ELSEVIER

Autonomic Neuroscience: Basic and Clinical 137 (2007) 94–101

AUTONOMIC NEUROSCIENCE: Basic & Clinical

www.elsevier.com/locate/autneu

Higher heart rate and reduced heart rate variability persist during sleep in chronic fatigue syndrome: A population-based study☆

Roumiana S. Boneva [a,*], Michael J. Decker [b,c], Elizabeth M. Maloney [a], Jin-Mann Lin [a], James F. Jones [a], Helgi G. Helgason [c], Christine M. Heim [d], David B. Rye [b], William C. Reeves [a]

[a] *Chronic Viral Diseases Branch, National Center for Zoonotic, Vector-borne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA*

[b] *Department of Neurology, Emory University School of Medicine, Atlanta, GA, USA*

[c] *Fusion Sleep, 4265 Johns Creek Parkway, Suite A, Suwanee, Georgia 30024, USA*

[d] *Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA*

Received 22 March 2007; received in revised form 8 August 2007; accepted 20 August 2007

Abstract

Autonomic nervous system (ANS) dysfunction has been suggested in patients with chronic fatigue syndrome (CFS). In this study, we sought to determine whether increased heart rate (HR) and reduced heart rate variability (HRV) parameters observed in CFS patients during wakefulness persist during sleep. To this end, we compared heart rate (HR) and HRV as indicators of ANS function in CFS subjects and non-fatigued (NF) controls in a population-based, case-control study. Thirty subjects with CFS and 38 NF controls, matched for age-, sex- and body mass index, were eligible for analysis. Main outcome measures included mean RR interval (RRI), HR, and HRV parameters derived from overnight ECG. Plasma aldosterone and norepinephrine levels, medicines with cardiovascular effect, and reported physical activity were examined as covariates. General Linear Models were used to assess significance of associations and adjust for potential confounders. Compared to controls, CFS cases had significantly higher mean HR (71.4 vs 64.8 bpm), with a shorter mean RRI [840.4 (85.3) vs 925.4 (97.8) ms] ($p<0.0004$, each), and reduced low frequency (LF), very low frequency (VLF), and total power (TP) of HRV ($p<0.02$, all). CFS cases had significantly lower plasma aldosterone ($p<0.05$), and tended to have higher plasma norepinephrine levels. HR correlated weakly with plasma norepinephrine ($r=0.23$, $p=0.05$) and moderately with vitality and fatigue scores ($r=-0.49$ and 0.46, respectively, $p<0.0001$). Limitation in moderate physical activity was strongly associated with increased HR and decreased HRV. Nevertheless, among 42 subjects with similar physical activity limitations, CFS cases still had higher HR (71.8 bpm) than respective controls (64.9 bpm), $p=0.023$, suggesting that reduced physical activity could not fully explain CFS-associated differences in HR and HRV. After adjusting for potential confounders case-control differences in HR and TP remained significant ($p<0.05$). Conclusion: the presence of increased HR and reduced HRV in CFS during sleep coupled with higher norepinephrine levels and lower plasma aldosterone suggest a state of sympathetic ANS predominance and neuroendocrine alterations. Future research on the underlying pathophysiologic mechanisms of the association is needed. Published by Elsevier B.V.

Keywords: Chronic fatigue syndrome; Heart rate; Heart rate variability; Aldosterone; Norepinephrine; Population-based; Case-control; SF-36; Multiple fatigue inventory

1. Introduction/background

Chronic fatigue syndrome (CFS) presents a diagnostic and management challenge for physicians. A diagnosis of CFS is derived from self-reported symptoms, which are not explained by a differential diagnosis of known medical conditions. Symptoms include clinically evaluated, unexplained, persistent or relapsing fatigue of at least 6-months' duration, that is not

☆ Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency. Names of instruments in this report are for identification purposes only and are not in any way endorsed by the funding agency. At the time of this study, both Drs. Decker and Rye were on the Medcare speaker's bureau.

* Corresponding author. Mail Stop A-15, Chronic Viral Diseases Branch, National Center for Zoonotic, Vector-borne and Enteric Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, GA 30329, USA. Tel.: +1 404 639 3921; fax: +1 404 639 2779.

E-mail address: rboneva@cdc.gov (R.S. Boneva).

1566-0702/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.autneu.2007.08.002

substantially alleviated by rest, and results in substantial reduction in previous levels of activity. In addition, CFS diagnosis requires the presence of at least 4 of 8 case-defining symptoms (unusual post-exertional malaise, unrefreshing sleep, significant impairment in memory or concentration, headache, muscle pain, joint pain, sore throat and tender lymph nodes) (Fukuda et al., 1994). There are no pathognomonic physical signs or diagnostic laboratory abnormalities. The pathophysiology of CFS remains unknown, and as yet there is no definitive treatment. Current therapy is directed toward relieving symptoms and improving function (Afari and Buchwald, 2003).

Disorders of the autonomic nervous system (ANS) share many clinical features of CFS and may account for some cases (Jones et al., 2005). Indexes of heart rate variability (HRV) derived from a continuous electrocardiographic (ECG) recording can be used as indicators of the overall output of the sympathetic and parasympathetic branches of the ANS to the sino-atrial node of the heart. Several studies have found differences in heart rate (HR) and HRV in CFS cases compared to controls (Freeman and Komaroff, 1997; De Becker et al., 1998; Stewart et al., 1998; Stewart, 2000; LaManca et al., 1999; Soetekouw et al., 1999; Winkler et al., 2004; Yamamoto et al., 2003; Yoshiuchi et al., 2004). The majority of the existing studies on HRV in CFS included patients from referral clinics and, therefore, have some recruitment bias. Studies examining heart rate and HRV in CFS in a population-based fashion have not been conducted. Perhaps of greater significance, all of the preceding studies were conducted during wakefulness, and usually under different challenges (standing, head-up tilt test, forced/paced breathing, treadmill, Valsalva maneuver). Therefore, the role of potential confounders such as environmentally induced stress responses, anxiety, etc. cannot be excluded. Many CFS subjects receive medications (Jones et al., 2003) that may affect HR and HRV. Existing studies either excluded subjects receiving cardiovascular- or ANS-acting medications or, conversely, did not discuss medication use at all and, therefore, did not control for such. Finally, none of the studies included long-term ECG recordings at rest or during sleep, when environmental stressors are removed or assessment of baseline plasma norepinephrine. In this study, we used a population-based, case-control study design to determine whether previously described reduced HRV in CFS subjects persists during sleep.

2. Hypothesis

We hypothesized that decreased HRV observed in CFS reflects a perturbation in autonomic function that persists during sleep.

3. Methods

3.1. Study design

This was a population-based, case-control study, approved by the Centers for Disease Control and Prevention (CDC) and Emory University Institutional Review Boards. All subjects gave informed consent. Two hundred twenty seven adults from the general population of Wichita, Kansas, who were identified during a 4-year CFS surveillance study as either having CFS or being non-fatigued (NF), participated in a 2-day in-hospital study conducted between January–July 2003. This study was described in detail elsewhere (Reyes et al., 2003; Heim et al., 2006; Reeves et al., 2006). Briefly, participants answered several questionnaires that measured symptoms, functioning and fatigue [CDC Symptom Inventory, Short Form Health Survey (SF-36), Multidimensional Fatigue Inventory (MFI)]. They completed the Diagnostic Interview Schedule (DIS) (Robbins et al., 1995) and underwent medical history, physical examination, and laboratory testing, which were all used by a physician panel to identify conditions exclusionary for CFS.

As a result of these evaluations there were: (i) 58 subjects who met the 1994 CDC clinical case definition for CFS; (ii) 55 non-fatigued (NF) controls randomly selected and sex-, race-, age- and BMI-matched to CFS cases; (iii) 59 persons with medically unexplained fatigue of 6 months or longer but having "insufficient" number of symptoms to meet the CFS case definition (called ISF). Other 27 persons with CFS and 28 persons with ISF who had melancholic depression, as determined by DIS, were not included in our analyses. Remaining participants were subsequently reclassified based on cut off values from the SF-36, MFI and the CDC Symptom Inventory questionnaires, which resulted in 43 who met criteria for CFS, 61 classified as ISF, and 60 NF controls (Heim et al., 2006; Reeves et al., 2005).

3.2. Data collection

Demographic information, including age, sex, and race/ethnicity was obtained by telephone interview prior to the clinic appointment and confirmed at time of exam. Race was self-selected by subjects from either: White, African American, Native American/Alaskan, Asian/Asian Pacific or mixed, and ethnicity included either Hispanic or non-Hispanic. Height and weight were measured at clinic. Body mass index (BMI) was computed as the ratio of weight in kilograms to height in meters squared. Participants were instructed to bring to clinic all their currently used medications (prescription and over-the-counter). A nurse recorded the name, dose, and time of administration of every medication as reported by the patient. Patients continued their medications during the clinic stay.

3.3. Laboratory

Blood and urine for standard laboratory and for endocrine tests was collected at awakening in the morning of either day 2 or day 3 of the clinic stay (day 1 being the late afternoon when patients were admitted). Clinical laboratory tests were performed in licensed commercial laboratories (Quest Diagnostics and Esoterix Inc). Blood for aldosterone and norepinephrine (NE) was drawn in the morning, upon

wakening. For aldosterone, 1 ml of serum was separated and immediately frozen. Blood for norepinephrine was drawn in an EDTA tube and, immediately after collection, centrifuged in a cold (4 °C) centrifuge. One milliliter of plasma was separated and frozen immediately. Samples were shipped frozen to Esoterix Laboratory Services for testing. Aldosterone levels were measured by radioimmunoassay after solvent extraction. Norepinephrine plasma levels were determined by high performance liquid chromatography.

We calculated osmolality by the formula (1.89 * sodium [mmol/l]) + (1.38 * potassium [mmol/l]) + (1.08 * glucose [mg/dl]/18) + (1.03 * BUN [mg/dl]/28) + 7.45, which has been shown to generate the closest values to measured osmolality (Bhagat et al., 1984).

Polysomnography (PSG) was performed with the Embla N-7000 diagnostic system (Medcare, Iceland), which consists of a small (approximately 10 × 7.5 × 15 cm) digital amplifier that interfaces with a standard Pentium-based computer for real time data collection and review. It has 40-channel capability with all channels fully configurable for sensitivities, sampling rates and filters. We employed a sampling rate of 200 Hz. The system employs a Windows platform and uses proprietary software (Somnologica). The Embla N-7000 (Medcare) relies upon standard gold electrodes for recording of electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) for sleep staging and appreciation of sleep structure. Respiration was recorded with standard sensors for airflow, respiratory effort, body position, and snoring sounds (Pro-Tech, Inc., Woodwinville, WA). A pressure transducer (Pro-tech, model PTAF2) attached to a cannula worn in the nasal nares was used to aid in identification of episodes of elevated upper airway resistance. Built-in oximetry provided continuous co-monitoring of arterial oxygen saturation via a standard non-invasive finger probe. The following signals were recorded: central (C3-A2//C4-A1) EEG, left and right monopolar EOG, surface mentalis EMG, ECG (lead II), respiratory airflow (recorded with both thermistors and pressure transducers), respiratory effort (rib cage and abdomen), and 4 separate channels of surface EMG from both legs recorded unilaterally. Impedance at the time of evening hook-up will be less than 5000 Ω on all EMG channels. Subjects had continuous PSG recordings during night 1 and night 2 (from ~22:30 h to 7:00 h the following morning). Recorded data were scanned visually and artifacts were manually removed. ECG recordings from the patients' second night were analyzed as the first night was considered an 'adaptation night'.

The mean RRI [in milliseconds (ms)] and mean HR [bpm] (HR = 60,000 ms divided by RRI) were calculated from the mean cycle length of all normal RRI (i.e., abnormal QRS complexes were excluded from calculations). The following time domain heart rate variability parameters (Task force, 1996) were derived from the recordings: SDNN — the standard deviation of the N–N interval (the N–N interval refers to 'normal-to-normal' RRI); SDANN — the standard deviation of the average N–N intervals calculated over short (5-minute) periods; SDNN index — the mean of the 5-minute standard deviations of N–N intervals (calculated over the whole duration of the recording), which measures the variability due to cycles shorter than 5 min; RMSSD — the square root of the mean squared differences of successive N–N intervals; NN50 — the number of successive N–N intervals differing by more than 50 ms; pNN50 — the proportion derived by dividing NN50 by the total number of all N–N intervals. The following frequency domain measures were calculated: power in low frequency range (0.04–0.15 Hz) (LF [ms^2]); power in the very low frequency range (≤0.004 Hz) (VLF [ms^2]); power in the high frequency range (0.15–0.4 Hz) (HF [ms^2]); total power, measured as the variance of the N–N interval over the temporal segment (TP [ms^2]). We also analyzed HR measurements obtained for 29 of the 30 CFS and all NF subjects using data collected at daytime baseline, after resting for 30 min in supine position (Jones et al., 2005).

Standard PSG variables were analyzed previously and sleep data have been published separately (Reeves et al., 2006). Briefly, there were no major differences in sleep architecture between CFS subjects and NF controls with the exception of a significantly higher mean frequency of obstructive apnea per hour in persons with CFS. Other characteristics of sleep architecture did not differ significantly between persons with CFS and controls (Reeves et al., 2006). Patients with respiratory disturbance index (RDI) ≥10 events per hour, indicative of sleep hypopnea/apnea, were not included in our analyses because said events, per se, elicit ANS response (Ferini-Strambi et al., 1992).

4. Statistical analysis

We used a *t*-test to compare continuous variables such as age, BMI, HR and HRV parameters. Case-control differences in categorical variables (e.g., sex, medication use) were analyzed using chi-square or Fisher's exact test. For continuous variables that did not pass the normality distribution tests (Kolmogorov–Smirnov and Levin's tests), logarithmic transformation was used to achieve normal distribution for the purposes of the analysis. When log transformation did not result in normal distribution, Wilcoxon rank test was used to compare values. Spearman correlation coefficients were used

Table 1
Distribution of demographic characteristics and body mass index (BMI) among CFS cases and non-fatigued (NF) controls

Characteristics	CFS (n=30)	NF (n=38)	p-value
Age in years (mean±SD)	49.5±7.8	50.5±8.8	0.60
Sex [n (%)]			
Female	26 (86.7)	31 (81.6)	0.74
Male	4 (13.3)	7 (18.4)	
Race [n (%)]			
White	27 (90)	34 (89.5)	0.79
Black, other	3 (10)	3 (7.9)	
Missing	0	1 (2.6)	
BMI (mean±SD)	28.5±4.0	28.1±4.5	0.93

Table 2
Frequency of medication use among CFS patients and non-fatigued (NF) controls

Type of medication	CFS		NF		*p* value
	Medication use				
	Yes	No	Yes	No	
	n (%)	*n* (%)	*n* (%)	*n* (%)	
Antidepressants	8 (26.7)	22 (73.3)	4 (10.5)	34 (89.5)	0.08
Beta blockers	2 (6.7)	28 (93.3)	3 (7.9)	35 (92.1)	0.85
Calcium channel blockers	3 (10.0)	27 (90)	2 (5.3)	36 (94.7)	0.46
Sympathomimetics	3 (10.0)	27 (90.0)	3 (7.9)	35 (92.1)	0.76
Antihypertensive medicines [a]	4 (13.3)	26 (86.7)	5 (13.2)	33 (86.8)	0.99

[a] Includes diuretics, ACE inhibitors, angiotensin II receptor blockers; does not include beta blockers or calcium channel blockers.

to measure correlations of scores from the SF-36 and MFI subscales with HR and HRV parameters.

General linear models were used to examine the association between HRV parameters (dependent variable) and the primary independent variable of case (CFS) — control (NF) status. To rule out spurious associations between CFS and HR/HRV and to assess effects of other factors, we controlled for several sets of covariates by including them in the regression model. These were: (a) age, sex and BMI (the pre-clinical match factors) — to account for breaking the matched pairs due to reclassification and exclusions after the clinical evaluation; (b) medications with cardiovascular effect (antidepressants, anti-hypertensives (ACE inhibitors and diuretics), beta blockers, calcium channel blockers, and sympathetomimetics) — to evaluate possible medication effect on HR and HRV (Task force, 1996); (c) sleep parameters that were significantly associated with either CFS or with HRV (number of obstructive apnea episodes per hour (Reeves et al., 2006), periodic leg movements (PLM) with arousal, and number of central apnea events per hour); (d) plasma baseline levels of norepinephrine and aldosterone (for 6.5% of subjects with aldosterone values <1 ng/dl (lower limit of detection), the value of 0.5 was imputed); and (e) physical activity level — as it can also influence HR and HRV, because by definition CFS subjects have reduced physical activity. Therefore, in addition to the above analyses, we also conducted a separate restricted analysis of the association between HR/HRV and CFS only among cases and controls who reported the same level of moderate physical activity in the physical function section of the SF-36.

Statistical significance of two-tailed tests was determined by an alpha level of 0.05. SAS Version 9.0, was used to conduct all analyses (SAS Institute, Cary, N.C).

5. Results

There were 43 subjects classified as CFS, 61 subjects with ISF and 60 non-fatigued (NF) controls who did not have exclusionary medical or psychiatric conditions as determined by review of DIS data, medical history, physical exam, and laboratory test results (see Reyes et al., 2003; Heim et al., 2006; Reeves et al., 2006). After excluding subjects with RDI score ≥10 or inadequate ECG recording, there were 30 of 43 (69.8%) CFS cases, 44 of 61 (72.1%) subjects with ISF, and 38 of 60 (63.3%) NF controls eligible for analysis. Comparisons focus on the 30 CFS cases and 38 NF controls.

CFS subjects and NF controls were similar with respect to their distributions of age, sex, race and BMI (Table 1). Use of beta blockers, calcium channel blockers, anti-hypertensive medications, and antidepressants did not differ significantly between CFS cases and NF controls (Table 2).

Table 3
Distribution of mean values for RR interval, heart rate, and heart rate variability parameters among CFS cases and non-fatigued controls

HRV parameter	CFS (*n*=30)	NF (*n*=38)	*p* value		
	Mean (SD)*		Unadjusted	Adjusted for age, sex, and BMI	Adjusted for age, sex, BMI and specific medications[†]
HR (bpm)	72.1 (7.6)	65.5 (7.0)	<0.001	<0.001	0.0025 (a)
RR interval (ms)	840.4 (85.3)	925 (97.8)	<0.001	<0.001	0.002 (a)
NN50	3519.3 (4028.1)	3707.1 (3685.7)	0.69	0.73	0.93 (a)
pNN50%	11.9 (14.7)	13.5 (13.8)	0.53	0.58	0.79 (a)
SDNN	81.6 (29.7)	99.4 (57.7)	0.14	0.16	0.26 (a)
SDANN	62.3 (43.5)	71.5 (59.2)	0.30	0.37	0.71(a, b)
RMSSD	51.1 (40.8)	66.6 (87.5)	0.73	0.73	0.98 (a, b)
SDNN index	59.3 (26.9)	76.5 (54.7)	0.14	0.15	0.27 (a, b)
HRV triangular index	15.6 (5.8)	16.8 (5.8)	0.35	0.39	0.61 (a)
HF power	1493.2 (1062.5)	1668.6 (1276.0)	0.65	0.63	0.96 (a)
LF power	3189.1 (1179.6)	4035.9 (1590.7)	0.02	0.02	0.04 (a, ah)
VLF power	5464.9 (2424.8)	7328.8 (2891.7)	0.006	0.007	0.01 (a, c)
Total power	10296.7 (3326.9)	13208.1 (4278.1)	0.003	0.003	0.006 (a)
LF/HF ratio	2.85 (1.68)	3.42 (2.09)	0.23	0.32	0.24 (a)

*SD, standard deviation; The *p*-value for differences between CFS and NF were derived from general linear models. [†]Medications: (a) antidepressants; (b) beta blockers; (c) calcium channel blockers; (ah) anti-hypertensives.

Table 4
Mean values and standard deviations of heart rate variability (HRV) parameters by sex in the combined group of CFS cases and NF controls

HRV parameter	Female ($n=57$)	Male ($n=11$)	p
	Mean (SD)		
Heart rate (bpm)	69.1 (7.9)	65.0 (7.1)	0.12
R–R interval (ms)	879.3 (101.0)	932.3 (94.3)	0.12
Low frequency power (ms^2)	3378.0 (1183.0)	5135.6 (1978.4)	<0.001
Very low frequency power (ms^2)	5999.6 (2525.5)	9133.1 (3007.0)	<0.001
Total power (ms^2)	11087.5 (3584.9)	16256 (4173.3)	<0.001
RMSSD	60.2 (75.5)	57.4 (41.1)	0.54
NN50	3523.0 (3831.8)	4149.0 (3845.7)	0.19
pNN50	12.3 (13.9)	15.2 (15.3)	0.17
SDNN	90.1 (51.4)	99.1 (23.6)	0.19
SDANN	65.7 (53.1)	76.5 (52.1)	0.28
SDNN index	67.5 (48.2)	76.5 (24.4)	0.15
HRV triangular index	16.0 (6.0)	17.8 (4.1)	0.19
HF (ms^2)	1537.4 (1149.3)	1870.2 (1360.3)	0.31
LF/HF ratio	3.01 (1.73)	3.98 (2.69)	0.13

We compared HRV parameters between CFS and NF groups. Although the mean RRI (and heart rate) were within normal limits for all study subjects, the mean RRI in CFS patients was significantly shorter (840.4 ms) than in the NF controls (925.0 ms) ($p=0.0004$, Table 3). A corollary finding was that CFS subjects had a higher mean HR (72.1±7.6) compared to controls (65.5±7.0), ($p=0.0004$, Table 3). Similarly, during wakefulness, after a 30-minute period in recumbent position, CFS cases had a significantly higher mean HR at baseline (79.2±9.6 bpm), compared to the NF controls (72.2±8.7, $n=36$) ($p=0.003$). Mean values for LF, VLF, and TP were also significantly lower in cases than controls (Table 4, $p=0.02$, $p=0.006$, $p=0.003$, respectively). The remaining HRV parameters were also lower in CFS subjects than in NF controls, but the differences did not reach statistical significance (Table 3).

In our study sample, women had significantly lower mean LF, VLF and TP compared to men (Table 4); HRV parameters did not vary significantly by age or BMI. To adjust for potential confounding that may account for the association between CFS and reduced HRV, we examined these covariates by including them in the statistical model. Controlling for sex, age, and BMI did not alter the association of CFS with shorter RRI (i.e., higher HR), LF, VLF, and TP (Table 3).

Users of antidepressants had significantly faster mean HR, shorter mean RRI, and lower SDANN, HF power, and LF power (data not shown, all $p<0.05$). Users of beta blockers had significantly lower mean SDANN ($p=0.03$) compared to non-users. After controlling for medication use (antidepressants alone, or antidepressants and an additional cardiovascular medication, where indicated) CFS remained significantly associated with higher HR (shorter RRI), and lower LF, VLF and total power ($p<0.01$, $p=0.04$, $p=0.01$, $p<0.01$, respectively) (Table 3).

We further examined whether additional covariates (serum osmolality, plasma aldosterone and norepinephrine levels) accounted for the relationship between CFS and reduced HRV parameters. Osmolality did not differ between CFS and NF controls (283.4±3.7 vs 282.9±2.9 mmol/l, respectively) and was not considered in further analyses. CFS subjects had significantly lower aldosterone (5.23±4.95 ng/dl) compared to controls (7.07±4.00 ng/dl), $p=0.012$. Mean norepinephrine levels in CFS subjects were higher than in NF (449.7±264.4 vs 409.5±210.0, respectively) but not statistically different. Norepinephrine levels, however, were weakly correlated with HR, (i.e. higher norepinephrine correlated with higher HR) in the combined group of CFS, ISF and NF subjects ($r=0.23$,

Table 5
Correlation of HRV parameters with health, function, and impairment scores from the MFI and SF scales

SF-36 and MFI variables	SF and MFI score, by group		Spearman coefficients of correlation between HR and HRV parameters and individual SF or MFI scores (with respective p value)							
	Mean±SD		HR		LF		VLF		TP	
SF-36 subscales	CFS, $n=30$	NF, $n=38$	r	p	r	p	r	p	r	p
General health	52.9 (21.3)	83.2 (15.2)	−0.38	0.002	NS*	NS	0.24	0.046	NS	NS
Vitality	18.5 (12.2)	70.8 (14.3)	−0.49	<0.001	0.21	0.09	0.27	0.024	0.27	0.02
Physical function	55.0 (21.5)	90.1 (12.0)	−0.39	0.001	0.31	0.01	0.41	0.001	0.25	0.04
Role physical	23.3 (32.1)	85.5 (28.3)	−0.35	0.004	NS	NS	0.30	0.014	0.25	0.04
Social function	50.8 (23.7)	94.7 (10.7)	−0.32	0.008	NS	NS	NS	NS	NS	NS
Mental Health	64.4 (20.0)	86.4 (8.9)	−0.31	0.009	NS	NS	NS	NS	NS	NS
Bodily pain	42.1 (17.1)	77.7 (17.8)	−0.39	0.001	0.20	0.10	0.39	0.006	0.33	0.01
MFI subscales										
General fatigue	17.6 (2.0)	8.2 (2.6)	0.46	<0.001	NS	NS	NS	NS	−0.24	<0.05
Physical fatigue	14.4 (3.1)	6.7 (2.0)	0.43	<0.001	NS	NS	NS	NS	NS	NS
Mental fatigue	14.3 (4.2)	7.0 (2.6)	0.24	0.049	NS	NS	NS	NS	NS	NS
Activity reduction	14.8 (3.2)	5.6 (1.7)	0.41	<0.001	0.19	0.11	−0.27	0.027	−0.25	0.04
Motivation reduction	12.5 (4.1)	6.1 (1.8)	0.37	0.002	NS	NS	NS	NS	NS	NS

*Correlation coefficients <0.20 and p values >0.11 are marked as NS. Higher scores in SF-36 reflect better health and function; higher MFI scores correspond to more fatigue or impairment.

$p=0.05$). In addition, in a linear regression model, HR (and RRI, respectively) was significantly associated with a dichotomous (high vs. low) measure of norepinephrine (high norepinephrine ≥ 410 pg/dl, the laboratory's upper normal limit), $p=0.039$. After adjusting for norepinephrine, sex and relevant medications, the differences in HR, RRI, LF, VLF and TP levels between CFS and NF remained statistically significant ($p=0.0004$, $p=0.048$, $p=0.014$, $p=0.007$, respectively). After adjustment for aldosterone, the case-control difference in HR, RRI, VLF and TP remained statistically significant ($p=0.001$, $p=0.014$, $p=0.008$, respectively), while LF was no longer significantly associated with CFS ($p=0.055$).

Among the examined sleep parameters, only adjusting for mean hourly number of periodic leg movements with arousal diminished the statistical significance of the association of CFS with lower LF and VLF power ($p=0.07$ and $p=0.09$, respectively).

HR correlated significantly with all the subscale scores from the SF-36 (negatively) and the MFI (positively), indicating that a higher HR was associated with more impairment and worse fatigue. The correlations of HR with SF-36 subscales measuring vitality ($r=-0.49$), general and physical fatigue, activity reduction (all $r>0.40$, $p<0.005$), bodily pain, and physical function ($r=-0.39$, $p=0.01$, for both) were stronger than the correlation with mental fatigue ($r=-0.24$, $p=0.049$) (Table 5).

In a separate model, we explored the associations between individual SF-36 subscales measuring physical health, as independent variables, and each HRV parameter, as the dependent variable. Limitation in moderate physical activity remained the only variable significantly associated with HR and HRV. Limitation in moderate physical activity was significantly associated with higher HR (shorter RRI) $p<0.001$, and with lower LF, VLF and TP ($p=0.031$, $p=0.006$, and $p=0.002$, respectively). Finally, to establish whether, after accounting for level of limitation in moderate physical activity, CFS was still associated with higher HR and lower HRV, we restricted the analysis to 36 CFS cases and 6 controls who reported being equally limited (i.e., "limited a lot") in moderate physical activity. Among this subset of subjects, patients with CFS still had significantly higher mean HR compared to controls (71.2 bpm vs. 64.9 bpm), $p=0.02$, with shorter RR interval (884.0 ms vs 933.9 ms, $p=0.03$). Their mean LF, VLF and TP were also lower compared to controls but no longer statistically different (Table 6).

Table 6
Heart rate and frequency domain heart rate variability parameters among 42 subjects who reported being "a lot limited" in their moderate physical activities

Variable	Mean (SD)		p
	CFS ($n=36$)	NF ($n=6$)	
HR	71.82 (7.9)	64.9 (6.5)	0.023
RR_interval	884.0 (93.1)	933.9 (93.0)	0.034
LF	3286.0 (1177.4)	4115.6 (1597.0)	0.23
VLF	6702.2 (1978.6)	7455.7 (2905.6)	0.55
TP	11667.3 (3060.5)	13467.8 (4231.9)	0.33

p values are derived from the general linear model.

In separate general linear models in which we regressed either CFS or limitation in moderate physical activity on HRV parameters, each explained a small part of the variations in HR (17% and 20%, respectively), LF (18% and 19%, respectively), VLF (11% and 14%, respectively) and TP (12.5 and 17%, respectively). A model that included CFS, limitation in moderate physical activity, and sex as independent variables, explained 23.6%, 27.5%, 28.5% and 35.6% of the variance in HR (RRI), LF, VLF and TP, respectively (data not shown).

6. Discussion

We demonstrate that subjects meeting the CFS case definition have increased HR with reduced LF, VLF, and TP components of HRV that persist during sleep. This is observed in the presence of higher baseline norepinephrine levels and lower baseline aldosterone levels in the CFS group compared to controls.

Our novel findings concur with previous reports of reduced HRV in CFS subjects during wakefulness, under challenge conditions (De Becker et al., 1998; Stewart et al., 1998; LaManca et al., 1999; Cordero et al., 1996; Duprez et al., 1998; Yamamoto et al., 2003). Our findings further enhance those results by demonstrating that increased HR and diminished HRV in CFS subjects persist during sleep. We also demonstrate that the higher HR and lower HRV do not appear to be due to differential use of medications (antidepressants, beta blockers, sympathomimetics, calcium channel blockers) known to alter HRV (Task force, 1996), nor can be fully accounted for by CFS subjects' decreased physical activity. In addition, this study is the first to show that increased HR in CFS subjects correlated with standardized and validated measures of fatigue and impairment (the MFI and SF-36 instruments).

We acknowledge several limitations of our study. First, we did not include an objective measure of physical fitness. By default, CFS cases are limited in their physical activity. Therefore, our study cannot definitively determine whether the differences in HR and HRV between CFS subjects and controls correspond to differences in physical activity and fitness. This has also been the limitation of other studies of heart rate variability in CFS and needs to be addressed in the future. However, in a subset of our sample, we demonstrate that for equal self-reported limitations in moderate physical activity CFS subjects still had a significantly higher HR compared to controls. Similar to the entire sample, in this subset LF, VLF and TP in CFS cases were still lower (numerically, though not statistically) than in controls. Second, we may have introduced bias if the CFS cases eliminated due to inadequate ECG recordings were more healthy than the CFS cases who remained in our analysis. However, we have no reason to suspect that this bias

operated differently in cases and controls, therefore the effect on our analysis would be to bias the results toward the null. Third, small sample sizes may limit our ability to generalize findings to all CFS patients but we attempted to address this limitation by describing our sample's demographics and BMI, demonstrating that the CFS patients in this analysis were of similar age, sex and BMI as CFS patients described in other studies.

We appreciate that occult coronary artery disease or heart disease could contribute to the reduced HRV. All subjects in our study underwent physical examination and laboratory tests to rule out fatiguing illnesses with known cause and subjects with fatigue due to known or suspected heart disease or other acute or chronic condition were excluded from our study. In addition, use of cardiovascular medications (as a surrogate measure of cardiovascular disease) did not differ significantly between CFS cases and NF controls.

HRV is reduced in a number of psychiatric conditions including depression (Birkhofer et al., 2005), which is a relatively common co-morbid condition in CFS. Subjects with major depression with melancholic features were excluded from the study based on detailed psychiatric interview, while those without melancholic features were not. Even after controlling for antidepressant use, the association between CFS and increased HR with reduced HRV remained significant, which demonstrates that CFS, per se, was associated with lower HRV and increased HR.

It is plausible that the increased HR we observed in CFS may reflect a homeostatic attempt to maintain adequate cardiac output in the presence of a lower blood volume. At least one study has reported lower blood volume in CFS patients (Farquhar et al., 2002). While we did not measure blood volume directly, we were able to rule out hypertonic dehydration (hyperosmolality) in CFS by demonstrating that mean osmolality in CFS subjects and controls were similar.

CFS cases in our study had a lower mean aldosterone level than controls. This may be a side effect of nonsteroid anti-inflammatory drug use (Zawada, 1982), which is common among CFS subjects. Regardless, the mechanism underlying lower aldosterone levels, observed in our CFS subjects, remains unexplained at present and deserves further attention.

CFS was associated with higher norepinephrine levels and there was a weak but significant correlation between high norepinephrine levels and higher HR in our study. Higher NE levels have been found to correlate negatively with low frequency power (Kurata et al., 1997) and TP (Eller, 2007) and our study is in agreement with those findings as our CFS subjects had reduced LF and TP. Plasma norepinephrine levels correlate positively with HR, especially under stress conditions when the sympathetic nervous system dominates. Thus, higher norepinephrine in conjunction with increased HR and reduced HRV in CFS patients may reflect an increased sympathetic output to the heart (or, alternatively, a decreased norepinephrine clearance). Antidepressants, which were more frequently used by CFS subjects, may also increase plasma norepinephrine levels (Veith et al., 1983). However, adjusting for antidepressant use did not change the association between CFS and HR/HRV substantially. Higher plasma norepinephrine can also be found in congestive heart failure (Esler and Kaye, 1998) but, as already discussed, subjects were screened for fatiguing illnesses with known cause at physical exam and heart failure was an exclusionary condition.

LF power, VLF, and TP were all significantly lower in CFS cases compared to NF controls. When we included periodic leg movement with arousal as a covariate in the model, the association of CFS with lower LF and VLF became statistically insignificant ($p=0.07$ and $p=0.09$, respectively). VLF is thought to reflect, among other things, state of arousal (Taylor et al., 1998). Therefore, it is possible that the reduced LF and VLF domains of HRV in CFS cases may be partially due to more arousals episodes during sleep.

The fact that the significance of the association between CFS and LF power diminished after including aldosterone in the model implies that plasma aldosterone levels may influence LF power, possibly by regulation of blood volume.

Because CFS, reduced physical activity, and sex explained only less than or approximately a third of the variance in HR and the frequency domains of HRV, there may be additional factors associated with CFS and with reduced HRV. Eller (2007) found that reduced high frequency and total power components of heart rate variability are associated with risk factors such as waist-to-hip ratio, glycosylated hemoglobin, and norepinephrine (Eller, 2007). Compared to their non-fatigued controls, our CFS subjects had a higher allostatic load (one component of which included waist-to-hip ratio) (Maloney et al., 2006). BMI is known to be highly correlated with waist-to-hip ratio. After we controlled for BMI in our analyses, CFS remained significantly associated with higher HR and lower HRV. It may be worth it, in a future study, to further examine whether the higher HR and the lower HRV in CFS are associated with higher glycosylated hemoglobin and other features of metabolic syndrome.

Heart rate variability declines with age (Bonnemeier et al., 2003). With their higher mean HR and lower heart rate variability, CFS subjects in our study appeared 'physiologically older' than their age-, sex-, race-, and BMI-matched controls.

A large, prospective, population-based study of middle-aged men and women (Dekker et al., 2000), found that high heart rate and, especially, low HRV at baseline were predictive of coronary heart disease and increased mortality rates (cardiovascular and all causes) at follow-up. For HRV, this relation could not be attributed to cardiovascular risk factors or to underlying disease suggesting that low HRV is an indicator of poor general health (Dekker et al., 2000). Whether, the CFS subjects' higher HR with lower HRV places them at increased risk of cardiovascular (and other) morbidity and mortality remains to be seen but at least one study suggests that such risk may exist (Jason et al., 2006).

HR and HRV measure the overall input of the sympathetic and parasympathetic branches of the autonomic nervous

system to the sino-atrial node of the heart. HR and its variability are viewed as a quantifiable measure of the subject's "efforts" in response to environmental demands (Pagani and Lucini, 1999). From this view point, CFS subjects in our study appear to experience greater physiologic effort at rest (during sleep) than NF controls.

In summary, our novel observations of higher HR and lower HRV during sleep in CFS, coupled with higher baseline plasma norepinephrine and lower aldosterone suggest a state of sympathetic ANS predominance with perturbed neuroendocrine activity. Future research is needed to understand the underlying pathophysiologic mechanism of this dysfunction.

References

Afari, N., Buchwald, D., 2003. Chronic fatigue syndrome: a review. Am. J. Psychiatr. 160, 221–236.

Bhagat, C.I., Garcia-Webb, Fletcher, E., Beilby, J.P., 1984. Calculated vs measured plasma osmolalities revisited. Clin. Chem. 30, 1703–1705.

Birkhofer, A., Schmidt, G., Forstl, H., 2005. Heart and brain — the influence of psychiatric disorders and their therapy on the heart rate variability. Fortschr. Neurol. Psychiatr. 73, 192–205.

Bonnemeier, H., Richardt, G., Potratz, J., et al., 2003. Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. J. Cardiovasc. Electrophysiol. 14, 791–799.

Cordero, D.L., Sisto, S.A., Tapp, W.N., et al., 1996. Decreased vagal power during treadmill walking in patients with chronic fatigue syndrome. Clin. Auton. Res. 6, 329–333.

De Becker, P., Dendale, P., De Meirleir, K., et al., 1998. Autonomic testing in patients with chronic fatigue syndrome. Am. J. Med. 105, 22S–26S.

Dekker, J.M., Crow, R.S., Folsom, A.R., Hannan, P.J., Liao, D., Swenne, C.A., Schouten, E.G., 2000. Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis risk in communities. Circulation 102 (11), 1239–1244 (Sep 12).

Duprez, D.A., De Buyzere, M.L., Drieghe, B., et al., 1998. Long- and short-term blood pressure and RR-interval variability and psychosomatic distress in chronic fatigue syndrome. Clin. Sci. (Lond). 94, 57–63.

Eller, N.H., 2007. Total power and high frequency components of heart rate variability and risk factors for atherosclerosis. Auton. Neurosci. Basic Clin. 131, 123–130.

Esler, M., Kaye, D., 1998. Increased sympathetic nervous system activity and its therapeutic reduction in arterial hypertension, portal hypertension and heart failure. J. Auton. Nerv. Syst. 72, 210–219.

Farquhar, W.B., Hunt, B.E., Taylor, J.A., et al., 2002. Blood volume and its relation to peak O(2) consumption and physical activity in patients with chronic fatigue. Am. J. Physiol. Heart Circ. Physiol. 282, H66–H71.

Ferini-Strambi, L., Zucconi, M., Oldani, A., Smirne, S., 1992. Heart rate variability during sleep in snorers with and without obstructive sleep apnea. Chest 102, 1023–1027.

Freeman, R., Komaroff, A., 1997. Does the chronic fatigue syndrome involve the autonomic nervous system? Am. J. Med. 102, 357–364.

Fukuda, K., Straus, S.E., Hickie, I., et al., 1994. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International chronic fatigue syndrome study group. Ann. Intern. Med. 121, 953–959.

Heim, C., Wagner, D., Maloney, E.M., et al., 2006. Early adverse experience and risk for chronic fatigue syndrome: results from a population-based study. Arch. Gen. Psychiatry 63, 1258–1266.

Jason, L.A., Corradi, K., Gress, S., Williams, S., Torres-Harding, S., 2006. Causes of death among patients with chronic fatigue syndrome. Health Care Women Int. 27, 615–626.

Jones, J.F., Nicholson, A., Nisenbaum, R., et al., 2005. Orthostatic instability in persons with chronic fatigue syndrome and healthy control subjects in the general population. Am. J. Med. 118 , 1415.e19–1415.e28.

Jones, J.F., Nisenbaum, R., Reeves, W.C., 2003. Medication use by persons with chronic fatigue syndrome: results of a randomized telephone survey in Wichita, Kansas. Health Qual. Life Outcomes 1, 74. doi:10.1186/1477-7525-1-74.

Kurata, C., Shouda, S., Mikami, T., et al., 1997. Comparison of [123I] metaiodobenzylguanidine kinetics with heart rate variability and plasma norepinephrine level. J. Nucl. Cardiol. 4, 515–523.

LaManca, J.J., Peckerman, A., Walker, J., et al., 1999. Cardiovascular response during head-up tilt in chronic fatigue syndrome. Clin. Physiol. 19, 111–220.

Maloney, E.M., Gurbaxani, B.M., Jones, J.F., de Souza Coelho, L., Pennachin, C., Goertzel, B.N., 2006. Chronic fatigue syndrome and high allostatic load. Pharmacogenomics 7, 467–473.

Pagani, M., Lucini, D., 1999. Chronic fatigue syndrome: a hypothesis focusing on the autonomic nervous system. Clin. Sci. (Lond). 96, 117–125.

Reeves, W.C., Wagner, D., Nisenbaum, R., et al., 2005. Chronic fatigue syndrome—a clinically empirical approach to its definition and study. BMC Med. 3, 19.

Reeves, W.C., Heim, C., Maloney, E., et al., 2006. Sleep characteristics of persons with chronic fatigue syndrome and non-fatigued controls: results from a population-based study. BMC Neurol. 6, 41.

Reyes, M., Nisenbaum, R., Hoaglin, D.C., et al., 2003. Prevalence and incidence of chronic fatigue syndrome in Wichita, Kansas. Arch. Intern. Med. 163, 1530–1536.

Robbins, L., Cottler, L., Bucholz, K., Compton, W., 1995. Diagnostic Interview Schedule for DSM-IV (DIS-IV). Washington University, St. Louis, MO.

Soetekouw, P.M., Lenders, J.W., Bleijenberg, G., et al., 1999. Autonomic function in patients with chronic fatigue syndrome. Clin. Auton. Res. 9, 334–340.

Stewart, J., Weldon, A., Arlievsky, N., et al., 1998. Neurally mediated hypotension and autonomic dysfunction measured by heart rate variability during head-up tilt testing in children with chronic fatigue syndrome. Clin. Auton. Res. 8, 221–230.

Stewart, J.M., 2000. Autonomic nervous system dysfunction in adolescents with postural orthostatic tachycardia syndrome and chronic fatigue syndrome is characterized by attenuated vagal baroreflex and potentiated sympathetic vasomotion. Pediatr. Res. 48, 218–226.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Eur. Heart J. 17, 354–381.

Taylor, J.A., Carr, D.L., Myers, C.W., Eckberg, D.L., 1998. Mechanisms underlying very-low-frequency RR-interval oscillations in humans. Circulation 98, 547–555.

Veith, R.C., Raskind, M.A., Barnes, R.F., Gumbrecht, G., Ritchie, J.L., Halter, J.B., 1983. Tricyclic antidepressants and supine, standing, and exercise plasma norepinephrine levels. Clin. Pharmacol. Ther. 33, 763–769.

Winkler, A.S., Blair, D., Marsden, J.T., et al., 2004. Autonomic function and serum erythropoietin levels in chronic fatigue syndrome. J. Psychosom. Res. 56, 179–183.

Yamamoto, Y., LaManca, J.J., Natelson, B.H., 2003. A measure of heart rate variability is sensitive to orthostatic challenge in women with chronic fatigue syndrome. Exp. Biol. Med. 228, 167–174.

Yoshiuchi, K., Quigley, K.S., Ohashi, K., et al., 2004. Use of time-frequency analysis to investigate temporal patterns of cardiac autonomic response during head-up tilt in chronic fatigue syndrome. Auton. Neurosci. 113, 55–62.

Zawada Jr., E.T., 1982. Renal consequences of nonsteroidal antiinflammatory drugs. Postgrad. Med. 71, 223–230.