A., . . . Reeves, W. C. (1994). The Chronic Fatigue Syndrome - a Comprehensive Approach to Its Definition and Study. *Annals of Internal Medicine, 121*(12), 953-959. doi:10.7326/0003-4819-121-12-199412150-00009
Geiss, A., Rohleder, N., & Anton, F. (2012). Evidence for an association between an enhanced reactivity of interleukin-6 levels and reduced glucocorticoid sensitivity in patients with fibromyalgia. *Psychoneuroendocrinology, 37*(5), 671-684. doi:10.1016/j.psyneuen.2011.07.021
Groven, N., Fors, E. A., Iversen, V. C., White, L. R., & Reitan, S. K. (2018). Association between cytokines and psychiatric symptoms in chronic fatigue syndrome and healthy controls. *Nord J Psychiatry, 72*(8), 556-560. doi:10.1080/08039488.2018.1493747

Gur, A., Cevik, R., Nas, K., Colpan, L., & Sarac, S. (2004). Cortisol and hypothalamic-pituitary-gonadal axis hormones in follicular-phase women with fibromyalgia and chronic fatigue syndrome and effect of depressive symptoms on these hormones. *Arthritis Res Ther, 6*(3), R232-238. doi:10.1186/ar1163

Harvey, A. (2001). Insomnia: Symptom or diagnosis *Clinical Psychology Review, 21*(7), 1037-1059.

Heidari, F., Afshari, M., & Moosazadeh, M. (2017). Prevalence of fibromyalgia in general population and patients, a systematic review and meta-analysis. *Rheumatol Int, 37*(9), 1527-1539. doi:10.1007/s00296-017-3725-2

Heim, C., Ehlert, U., & Hellhammer, D. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. . *Psychoneuroendocrinology, 25*, 1-35.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., . . . Nemeroff, C. B. (2000). Pituitary-Adrenal and Autonomic Responses to Stress in Women After Sexual and Physical Abuse in Childhood. *JAMA, 284*.

Hirotsu, C., Tufik, S., & Andersen, M. L. (2015). Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. *Sleep Sci, 8*(3), 143-152. doi:10.1016/j.slsci.2015.09.002

Hoffman, D. L., & Dukes, E. M. (2008). The health status burden of people with fibromyalgia: a review of studies that assessed health status with the SF-36 or the SF-12. *Int J Clin Pract, 62*(1), 115-126. doi:10.1111/j.1742-1241.2007.01638.x

Hucklebridge, F. H., Clow, A., Abeyguneratne, T., Huezo-Diaz, P., & Evans, P. (1999). The awakening cortisol response and blood glucose levels *Life Sciences, 64*, 931-937.

Jason, L. A., Torres-Harding, S. R., Jurgens, A., & Helgerson, J. (2011). Comparing the Fukuda et al. Criteria and the Canadian Case Definition for Chronic Fatigue Syndrome. *Journal of Chronic Fatigue Syndrome, 12*(1), 37-52. doi:10.1300/J092v12n01_03

Jensen, K. B., Berna, C., Loggia, M. L., Wasan, A. D., Edwards, R. R., & Gollub, R. L. (2012). The use of functional neuroimaging to evaluate psychological and other non-pharmacological treatments for clinical pain. *Neurosci Lett, 520*(2), 156-164. doi:10.1016/j.neulet.2012.03.010

Jerjes, W. K., Cleare, A. J., Wessely, S., Wood, P. J., & Taylor, N. F. (2005). Diurnal patterns of salivary cortisol and cortisone output in chronic fatigue syndrome. *J Affect Disord, 87*(2-3), 299-304. doi:10.1016/j.jad.2005.03.013

Johnston, S., Brenu, E. W., Staines, D. R., & Marshall-Gradisnik, S. (2013). The adoption of chronic fatigue syndrome/myalgic encephalomyelitis case definitions to assess prevalence: a systematic review. *Ann Epidemiol, 23*(6), 371-376. doi:10.1016/j.annepidem.2013.04.003

Kallestad, H., Jacobsen, H. B., Landro, N. I., Borchgrevink, P. C., & Stiles, T. C. (2015). The role of insomnia in the treatment of chronic fatigue. *J Psychosom Res, 78*(5), 427-432. doi:10.1016/j.jpsychores.2014.11.022

Kaplan, N. M. (1988). The adrenal glands In S. R. Ojeda (Ed.), *Textbook of Endocrine Physiology*. New York: Oxford University Press

Kudielka, B. M., Bellingrath, S., & Hellhammer, D. (2006). Cortisol in burnout and vital exhaustion: an overview *G Ital Med Lav Erg, 28*, 34-42.

Kudielka, B. M., Broderick, J. E., & Kirschbaum, C. (2003). Compliance With Saliva Sampling Protocols: Electronic Monitoring Reveals Invalid Cortisol Daytime Profiles in Noncompliant Subjects. *Psychosomatic Medicine, 65*(2), 313-319. doi:10.1097/01.Psy.0000058374.50240.Bf

Landis, C. A. (2011). Sleep, pain, fibromyalgia, and chronic fatigue syndrome. In P. M. a. S. Chokroverty (Ed.), *Handbook of Clinical Neurology* (Vol. 98): Elsevier B.V.

Landmark, T., Romundstad, P. R., Borchgrevink, P. C., Kaasa, S., & Dale, O. (2013). Longitudinal associations between exercise and pain in the general population--the HUNT pain study. *PLoS One, 8*(6), e65279. doi:10.1371/journal.pone.0065279

Lattie, E. G., Antoni, M. H., Fletcher, M. A., Penedo, F., Czaja, S., Lopez, C., . . . Klimas, N. (2012). Stress management skills, neuroimmune processes and fatigue levels in persons with chronic fatigue syndrome. *Brain Behav Immun, 26*(6), 849-858. doi:10.1016/j.bbi.2012.02.008

Leproult, R., Copinschi, G., Buxton, O., & Van Cauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening *Sleep, 20*, 865-870.

Manber, R., Edinger, J. D., Gress, J. L., San Pedro-Salcedo, G., Kuo, T. F., & Kalista, T. (2008). Cognitive Behvaioral Therapy for Insomnia Enhances Depression Outcome in Patients with Comorbid Major Depressive Disorder and Insomnia. *Sleep, 31*(4).

Mariman, A. N., Vogelaers, D. P., Hanoulle, I. P., Delesie, L. M., & Pevernagie, D. A. (2012). Subjective sleep quality and daytime sleepiness in a large sample of patients with Chronic Fatigue Syndrome (CFS). *Acta Clinica Belica, 67:1*, 19-24. doi:10.2143/ACB.67.1.2062621

Mariman, A. N., Vogelaers, D. P., Tobback, E., Delesie, L. M., Hanoulle, I. P., & Pevernagie, D. A. (2013). Sleep in the chronic fatigue syndrome. *Sleep Med Rev, 17*(3), 193-199. doi:10.1016/j.smrv.2012.06.003

Markkula, R., Jarvinen, P., Leino-Arjas, P., Koskenvuo, M., Kalso, E., & Kaprio, J. (2009). Clustering of symptoms associated with fibromyalgia in a Finnish Twin Cohort. *Eur J Pain, 13*(7), 744-750. doi:10.1016/j.ejpain.2008.09.007

McCain, G. A., & Tilbe, K. S. (1989). Diurnal hormone variation in fibromyalgia syndrome: a comparison with rheumatoid arthritis. *J Rheumatol Suppl., 19*, 154-157.

McCue, P., Buchanan, T., & Martin, C. R. (2006). Screening for psychological distress using internet administration of the Hospital Anxiety and Depression Scale (HADS) in individuals with chronic fatigue syndrome. *Br J Clin Psychol, 45*(Pt 4), 483-498. doi:10.1348/014466505X82379

McEwen, B. S. (2006). Stress, Adaptation, and Disease. Allostasis and Allostatic load. *Neuroimmunomodulation: Molecular aspects, integrative systems, and clinical advances, 840*(1), 33-44.

Mikkelsen, S., Forman, J. L., Fink, S., Vammen, M. A., Thomsen, J. F., Grynderup, M. B., . . . Bonde, J. P. (2017). Prolonged perceived stress and saliva cortisol in a large cohort of Danish public service employees: cross-sectional and longitudinal associations. *Int Arch Occup Environ Health, 90*(8), 835-848. doi:10.1007/s00420-017-1241-z

Miller, R., Stalder, T., Jarczok, M., Almeida, D. M., Badrick, E., Bartels, M., . . . Kirschbaum, C. (2016). The CIRCORT database: Reference ranges and seasonal changes in diurnal salivary cortisol derived from a meta-dataset comprised of 15 field studies. *Psychoneuroendocrinology, 73*, 16-23. doi:10.1016/j.psyneuen.2016.07.201

Milrad, S. F., Hall, D. L., Jutagir, D. R., Lattie, E. G., Czaja, S. J., Perdomo, D. M., . . . Antoni, M. H. (2018). Depression, evening salivary cortisol and inflammation in chronic fatigue syndrome: A psychoneuroendocrinological structural regression model. *Int J Psychophysiol, 131*, 124-130. doi:10.1016/j.ijpsycho.2017.09.009

Milrad, S. F., Hall, D. L., Jutagir, D. R., Lattie, E. G., Ironson, G. H., Wohlgemuth, W., . . . Antoni, M. H. (2017). Poor sleep quality is associated with greater circulating pro-inflammatory cytokines and severity and frequency of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) symptoms in women. *J Neuroimmunol, 303*, 43-50. doi:10.1016/j.jneuroim.2016.12.008

Moldofsky, H., Scarisbrick, P., England, R., & Smythe, H. (1975). Musculoskeletal Symtoms and Non-REM Sleep Disturbances in Patients with «Fibrositis Syndrome» and healthy subjects. . *Psychosom Med, 37* (4), 341-351.

Morin, C. M. (1993). *Insomnia. Psychological assessment and management.* . New York, US: Guilford Press.

Morin, C. M., Belleville, G., Bélanger, L., & Ivers, H. (2011). The Insomnia Severity Index: Psychometric Indicators to Detect and Evaluate Treatment Responses. *Sleep, 34*(5), 601-608.

Morin, C. M., & Ware, J. C. (1996). Sleep and psychopathology. *Applied & Preventive Psychology, 5*, 211-224.

Morriss, R. K., Wearden, A. J., & Mullis, R. (1998). Exploring the validity of the chalder fatigue scale in chronic fatigue syndrome. *Journal of Psychosomatic Research, 45*(5), 411-417.

Nacul, L. C., Lacerda, E. M., Campion, P., Pheby, D., Drachler Mde, L., Leite, J. C., . . . Molokhia, M. (2011). The functional status and well being of people with myalgic encephalomyelitis/chronic fatigue syndrome and their carers. *BMC Public Health, 11*, 402. doi:10.1186/1471-2458-11-402

Nasjonal veileder. Pasienter med CFS/ME: Utredning, diagnostikk, behandling, rehablitering, pleie og omsorg. (2015). Retrieved from Norwegian Directorate of Health

Nater, U. M., Youngblood, L. S., Jones, J. F., Unger, E. R., Miller, A. H., Reeves, W. C., & Heim, C. (2008a). Alterations in diurnal salivary cortisol rhythm in a population-based sample of cases with chronic fatigue syndrome. *Psychosomatic Medicine, 70*(3), 298-305. doi:10.1097/PSY.0b013e3181651025

Nater, U. M., Youngblood, L. S., Jones, J. F., Unger, E. R., Miller, A. H., Reeves, W. C., & Heim, C. (2008b). Alterations in diurnal salivary cortisol rhythm in a population-based sample of cases with chronic fatigue syndrome. *Psychosom Med, 70*(3), 298-305. doi:10.1097/PSY.0b013e3181651025

Nicassio, P. M., Moxham, E. G., Schuman, C. E., & Gevirtz, R. N. (2002). The contribution of pain, reported sleep quality, and depressive symptoms to fatigue in fibromyalgia. *Pain, 100*, 271-279.

Niitsu, K., Rice, M. J., Houfek, J. F., Stoltenberg, S. F., Kupzyk, K. A., & Barron, C. R. (2019). A Systematic Review of Genetic Influence on Psychological Resilience. *Biol Res Nurs, 21*(1), 61-71. doi:10.1177/1099800418800396

O'Connor, M. F., Bower, J. E., Cho, H. J., Creswell, J. D., Dimitrov, S., Hamby, M. E., . . . Irwin, M. R. (2009). To assess, to control, to exclude: effects of biobehavioral factors on circulating inflammatory markers. *Brain Behav Immun, 23*(7), 887-897. doi:10.1016/j.bbi.2009.04.005

Palesh, O., Zeitzer, J. M., Conrad, A., Giese-Davis, J., Mustian, K. M., Popek, V., . . . Spiegel, D. (2008). Vagal regulation, cortisol and sleep disruption in Women with Metastatic Breast Cancer. . *Journal of Clinical Sleep Medicine, 4*, 441-449.

Papadopoulos, A. S., & Cleare, A. J. (2011). Hypothalamic-pituitary-adrenal axis dysfunction in chronic fatigue syndrome. *Nat Rev Endocrinol, 8*(1), 22-32. doi:10.1038/nrendo.2011.153

Park, J., & Gilmour, H. (2017). Medicallt unexplained physical symptoms (MUPS) among adults in Canada: Comorbidity, health care use and employment In S. Canada (Ed.), *Health Matters* (pp. 3-8). Ottawa Statistics Canada

Pruessner, J. C., Wolf, O. T., Hellhammer, D., Buske-Kirschbaum, A., von Auer, K., Jobst, S., . . . Kirschbaum, C. (1997). Free cortisol levels after awakening: A reliable Biological Marker for the Assesment of Adrenocortical Activity *Life Sciences, 61*(26), 2539-2549.

Reeves, W. C., Heim, C., Maloney, E. M., Youngblood, L. S., Unger, E. R., Decker, M. J., . . . Rye, D. B. (2006). Sleep characteristics of persons with chronic fatigue syndrome and non-fatigued controls: results from a population-based study. *BMC Neurol, 6*, 41. doi:10.1186/1471-2377-6-41

Riva, R., Mork, P. J., Westgaard, R. H., & Lundberg, U. (2012). Comparison of the cortisol awakening response in women with shoulder and neck pain and women with fibromyalgia. *Psychoneuroendocrinology, 37*(2), 299-306. doi:10.1016/j.psyneuen.2011.06.014

Roberts, A. D., Papadopoulos, A. S., Wessely, S., Chalder, T., & Cleare, A. J. (2009). Salivary cortisol output before and after cognitive behavioural therapy for chronic fatigue syndrome. *J Affect Disord, 115*(1-2), 280-286. doi:10.1016/j.jad.2008.09.013

Rodenbeck, A., Huether, G., Rüther, E., & Hajak, G. (2002). Interactions between evening and nocturnal cortisol secretion and sleep parameters in patients with severe chronic primary insomnia *Neurosci Lett, 324*, 159-163.

Rodriguez-Pinto, I., Agmon-Levin, N., Howard, A., & Shoenfeld, Y. (2014). Fibromyalgia and cytokines. *Immunol Lett, 161*(2), 200-203. doi:10.1016/j.imlet.2014.01.009

Roerink, M. E., Roerink, S., Skoluda, N., van der Schaaf, M. E., Hermus, A., van der Meer, J. W. M., . . . Nater, U. M. (2018). Hair and salivary cortisol in a cohort of women with chronic fatigue syndrome. *Horm Behav, 103*, 1-6. doi:10.1016/j.yhbeh.2018.05.016

Ryan, R., Booth, S., Spathis, A., Mollart, S., & Clow, A. (2016). Use of Salivary Diurnal Cortisol as an Outcome Measure in Randomised Controlled Trials: a Systematic Review. *Ann Behav Med, 50*(2), 210-236. doi:10.1007/s12160-015-9753-9

Sapolsky, R. M., Romero, M. L., & Munck, A. U. (2000). How Do Glucocorticoids influence stress responses? Integrating Permissive, Supressive, Stimulatory, and Preparative Actions. *Endocrine Reviews, 21*, 55-89.

Scheibenbogen, C., Freitag, H., Blanco, J., Capelli, E., Lacerda, E., Authier, J., . . . Murovska, M. (2017). The European ME/CFS Biomarker Landscape project: an initiative of the European network EUROMENE. *J Transl Med, 15*(1), 162. doi:10.1186/s12967-017-1263-z

Sheehan, D. V., Lecrubier, Y., Sheehan, K., Amorim, P., Janavs, J., Weiller, E., . . . Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The clinical of Clinical Psychiatry, 59*, 22-33.

Sluka, K. A., & Clauw, D. J. (2016). Neurobiology of fibromyalgia and chronic widespread pain. *Neuroscience, 338*, 114-129. doi:10.1016/j.neuroscience.2016.06.006

Sotzny, F., Blanco, J., Capelli, E., Castro-Marrero, J., Steiner, S., Murovska, M., . . . European Network on, M. C. (2018). Myalgic Encephalomyelitis/Chronic Fatigue Syndrome - Evidence for an autoimmune disease. *Autoimmun Rev, 17*(6), 601-609. doi:10.1016/j.autrev.2018.01.009

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *The Lancet, 354*(9188), 1435-1439. doi:10.1016/s0140-6736(99)01376-8

Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., & Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals *Psychoneuroendocrinology, 26*, 295-306.

Strickland, P. M., R., Wearden, A., Deakin, B. (1997). A comparison of salivary cortisol in chronic fatigue syndrome, community depression and healthy controls. . *Journal of Affective Disorders, 47*, 191-194.

Sutton, B. C., & Opp, M. R. (2014). Musculoskeletal sensitization and sleep: chronic muscle pain fragments sleep of mice without altering its duration. *Sleep, 37*(3), 505-513. doi:10.5665/sleep.3486

Tak, L. M., Cleare, A. J., Ormel, J., Manoharan, A., Kok, I. C., Wessely, S., & Rosmalen, J. G. (2011). Meta-analysis and meta-regression of hypothalamic-pituitary-adrenal axis activity in functional somatic disorders. *Biol Psychol, 87*(2), 183-194. doi:10.1016/j.biopsycho.2011.02.002

Taylor, A. K., Loades, M., Brigden, A. L., Collin, S. M., & Crawley, E. (2017). 'It's personal to me': A qualitative study of depression in young people with CFS/ME. *Clin Child Psychol Psychiatry, 22*(2), 326-340. doi:10.1177/1359104516672507

Theadom, A., & Cropley, M. (2010). 'This constant being woken up is the worst thing' - experiences of sleep in fibromyalgia syndrome. *Disabil Rehabil, 32*(23), 1939-1947. doi:10.3109/09638281003797331

Tomas, C., Newton, J., & Watson, S. (2013). A Review of Hypothalamic-Pituitary-Adrenal Axis Function in Chronic Fatigue Syndrome. *ISRN Neuroscience, 2013*, 1-8. doi:10.1155/2013/784520

Torres-Harding, S., Sorenson, M., Jason, L., Maher, K., Fletcher, M., Reynolds, N., & Brown, M. (2008). The associations between basal salivary cortisol and illness symptomatology in chronic fatigue syndrome *J Appl Biobehav Res, 1*(13), 157-160.

Turner-Cobb, J. M., Osborn, M., da Silva, L., Keogh, E., & Jessop, D. S. (2010). Sex differences in hypothalamic-pituitary-adrenal axis function in patients with chronic pain syndrome. *Stress, 13*(4), 292-300. doi:10.3109/10253890903524785

Van Houdenhove, B., Kempke, S., & Luyten, P. (2010). Psychiatric aspects of chronic fatigue syndrome and fibromyalgia. *Curr Psychiatry Rep, 12*(3), 208-214. doi:10.1007/s11920-010-0105-y

Vollmer-Conna, U., Cameron, B., Hadzi-Pavlovic, D., Singletary, K., Davenport, T., Vernon, S., . . . Dubbo Infective Outcomes Study, G. (2007). Postinfective fatigue syndrome is not associated with altered cytokine production. *Clin Infect Dis, 45*(6), 732-735. doi:10.1086/520990

Wang, H., Moser, M., Schiltenwolf, M., & Buchner, M. (2008). Circulating Cytokine Levels Compared to Pain in Patients with Fibromyalgia - A Prospective Longitudinal Study Over 6 Months *The Journal of Rheumatology, 38*, 1366-1370.

Ware, J., & Sherbourne, C. D. (1992). The MOS 36-item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item Selection. *Medical Care, 30*, 473-483.

Ware, J. C., Kosinski, M., & Keller, S. D. (1995). *SF-36 Physical and Mental Health Summary Scales: A User´s Manual* Boston, MA: Health Assessment Lab.

Wessely, S., Nimnuan, C., & Sharpe, M. (1999). Functional somatic syndromes: one or many? *The Lancet, 354*(9182), 936-939. doi:10.1016/s0140-6736(98)08320-2

Wingenfeld, K., Nutzinger, D., Kauth, J., Hellhammer, D., & Lautenbacher, S. (2010). Salivary cortisol release and hypothalamic pituitary adrenal axis feedback sensitivity in fibromyalgia is associated with depression but not with pain. . *J Pain, 11*, 1195-1202. Retrieved from https://www.sciencedirect.com/science/article/pii/S1526590010003500?via%3Dihub

Wolfe, F., Brahler, E., Hinz, A., & Hauser, W. (2013). Fibromyalgia prevalence, somatic symptom reporting, and the dimensionality of polysymptomatic distress: results from a survey of the general population. *Arthritis Care Res (Hoboken), 65*(5), 777-785. doi:10.1002/acr.21931

Wolfe, F., Clauw, D. J., Fitzcharles, M. A., Goldenerberg, D., Hauser, W., Katz, R. S., . . . Walitt, B. (2016). Revision to the 2010/2011 Fibromyalgia Diagnostic Criteria [abstract]. *Arthritis Rheumatol, 68*.

Wu, G., Feder, A., Cohen, H., Kim, J. J., Calderon, S., Charney, D. S., & Mathe, A. A. (2013). Understanding resilience. *Front Behav Neurosci, 7*, 10. doi:10.3389/fnbeh.2013.00010

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. . *Acta Psychiatr Scand., 67*(6), 361-370.

Aaron, L. A., Burke, M. M., & Buchwald, D. (2000). Overlapping Conditions Among Patients With Chronic Fatigue Syndrome, Fibromyalgia, and Tempoprpmandibular Disorder. *ARCH INTERN MED, 160*, 221-227.