

# Blunted Circadian Variation in Autonomic Regulation of Sinus Node Function in Veterans with Gulf War Syndrome

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**PURPOSE:** To test the hypothesis that subtle abnormalities of the autonomic nervous system underlie the chronic symptoms reported by many Gulf War veterans, such as chronic diarrhea, dizziness, fatigue, and sexual dysfunction.

**METHODS:** Twenty-two ill Gulf War veterans and 19 age-, sex-, and education-matched control veterans underwent measurement of circadian rhythm of heart rate variability by 24-hour electrocardiography, ambulatory blood pressure recording, Valsalva ratio testing, sympathetic skin response evaluation, sweat imprint testing, and polysomnography. Investigators were blinded to case- or control-group status.

**RESULTS:** High-frequency spectral power of heart rate variability increased normally 2.2-fold during sleep in controls but only 1.2-fold in ill veterans ( $P < 0.0001$ ). In ill veterans as compared with controls, it was lower at night ( $P = 0.0006$ ), higher during the

morning ( $P = 0.007$ ), but no different during the rest of the day ( $P = 0.8$ ). The mean heart rate of ill veterans also declined less at night ( $P = 0.0002$ ), and their corrected QT intervals tended to be longer over the full 24 hours ( $P = 0.07$ ), particularly at night ( $P = 0.03$ ). Blunting of the nocturnal heart rate dip in ill veterans was confirmed by 24-hour automatic ambulatory blood pressure monitoring ( $P = 0.05$ ) and polysomnography ( $P = 0.03$ ). These differences remained significant after adjusting for potential confounders. Cases and controls were similar on measures of sympathetic adrenergic and sudomotor function, sleep architecture, respiratory function, and circadian variation in blood pressure and body temperature.

**CONCLUSION:** Some symptoms of Gulf War syndrome may be due to subtle autonomic nervous system dysfunction. **Am J Med.** 2004;117:469–478. ©2004 by Elsevier Inc.

During or shortly after the 1991 Persian Gulf War, at least 100,000 of the approximately 700,000 U.S. military personnel who served developed symptoms of chronic “undiagnosed illness” (1,2). To date, 30% of living Gulf War veterans have been officially classified as at least partially disabled by service-connected conditions (3). Population-based epidemiologic studies have consistently shown 26% to 30% of the Gulf War–deployed force have symptomatic illness in excess of what is found in the nondeployed Gulf War–era military population (1).

Among the most common complaints are symptoms

suggesting autonomic dysfunction, such as chronic pathogen-free diarrhea resembling that in diabetic patients, dizziness and vertigo, night sweats, and sexual dysfunction (2,4–6). Moreover, nationwide rates of acute cholecystitis and cholecystectomy have reportedly risen from 1992 to 1997 in young, predominantly male Gulf War veterans, while falling in other groups of military veterans (7), suggesting the involvement of abnormal vagal (parasympathetic) control of gall bladder emptying. Conversely, orthostatic hypotension has not been common in the many clinical studies of Gulf War veterans (2), and audiovestibular testing in our original clinical case-control study suggested that dizzy spells in veterans represented attacks of vertigo from central vestibular dysfunction rather than postural hypotension (5,8).

While the cause of the illness remains controversial, evidence of chronic central nervous system damage from exposure to low-level sarin nerve gas has emerged. Exposure to low-level sarin in fallout from bombing and demolition of munitions during the 1991 Gulf War was widespread (9,10). All six epidemiologic studies that examined perceived nerve agent exposure found sarin exposure to be most strongly associated with chronic illness (11–16). Genetically low blood levels of paraoxonase, the enzyme that protects from nerve agent and pesticide exposure, have been associated with the illness (17,18). Ill Gulf War veterans have lower levels of N-acetylaspartate in basal ganglia as compared with

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This study was supported by the U.S. Army Medical Research and Materiel Command cooperative agreement no. DAMD17-97-2-7025 and DAMD01-1-0741; by U.S. Public Health Service grant MO1-RR00633; and by a grant from the Perot Foundation, Dallas, Texas.

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Manuscript submitted October 28, 2002, and accepted in revised form March 6, 2004.

controls, indicating brain cell damage (19–21). Rodents exposed repetitively to inhalation of sarin at concentrations too low to produce immediate toxic signs experience delayed onset of damage to muscarinic cholinergic receptors in basal ganglia (22), accompanied by subtle dysfunction of the autonomic nervous system (23).

The symptoms of autonomic dysfunction without orthostatic hypotension or other signs of sympathetic neural failure led us to hypothesize a subtle abnormality of the autonomic nervous system similar to that found in very early diabetic neuropathy. To test this hypothesis, we performed tests of parasympathetic and sympathetic nervous system regulation in the group of Gulf War veterans who were subjects in our previous investigations (5).

## METHODS

### *Sample*

Ill subjects included 22 male members of the 24th Reserve Naval Mobile Construction Battalion who developed typical symptoms of Gulf War syndrome during or shortly after serving in the 1991 Gulf War. Controls comprised 18 age-, sex-, and education-matched male subjects who served in the same battalion but who remained well. The case definition, methods of selection, and prior studies on these subjects have been published (4,5,8,11,17,19,20,24). The ill veterans and half of the controls had served in land-based combat support roles in Saudi Arabia, Kuwait, or Iraq during the 1991 war; by design, the remaining controls had not been deployed to the war zone (5). The ill veterans were similar to the controls in age (mean, 46.6 vs. 47.7 years), education level (12.8 vs. 12.7 years of education), height (179 vs. 176 cm), and body mass index (27.5 vs. 27.3 kg/m<sup>2</sup>) (5). None of the subjects was a shift worker or had recently traveled outside of the United States.

### *Clinical Research Protocol*

After discontinuing potentially interfering medications at least three half-lives before arrival, all subjects were admitted to our General Clinical Research Center in Parkland Hospital for 7 days, during which they maintained a sedentary, low-stress activity level and received a uniform, high-sodium (8 g/d) diet to maintain euvolemia. Subjects were allowed to continue smoking. All subjects gave written informed consent according to a protocol approved by the institutional review boards of our university and the U.S. Army Surgeon General (Fort Detrick, Maryland). All investigators were blinded to the subjects' case- or control-group status.

Autonomic control of cardiovagal function was tested by spectral analysis of a 24-hour Holter recording of the electrocardiogram (25–28). Recordings were performed during the hospital stay in 11 of the 22 ill subjects and in 9 of the 18 controls, and after discharge while off medications in 10 ill subjects and 8 controls. Full recordings

could not be obtained in 1 ill subject and 1 control, so they were excluded from the analysis of cardiovagal function. The R-R intervals between normal QRS complexes in 5-minute epochs every 15 minutes were analyzed in the frequency domain using the fast Fourier transform algorithm to produce the standard measures of high-frequency (0.15 to <0.40 Hz), low-frequency (0.04 to <0.14 Hz), and very low-frequency (0.003 to <0.04 Hz) spectral power, expressed in ms<sup>2</sup> (25–28). High-frequency spectral power is an index of vagal parasympathetic influence on cardiac rhythm (25–28) and is reproducible over time (29,30). Autonomic influences on cardiac repolarization were measured by heart rate-corrected QT interval (QTc) (31).

While subjects were in the hospital, ambulatory blood pressure and heart rate were measured every 20 minutes during the day and every 60 minutes during sleep for at least 24 hours with an automatic Space Labs model 90207 monitor (32,33).

Abstaining from alcohol for 40 hours and limiting daily caffeine intake equivalent to one cup of coffee before 11:00, each subject spent 4 consecutive nights in a sleep study unit where standard polysomnographic parameters, including pulse oximetry, were continuously recorded (34,35). While time of sleep onset varied, all subjects were awakened at 06:00. Data from the last 3 nights were analyzed. Automated blood pressure and Holter monitoring were not performed simultaneous with the sleep studies.

An experienced cardiologist measured postganglionic sympathetic nerve activity to skeletal muscle from a unipolar tungsten microelectrode (tip measuring 1 to 5 mm) that was inserted percutaneously and selectively into muscle nerve fascicles of the peroneal nerve using a microneurographic technique (36). With a central venous pressure catheter in place, sympathetic nerve activity was measured at baseline and under three levels of lower body negative pressure to test reflex mechanoreceptor and baroreceptor control of sympathetic nerve activity. The subject's lower body was enclosed in an airtight chamber with an opening on the left so that reflex changes in sympathetic nerve activity and blood flow could be measured in the left leg while lower body negative pressure was being applied to the right leg and pelvis to decrease central venous pressure without altering arterial blood pressure. Due to the inability to obtain a sympathetic nerve activity signal initially or to maintain the recording from dislodgment of the probe, development of a full bladder, or inability to tolerate prolonged recumbency, we were able to measure baseline sympathetic nerve activity in 15 ill veterans and 11 controls, and sympathetic nerve activity recordings at all three pressure levels of lower body negative pressure in 8 ill veterans and 7 controls.

We used standard procedures (37) to measure the Valsalva ratio, which tests the interaction of sympathetic and parasympathetic influences and is generally positive in fairly advanced autonomic disturbances (25,38–42). Sudomotor (sympathetic cholinergic) function was assessed by silastic sweat imprint (pure postganglionic) and sympathetic skin response (pre- and postganglionic) (37).

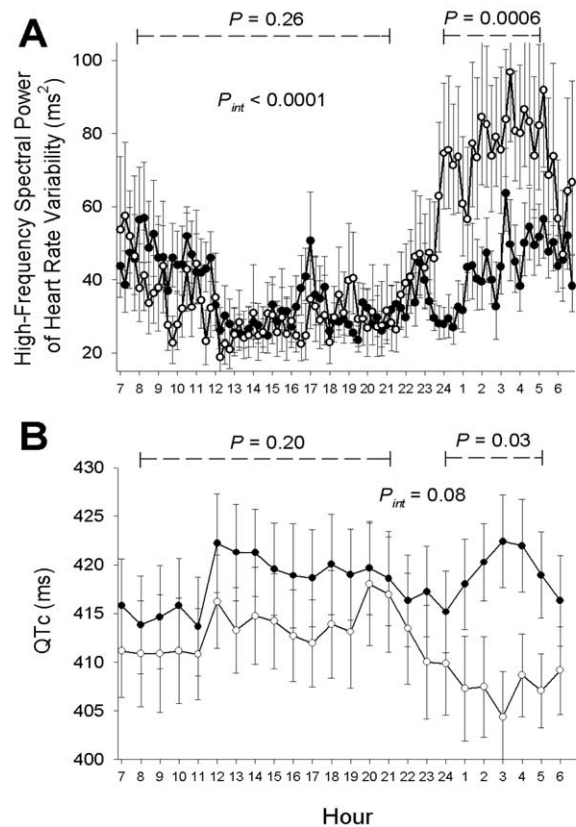
A psychiatrist interviewed all subjects following the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.

### Statistical Analysis

Studies involving repeated measurements over time (e.g., heart rate variability, heart rate, blood pressure), consecutive nights (e.g., sleep studies), or two or four extremities (e.g., sudomotor tests), or at different levels of a stimulus (e.g., microneurography experiments) were analyzed with repeated-measures mixed-effects models in which binary indicators such as clinical groups (case/control), time (day/night), and test conditions or covariates were treated as fixed effects, and subject as a random effect (43). Means ( $\pm$  SE) for outcome measures across epochs in a period or group were derived using the least squares method, adjusted for fixed and random effects in the mixed-effects models. All automated blood pressure measurements were analyzed in the mixed-effects models but were averaged by hour for graphical display. Tests of pre-stated hypotheses of circadian differences on Holter and automated blood pressure measures contrasted night (24:00 to 05:00) with day (08:00 to 21:00); intervals were chosen to avoid confounding by variation in times of sleep onset and waking. Analyses that were not predicted ahead of time contrasted morning (08:00 to 11:30) with the rest of the day (12:00 to 21:00).

To test for confounding, the repeated-measures mixed-effects models for high-frequency heart rate variability and heart rate measured by polysomnography were rerun, adjusting for age, body mass index, smoking, fasting glucose level, creatinine clearance rate, psychiatric diagnosis of alcohol abuse or depression, comorbid condition, medications taken at home, and medications continued in the hospital. The analysis of polysomnographic heart rate was also adjusted for the following simultaneously recorded measures: duration of sleep, sleep efficiency, rapid eye movement (REM) efficiency, percentage of sleep in REM, percentage of sleep in waking or movement, number of awakenings to stage 1 per hour, sleep apnea episodes per hour, and mean oxygen saturation by pulse oximetry (PSaO<sub>2</sub>) and respiratory rate in 5-minute epochs.

Statistical tests were performed with the MIXED, TTEST, NPAR1WAY, and FREQ procedures of SAS (release 8.2; SAS Institute, Cary, North Carolina). *P* values are two-sided. To avoid type I errors from multiple com-



**Figure 1.** Circadian variation in parameters of autonomic regulation of the cardiovascular system, measured by 24-hour Holter monitoring. Shown are high-frequency spectral power of heart rate variability, measured in 5-minute epochs every 15 minutes over 24 hours (A), and corrected QT interval (QTc), measured in 5-minute epochs every hour (B), in 21 ill Gulf War veterans (solid circles) and 19 matched control veterans (open circles). Error bars indicate  $\pm$  1 SEM. *P* values test the difference between ill veterans and controls during the day or night in the repeated-measures mixed-effects model, and the  $P_{int}$  values test day-night by group interaction.

parisons, the threshold for statistical significance was set at  $P \leq 0.01$  in the four primary family-wise tests (44) of autonomic function (parasympathetic control of heart rhythm, sympathetic adrenergic function, Valsalva ratio, and peripheral sudomotor function); at  $P \leq 0.005$  for parameters of sleep studies; and at  $P < 0.05$  for confirmatory tests of blunting of nocturnal heart rate dip from automated blood pressure and polysomnography measurements.

## RESULTS

### Circadian Variation of High-Frequency Heart Rate Variability

High-frequency spectral power of heart rate variability increased normally 2.2-fold during sleep in controls

**Table 1.** Baseline Model of Differences in Mean High-Frequency Spectral Power of Heart Rate Variability, Measured in 5-Minute Epochs Every 15 Minutes Over 24 Hours

Measurement Period	Control Veterans (n = 17)*	Ill Gulf War Veterans (n = 21)*	Difference	P Value
	Mean $\pm$ SE (ms <sup>2</sup> ) <sup>†</sup>			
During the day <sup>‡</sup>	31.1 $\pm$ 2.3	35.0 $\pm$ 2.5	-3.8 $\pm$ 3.2	0.26
At night	69.0 $\pm$ 6.7	41.3 $\pm$ 4.0	27.7 $\pm$ 7.6	0.0006

\* One ill subject and 1 control were excluded because the Holter recording could not be obtained.

<sup>†</sup> The model tests the effects seen in Figure 1A. Test of the day-night by group interaction was significant ( $P < 0.0001$ ).

<sup>‡</sup> In the morning, high-frequency spectral power was significantly lower in controls than in ill veterans (31.6  $\pm$  3.5 ms<sup>2</sup> vs. 48.8  $\pm$  5.5 ms<sup>2</sup>; difference = -17.0  $\pm$  5.5 ms<sup>2</sup>;  $P = 0.007$ ), but for the rest of the day there was no significant difference between the groups (29.3  $\pm$  2.6 ms<sup>2</sup> vs. 29.6  $\pm$  2.6 ms<sup>2</sup>; difference: -0.8  $\pm$  3.5 ms<sup>2</sup>;  $P = 0.8$ ; Figure 1A).

( $P < 0.0001$ ) but 1.2-fold in ill veterans ( $P < 0.09$ ; Figure 1, Table 1), a significant difference in circadian rhythm (day-night by group interaction,  $P < 0.0001$ ). Consequently, high-frequency heart rate variability was lower in ill veterans than controls at night ( $P = 0.0006$ ; Table 1), a difference that remained significant after adjusting for potential confounding variables (Table 2).

Over the entire daytime period, there was no significant difference between ill veterans and controls ( $P = 0.26$ ; Figure 1, Table 1). While high-frequency spectral power among controls fell normally in the early morning hours, that among ill veterans remained significantly higher throughout the morning, although by noon it fell to normal daytime levels, leaving no significant difference between groups for the rest of the day.

### Circadian Variation of QTc Interval

The QTc interval tended to be longer in ill veterans than controls during the full 24-hour period (mean  $\pm$  SEM difference = 9.1  $\pm$  4.9 ms,  $P = 0.07$ ; Figure 1B). This difference, which was not significant during the day (6.6  $\pm$  4.9 ms,  $P = 0.20$ ), increased at night when the QTc interval decreased in controls but increased in ill veterans (11.7  $\pm$  5.2 ms,  $P = 0.03$ ). Five (24%) of 21 ill veterans and 2 (11%) of 19 controls had maximal QTc intervals longer than 450 ms ( $P = 0.4$ ).

### Circadian Variation of Heart Rate

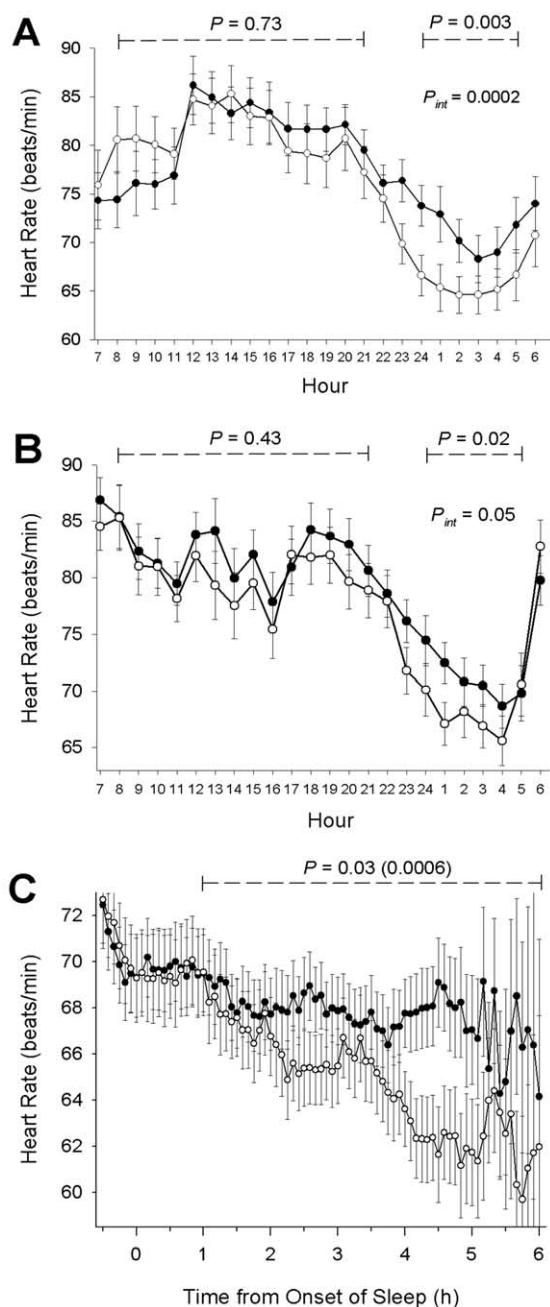
Heart rate declined less at night in ill veterans (mean change, 4.7  $\pm$  1.5 beats per minute) than in controls (13.5  $\pm$  1.6 beats per min,  $P = 0.0002$ ; Figure 2A). During the

**Table 2.** Effects of Adjusting for Potentially Confounding Conditions or Attributes of the Subjects

Potentially Confounding Attribute or Condition Adjusted for in the Model	Control Veterans	Ill Veterans	Difference in High Frequency Heart Rate Variability at Night, Adjusted for Confounders*	P Value*
	Number or Mean $\pm$ SD			
Baseline model <sup>†</sup>	17	21	27.7 $\pm$ 7.6	0.0006
Age (year)	43.3 $\pm$ 6.7	42.3 $\pm$ 8.9	30.1 $\pm$ 7.3	<0.0001
Body mass index (kg/m <sup>2</sup> )	27.2 $\pm$ 4.0	28.3 $\pm$ 3.9	25.8 $\pm$ 7.4	0.0011
Fasting glucose level (mg/dL)	101.4 $\pm$ 13.5	100.5 $\pm$ 13.0	29.0 $\pm$ 7.4	0.0003
Creatinine clearance (mL/min)	119.8 $\pm$ 14.7	127.2 $\pm$ 26.4	26.2 $\pm$ 7.5	0.0010
Smoking	10	13	28.3 $\pm$ 7.7	0.0005
Comorbid condition	4	10	21.2 $\pm$ 6.9	0.0037
Alcohol abuse	2	2	31.9 $\pm$ 10.1	0.0006
Major depressive disorder	2	13	30.2 $\pm$ 8.2	0.0006
Post-traumatic stress disorder	0	3	27.1 $\pm$ 7.6	0.0008
Took medication regularly at home	5	12	22.1 $\pm$ 7.2	0.0040
Continued medication in hospital	2	5	28.9 $\pm$ 8.4	0.0005
Holter monitoring in hospital	9	11	28.9 $\pm$ 8.4	0.0006
All of these characteristics	—	—	28.7 $\pm$ 10.5	0.0008

\* The test statistic (mean of controls minus mean of ill veterans) is one of the four comparisons generated by the group-time interaction.

<sup>†</sup> From Table 1. One ill subject and 1 control were excluded because the Holter recording could not be obtained.



**Figure 2.** Circadian variation in mean heart rate measured in 5-minute epochs every hour by Holter monitoring (A), every 20 minutes during the day and every 60 minutes at night by 24-hour automated blood pressure monitoring (B), and in all 5-minute epochs from 30 minutes before to 6 hours after the onset of sleep by polysomnographic recordings (C) on 3 consecutive nights in a sleep study unit in 21 ill Gulf War veterans (solid circles) and 19 matched control veterans (open circles). Error bars indicate  $\pm 1$  SEM. The  $P$  value in parentheses (C) is from the mixed-effects model that adjusted for respiratory rate, oxygen saturation by pulse oximetry (PSaO<sub>2</sub>), measures of sleep quality, and other covariates. Transient increases in heart rate every 1 to 2 hours in the control group in panel C are compatible with effects of rapid eye movement sleep reported in previous studies of normal subjects (45).

day, heart rate did not differ between the two groups (mean difference =  $0.8 \pm 2.3$  beats per minute,  $P = 0.73$ ), but at night it remained significantly higher in ill veterans (difference =  $8.0 \pm 2.5$  beats per minute,  $P = 0.003$ ).

Blunting of the nocturnal heart rate dip in ill veterans as compared with controls was confirmed by automated blood pressure monitoring ( $P = 0.05$ ; Figure 2B) and polysomnography ( $P = 0.03$ ; Figure 2C). Mean respiratory rate and PSaO<sub>2</sub> from polysomnography did not differ significantly between the groups over the night, and the group difference in heart rate dip remained significant after adjusting for mean respiratory rate, mean PSaO<sub>2</sub> values, measures of sleep quality, and other covariates ( $P = 0.0006$ ; Figure 2C).

### Circadian Variation of Blood Pressure

Automated blood pressure recording showed no significant group differences in the normal nocturnal dip in systolic ( $P = 0.15$ ) and diastolic ( $P = 0.92$ ) blood pressure.

### Test for Mixed Autonomic Dysfunction

Changes in heart rate during and following a standardized Valsalva maneuver tested during mid-day did not differ significantly between ill veterans and controls (mean Valsalva ratio,  $1.44 \pm 0.05$  vs.  $1.44 \pm 0.06$ ).

### Tests of Sympathetic Adrenergic Function

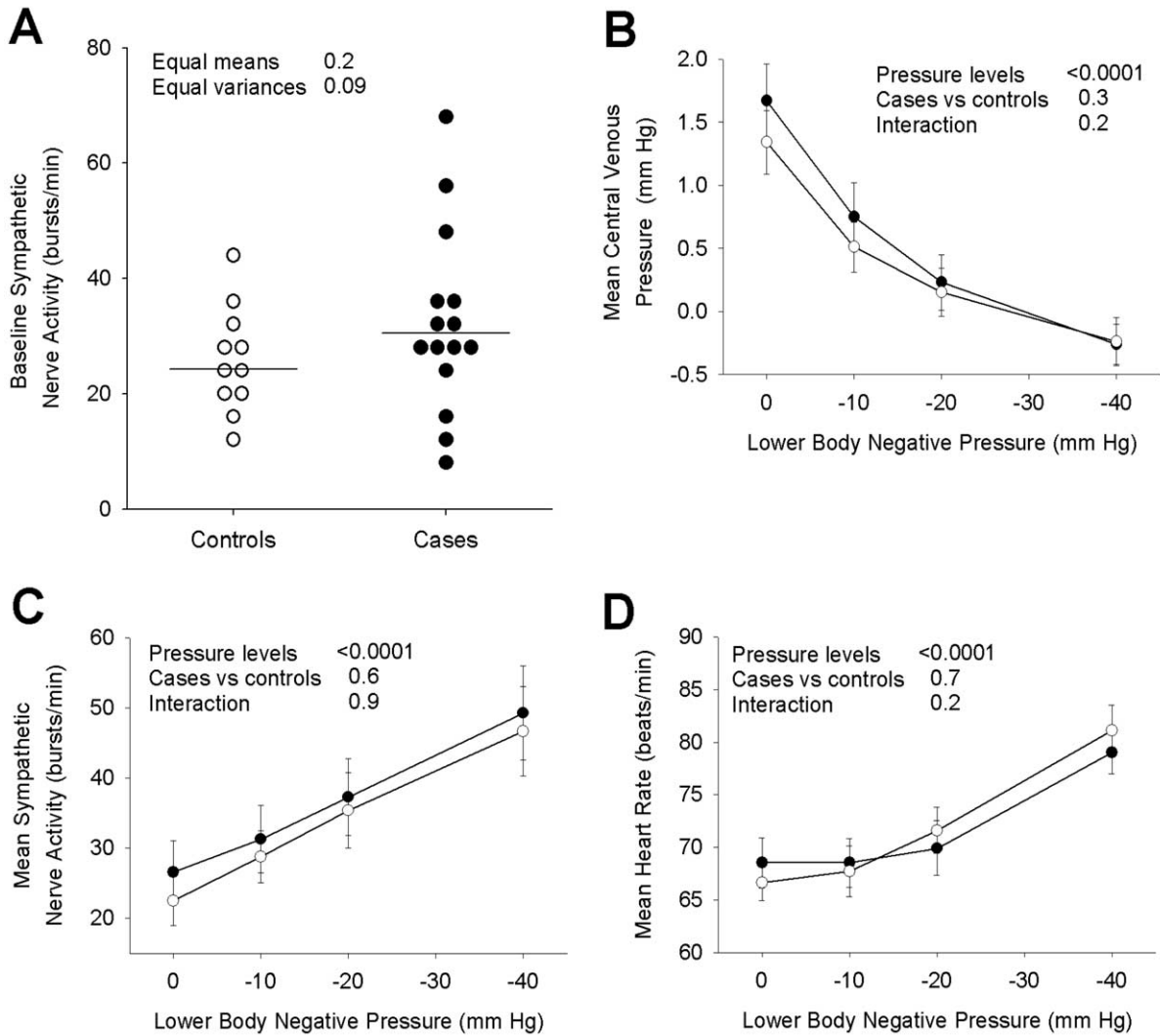
Baseline sympathetic nerve activity to skeletal muscle, measured at rest by microneurography in the early afternoon, tended to be higher and to have greater variance in ill veterans than controls, although these trends were not significant (Figure 3A). Progressive increases in lower body negative pressure, which reduced central venous pressure (Figure 3B), caused normal increases in sympathetic nerve activity (Figure 3C). At lower body negative pressures of  $-10$  and  $-20$  mm Hg, sympathetic nerve activity increased progressively while heart rate remained the same, indicating normal actuation of the low-pressure cardiopulmonary baroreflex affecting sympathetic nerve activity but not arterial blood pressure or pulse pressure (Figures 3C and 3D). At a lower body negative pressure of  $-40$  mm Hg, a further increase in sympathetic nerve activity was accompanied by a significant increase in heart rate, indicating normal actuation of the high-pressure sinoatrial baroreflexes that narrow pulse pressure.

### Tests of Sudomotor Function

The sympathetic skin response was present in both hands and both feet of all ill veterans and control subjects, except in both feet of 1 case subject (Table 3). The mean latency and amplitude of the sympathetic skin response, and the mean number of sweat droplets on the silastic imprint test, were similar in the two groups.

### Sleep Studies

The total sleep period of ill veterans averaged 35 minutes (9%) less than the mean total sleep period of 6.5 hours



**Figure 3.** Results of experiments testing reflexes of autonomic cardiovascular control. Shown are baseline sympathetic nerve activity to skeletal muscle measured by direct recording from the peroneal nerve by microneurography (A), and the effects of lower body negative pressure on central venous pressure (B), sympathetic nerve activity (C), and heart rate (D) in 15 ill Gulf War veterans (solid circles) and 11 matched control veterans (open circles). Error bars indicate  $\pm 1$  SEM. Inset numbers are *P* values.

among controls ( $P = 0.002$ ; Table 4). Although some of the other sleep parameters were outside normal limits (e.g., REM latency and percentage in stage 1 sleep), these parameters did not differ significantly between the groups. The number of central and obstructive sleep apneas per hour and the normal drop in tympanic membrane temperature during sleep also did not differ significantly between the groups.

## DISCUSSION

Our findings document an abnormality of the neuroregulation of high-frequency heart rate variability, most likely indicative of parasympathetic nervous system activity, in

members of a Naval Reserve construction battalion who became chronically ill during or shortly after service in the 1991 Gulf War (4). This abnormality was accompanied by blunting of the normal dip in heart rate during sleep (46), documented by three independent measurement modalities, and by a trend toward widened QTc interval, particularly during sleep. Additional tests, however, demonstrated no group differences in sympathetic nervous system function, sleep architecture, sleep apnea, or circadian variations in blood pressure or tympanic membrane temperature.

Measurement of circadian variation in heart rate variability by 24-hour Holter recording is a widely accepted measure of vagal parasympathetic regulation. Healthy subjects have a large increase in vagal parasympathetic tone at night, manifested by an increase in high-fre-

**Table 3.** Tests of Sudomotor Function

Measure	Ill Gulf War Veterans (n = 22)	Control Veterans (n = 18)	P Value*
	Mean ± SD		
Sympathetic skin response			
Latency (s) <sup>†</sup>			
Right hand	1.83 ± 0.28	1.69 ± 0.32	0.15
Left hand	1.86 ± 0.31	1.76 ± 0.23	0.24
Right foot <sup>‡</sup>	2.36 ± 0.33	2.29 ± 0.25	0.44
Left foot <sup>‡</sup>	2.43 ± 0.46	2.25 ± 0.39	0.19
Amplitude (μV) <sup>†</sup>			
Right hand	3041 ± 3010	3019 ± 2585	0.98
Left hand	2898 ± 3003	3153 ± 2300	0.77
Right foot <sup>‡</sup>	1628 ± 1307	2043 ± 1937	0.45
Left foot <sup>‡</sup>	1271 ± 940	2114 ± 1703	0.07
Sweat imprint test (sweat droplets per cm <sup>2</sup> ) <sup>†</sup>			
Right hand	206 ± 42	195 ± 49	0.49
Right foot	201 ± 47	202 ± 62	0.52

\* From the Student *t* test.

<sup>†</sup> The global hypothesis test for consistent differences across the extremities by a repeated measures mixed-effects model was not statistically significant.

<sup>‡</sup> Excluding 1 control subject who had no sympathetic skin response in both feet.

quency heart rate variability, coinciding with sleep and a precipitous drop to low daytime levels immediately upon waking (47,48). Blunting of this normal nocturnal increase is the earliest sign of impaired autonomic activity in progressive diseases of the peripheral autonomic nervous system, such as diabetic autonomic neuropathy (39,48), and in injuries of the central autonomic network, such as following hemispheric or brainstem strokes (49). Ewing et al's (47) demonstration that loss of the circadian variation in high-frequency heart rate variability was more sensitive than standard reflex tests in detecting early cardiac parasympathetic damage in diabetic patients has been replicated extensively (39-42,48,50-52). Our finding of blunted circadian variation in high-frequency heart rate variability along with normal values for several indexes of sympathetic nervous system function, including the Valsalva ratio (40), sympathetic skin response (53), sudomotor test (54), tests of baroreceptor function by microneurography (37,45,55), and circadian variation of blood pressure, in the same patients with Gulf War syndrome is compatible with an early, subtle abnormality of autonomic (mainly parasympathetic) regulation, similar to the very early stages of diabetic autonomic neuropathy. Our findings do not address whether the abnormality will progress in Gulf War veterans as it often does in diabetic patients.

Several alternative explanations for the findings were explored. First, if ill veterans had required several hours longer to fall asleep (longer sleep latency) than controls, this could have explained the lack of a rise in high-frequency heart rate variability at night. Likewise, frequent

prolonged episodes of hypoxia from sleep apnea could have stimulated sympathetic outflow and inhibited parasympathetic increases (57-59). However, we found that adjusting for PSaO<sub>2</sub> and respiratory rate did not explain the between-group difference in blunting of the normal nocturnal heart rate dip recorded simultaneously during polysomnography, and we found no substantial differences in sleep architecture or measures of sleep apnea during 3 nights of polysomnography in a sleep study unit, although the Holter recordings were not done on the same nights as the sleep unit studies.

Second, clinical depression has been shown to reduce the low frequency and very low-frequency components of heart rate variability, but it does not affect the high-frequency component controlled by vagal parasympathetic function (60-63), the component that we found to be abnormal in ill veterans. Adjusting for psychiatric diagnosis of depression also did not affect the significance of the between-group difference in high-frequency heart rate variability. Likewise, adjusting for post-traumatic stress disorder, an anxiety-related disorder, did not alter the results.

Third, one study has suggested that the dizziness and fatigue associated with Gulf War syndrome, as in chronic fatigue syndrome (64,65), may be associated with neurocardiogenic syncope (66). This type of syncope is characterized by paradoxical withdrawal of sympathetic outflow during orthostatic stress, such as head-up tilt table testing (67) or lower body negative pressure (68,69). Although we did not perform tilt table testing, 24-hour monitoring of blood pressure and heart rate during normal daily ac-

**Table 4.** Sleep Architecture, Sleep Apnea, and Tympanic Membrane Temperature on 3 Consecutive Nights in a Sleep Study Unit

Measure	Ill Gulf War Veterans (n = 22)	Control Veterans (n = 17)*	P Value
	Mean <sup>†</sup> ± SE		
Sleep architecture			
Total sleep period (min)	357.1 ± 7.7	392.9 ± 8.6	0.002
Sleep latency (min)	9.7 ± 1.9	8.8 ± 2.1	0.7
Sleep efficiency (%)	86.6 ± 1.4	85.8 ± 1.5	0.7
Stages of sleep (% of TSP)			
Stage 1	20.2 ± 2.0	22.4 ± 2.2	0.5
Stage 2	47.9 ± 2.0	47.7 ± 2.2	0.9
Stage SW (stages 3 and 4)	1.7 ± 0.6	1.2 ± 0.7	0.5
REM	20.5 ± 1.2	17.3 ± 1.3	0.06
Awake (including movement)	9.4 ± 0.9	9.8 ± 1.0	0.8
REM latency <sup>‡</sup> (min)	50.0 ± 3.5	58.9 ± 3.9	0.10
REM density per REM minute	2.8 ± 0.2	3.0 ± 0.2	0.5
REM efficiency	77.5 ± 2.2	78.1 ± 2.5	0.9
No. of arousals >1 min in TSP	20.7 ± 1.5	26.1 ± 1.7	0.01
Ascents to stage 1 per hour in TSP	4.1 ± 0.5	4.3 ± 0.6	0.8
Sleep apneas (per hour)			
Central apneas	1.8 ± 0.9	2.8 ± 0.9	0.4
Obstructive apneas	8.6 ± 2.1	12.5 ± 2.3	0.21
Total apneas	10.7 ± 2.5	15.2 ± 2.7	0.22
Tympanic membrane temperature (°C)			
Baseline 1 h before sleep	36.1 ± 0.1	36.3 ± 0.1	0.4
Nadir	34.6 ± 0.2	34.7 ± 0.3	0.7
Drop, baseline to nadir	-1.5 ± 0.2	-1.5 ± 0.2	0.9

\* One control subject was omitted from the sleep study because of a scheduling problem.

<sup>†</sup> Adjusting for linear effects of acclimation (changes over the 3 nights) and percentage body fat measured by submersion. Each subject slept in the sleep study unit for 4 consecutive nights, but data from the first night were not analyzed.

<sup>‡</sup> Includes awake time.

REM = rapid eye movement; SW = slow wave; TSP = total sleep period.

tivity as well as direct measurement of muscle sympathetic nerve activity during simulated orthostatic stress with lower body negative pressure revealed no episodes of neurocardiogenic syncope.

Fourth, it is always possible that subtle pre-existing differences between groups or characteristics of the study protocol might have introduced differences in the results. By adjusting for such measures, we ruled out confounding by age, body mass index, smoking, fasting blood glucose level, renal function, alcohol consumption, medications taken at home, medications continued during hospitalization for the study, and whether the Holter monitors were worn in the hospital or at home.

Our findings, although unable to attribute specific symptoms of Gulf War veterans directly to early autonomic dysfunction, suggest possible links to be explored in future research. Since autonomic discharge normally controls gastrointestinal motility, gall bladder emptying, and sexual function, diseases with abnormal autonomic function may involve chronic diarrhea, cholecystitis, and sexual dysfunction

(70–75). Our finding of subtle autonomic dysregulation provides a plausible explanation for some of the most troublesome complaints among Gulf War veterans, including chronic pathogen-free diarrhea alternating with constipation (4), the reported excessive and increasing rates of cholecystitis and cholecystectomies (7), and sexual complaints (4). Failure of nocturnally increased parasympathetic activity to return to the usual low baseline levels during the morning hours may explain the complaints of chronic morning fatigue (“unrefreshing sleep”).

Our findings indicating normal sympathetic nervous system function suggest that the complaints of dizziness and attacks of vertigo are not related to postural hypotension. This is compatible with our prior findings favoring vertigo attacks with a central vestibular origin (5,8), possibly related to neuronal damage in the brainstem or basal ganglia (19–21).

Reduced heart rate variability and prolonged QT interval are strong risk factors for increased mortality in the context of diabetes and symptomatic coronary artery disease, and in

the general population without known coronary artery disease (39,76), but additional studies will be required to determine whether they increase mortality in Gulf War veterans. The mean difference in QTc between the ill Gulf War veterans and controls, although statistically significant, appeared too small to indicate a clear mortality risk.

## ACKNOWLEDGMENT

The authors thank Laura Herbelin and Darwynn Cole for assistance with data collection, Patricia Thompson for logistical support, and Dr. Paul Solomon for helpful suggestions in the design of the study.

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