ABSTRACT Chronic fatigue/physical exhaustion (FPE) impacts combat readiness but is difficult to identify. We tested the hypothesis that resting heart rate variability (HRV), including both time- and frequency-domain assessments, would correlate with hydration status and aerobic capacity in military recruit-age men and women with varying fitness levels. Cardiac interbeat intervals were recorded using a heart R-R monitor during 20 minutes of quiet, supine rest with paced breathing (0.25 Hz). HRV metrics included average R-R interval (RRI avg), R-R interval standard deviation (RRISD), the percentage of adjacent R-R intervals varying by ≥50 ms (pNN50), and integrated areas of R-R interval spectral power at the high (0.15–0.4 Hz) (RRIHF) and low (0.04–0.15 Hz) (RRILF) frequencies. Treadmill maximal oxygen uptake (VO$_2$ max), segmental bioimpedance estimates of total body water (TBW), and urine specific gravity (USG) were also assessed. All dependent variables of interest were within expected ranges, although absolute ranges of individual values were considerable. RRI correlated with VO$_2$ max ($r = 0.49$; $p < 0.001$), with TBW ($r = 0.38$; $p < 0.001$), and inversely with USG ($r = -0.23$; $p = 0.02$). RRISD correlated with VO$_2$ max ($r = 0.21$; $p = 0.03$), but not with TBW or USG. pNN50 correlated inversely with USG ($r = -0.21$; $p = 0.03$) but not with VO$_2$ max or TBW. R-R interval spectral power at the high and low frequencies did not correlate with VO$_2$ max, TBW, or USG. We have demonstrated that fitness level and hydration status may affect cardiac function via changes in autonomic tone, highlighting the potential of field-based assessment of heart rate variability metrics to identify FPE and other aspects of combat readiness.

INTRODUCTION Intensive military training and missions accompanied by inconsistent and inadequate nutrition and sleep deprivation can last several weeks. Such training is conducive to the onset of chronic fatigue/physical exhaustion (FPE) and neuroendocrine changes reflecting diminished stress adaptability.1–6 Chronic FPE in a warfighter could compromise training and mission effectiveness and therefore must be readily identifiable.

It is not possible to continuously monitor neuroendocrine changes in the field. Therefore, development of a simple, non-invasive technique to assist in the identification of warfighters with FPE is required. Current work to develop a warfighter physiological status monitoring (WPSM) system includes assessment of hydration status, thermal load, cognitive function, and ballistic impact detection. Simple sensors to detect respiratory rate, body temperature, and heart rate will likely be incorporated into some future algorithm to provide insight into combat readiness,7–8 but exactly how these sensors should be incorporated has not been worked out in detail. Furthermore, it is well known that heart rate and respiratory rate provide little insight into injury severity or combat readiness.9,10

The autonomic nervous system (ANS) actively compensates for injury or fatigue by modulating the balance between parasympathetic and sympathetic cardiovascular control mechanisms. Understanding such ANS compensations can provide earlier and perhaps more sensitive feedback on physiological status. Although ANS function cannot be measured directly for field applications, it can be estimated through analysis of heart rate variability (HRV) and applied clinically.11,12 For example, frequency-domain analysis of HRV via spectral analysis has been used to classify trauma severity in intensive care unit patients.13,14 In the prehospital arena, frequency-domain analysis of HRV separated patients who would eventually die from those who would survive traumatic injury.15,16 However, it is not possible at this time to run real-time spectral analyses for practical use in the field.

A relatively new method for assessing ANS control of heart rate—heart rate volatility—has been developed recently as a practical, field-based monitoring strategy. The heart rate volatility measure is based on the standard deviation in integer 5-s heart rate recordings computed over 5-min intervals (HR$_{SD5}$). This method is highly correlated with actual HRV determination via multiple spectra and time-domain measures known to reflect ANS function.17 HR$_{SD5}$ has numerous advantages over traditional spectral waveform analyses. For example, the HR$_{SD5}$ integer data require significantly less computation and can be roughly computed in real time, while spectral analysis typically requires extensive offline computation. Together, these findings demonstrated that measurement of HRV characteristics is suitable for continuous real-time field assessment. Small, highly portable, and relatively inexpensive heart rate monitoring devices (e.g., two-lead ECG) such as those used by athletes during training and competition can be employed to collect the heart rate data for subsequent download to a computer via telemetry. Radespiel-Tröger et al.18 validated HRV field data from a relatively inexpensive
exercise heart rate monitor against clinically established reference HRV data.

Interbeat interval detectors will likely be incorporated into some future WPSM system, but exactly how these data should be interpreted remains unknown. In the current exploratory study, we tested the hypothesis that various applications of HRV, including simple cardiac interbeat interval data as well as both time- and frequency-domain assessments, would correlate with hydration status and aerobic capacity. We have chosen these latter physiological indicators because they represent a plasma volume that plays an important role in preventing or delaying the onset of FPE through plasma volume-related effects on ANS regulation of blood pressure.\textsuperscript{19,20}

MATERIALS AND METHODS

Subjects
Thirty-five male and 69 female (\(N = 104\)) nonsmoking and of military recruit-age subjects volunteered to participate in this study. Our subjects’ average characteristics (mean \(\pm\) SD) were 23.1 \(\pm\) 4.3 years for average age, their average height was 170.8 \(\pm\) 10.7 cm, and their average body weight was 67.2 \(\pm\) 11.5 kg. The average subject aerobic fitness, as determined by an incremental treadmill test, was 51.3 \(\pm\) 8.7 mL O\textsubscript{2} · kg body weight (BW)\textsuperscript{-1} · min\textsuperscript{-1} for males and 41.9 \(\pm\) 8.3 mL O\textsubscript{2} · kg BW\textsuperscript{-1} · min\textsuperscript{-1} for females (mean \(\pm\) SD). Before inclusion all subjects completed a medical evaluation (Department of Defense form 2807-1) to ensure they had no previous or current medical conditions that might preclude their participation. All subjects maintained their normal sleep patterns, refrained from exercise, and abstained from caffeine and other ANS stimulants including prescription or nonprescription drugs at least 24 hours before the study. Subjects received a verbal briefing and written descriptions of all procedures and risks associated with the study and were familiar with the laboratory, the protocol, and procedures. Subjects were encouraged to ask questions of the investigators, and then they signed an informed consent form that had been approved by the Institutional Review Board for the protection of human subjects in research from the University of Texas, San Antonio, Texas.

Experimental Protocol
Subjects reported to the laboratory in the morning of testing following an overnight fast. Subjects provided a sample of urine for immediate assessment of urine specific gravity (USG). Total body water (TBW) was assessed from segmental bioimpedance (Hydra 4200; Xitron Technologies, San Diego, California) and then subjects were instrumented with a commercial heart rate monitor (model RS810; Polar Electro, Kempele, Finland). Subjects were positioned supine, and then they breathed in time with a metronome set at a pace of 15 breaths per minute (0.25 Hz) for 20 minutes. We did not record respiratory rate, but subjects were monitored strictly during controlled breathing to ensure they did not deviate from the specified rate. Cooke et al.\textsuperscript{21} have shown previously that HRV tracks respiratory frequencies during metronomic breathing at various frequencies. A representative spectral analysis from one subject showing tight entrainment of HRV with the respiratory frequency of 0.25 Hz is shown in Figure 1.

Following the heart rate collection but while subjects remained in the supine position, a blood sample was obtained by standard venipuncture procedures. Plasma samples were recovered from whole blood using ethylenediaminetetraacetic acid (24 mg · mL\textsuperscript{-1}, pH 7.4) following centrifugation (15 min at 1,000 \(\times\) g) and stored at \(-80^\circ\text{C}\) for subsequent analysis. Plasma norepinephrine (NE) was measured using serial aliquots by enzyme immunoassay (Rocky Mountain Diagnostics, Colorado Springs, Colorado). Following heart rate and blood collection procedures, subjects underwent an incremental treadmill test to voluntary exhaustion. The volume of oxygen consumed was measured on a breath-by-breath basis with a metabolic cart (model 2400; ParvoMedics, Sandy, Utah). The highest VO\textsubscript{2} (mL O\textsubscript{2} · kg BW\textsuperscript{-1} · min\textsuperscript{-1}) obtained during the terminal treadmill stage was taken as that subject’s maximal oxygen uptake (VO\textsubscript{2} max).

Data Analysis
Individual heart beats were detected by the heart rate monitor and ECG and downloaded to a computer for off-line analysis. All analyses were performed with commercially available data analysis software (WinCPRS; Absolute Aliens, Turku, Finland). Using the 20 min of supine heart rate data, we were able to analyze the R-R interval as well as time- and frequency-domain components of HRV.

We calculated standard time-domain statistics such as average R-R intervals (RR\textsubscript{avg}), R-R interval standard deviations (RR\textsubscript{ISD}), and the percentage of adjacent interbeat intervals that varied by 50 ms or more (pNN50). Such time-domain statistics reflect both long- and short-term HRV mediated by both ANS and non-ANS sources. For representation

![FIGURE 1. R-R interval spectral power derived from a Fourier transform is shown for one subject during 20 min of supine controlled breathing at a frequency of 0.25 Hz.](image-url)
of HRV in the frequency domain, \( RRI_{\text{avg}} \) were replotted using linear interpolation and resampled at 5 Hz. Data then were passed through a low-pass impulse response filter with a cut-off frequency of 0.5 Hz. Data sets were submitted to a Fourier transform with a Hanning window. The magnitude of \( RRI_{\text{avg}} \) oscillations was quantified by calculating the power spectral density for the signal (total 0.04–0.4 Hz). Signal areas were separated into high-frequency (RRIHF) (0.15–0.4 Hz) and low-frequency (RRILF) (0.04–0.15 Hz) bands.\(^{12}\) Oscillations at the RRIHF represent modulation of HRV by parasympathetic efferent traffic, whereas oscillations at the RRILF represent modulation by a combination of factors that include influences of both parasympathetic and sympathetic efferent traffic. Table I summarizes the various HRV metrics we applied.

### Statistical Analysis

Pearson correlation coefficients were calculated among variables of interest. The probabilities associated with the correlation coefficients are presented as exact \( p \)-values.\(^{22}\) In an attempt to separate correlations of interest, we considered associations between variables to be weak if \( p \)-values were \( \geq 0.05 \) and strong if \( p \)-values were \( \leq 0.05 \). All data are expressed as means ± SEs unless specified otherwise.

### RESULTS

Descriptive statistics are shown for time- and frequency-domain HRV metrics, aerobic capacity, and estimates of hydration status in Table II.

Plasma NE was measured in 98 of the 104 subjects and was significantly different (\( p < 0.001 \)) between males (\( N = 31; 406.7 \pm 42.9 \text{ pg} \cdot \text{mL}^{-1} \)), luteal phase females (\( N = 31; 628.6 \pm 35.4 \text{ pg} \cdot \text{mL}^{-1} \)), and follicular phase females (\( N = 36; 214.6 \pm 25.3 \text{ pg} \cdot \text{mL}^{-1} \)). However, time- and frequency-domain variables and correlations with NE, \( VO_{2 \text{max}} \), TBW, and USG did not differ significantly between males, females in the luteal menstrual phase, and females in the follicular menstrual phase (data not shown). Therefore, we have reported pooled data from all subjects. Of our time-domain variables, \( RRI_{\text{avg}} \) correlated directly with \( VO_{2 \text{max}} \) (Fig. 2), as did RRISD (\( r = 0.21; \ p = 0.03 \)), while no correlation with \( VO_{2 \text{max}} \) was detected for pNN50. RRISD did not correlate with TBW or USG. No correlations were detected between pNN50 and TBW, but pNN50 correlated inversely with USG (\( r = -0.21; \ p = 0.03 \)).

### TABLE I. Heart Rate Variability Metrics Calculated in Both Time and Frequency Domains

<table>
<thead>
<tr>
<th>Metric (units)</th>
<th>Definition</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( RRI_{\text{avg}} ) (ms)</td>
<td>Average absolute time between each R-wave during 20-min supine test period</td>
<td>Associated with changes in vagal-cardiac nerve activity</td>
</tr>
<tr>
<td>RRISD (ms)</td>
<td>Standard deviation of consecutive RRI</td>
<td>A combination of ANS factors determining long-term heart rate variability</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>Percentage of adjacent RRI that varied by 50 ms or more</td>
<td>Associated with changes in vagal-cardiac nerve activity</td>
</tr>
<tr>
<td>RRIHF (ms(^2))</td>
<td>Power spectral density (integrative area under 0.15–0.04 Hz) of the high-frequency spectrum as derived from a Fourier transform</td>
<td>Changes are associated with changes in vagal-cardiac modulation</td>
</tr>
<tr>
<td>RRILF (ms(^2))</td>
<td>Power spectral density (integrative area under 0.04–0.15 Hz) of the low-frequency spectrum as derived from a Fourier transform</td>
<td>Changes are associated with changes in both vagal- and sympathetic-cardiac modulation</td>
</tr>
</tbody>
</table>

### TABLE II. Time- and Frequency-Domain Analyses of Heart Rate Variability Are Shown in Conjunction with Aerobic Capacity and Hydration Status

<table>
<thead>
<tr>
<th>Metric (units)</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( RRI_{\text{avg}} ) (ms)</td>
<td>1.040 ± 0.17</td>
<td>601–1,587</td>
</tr>
<tr>
<td>RRISD (ms)</td>
<td>104 ± 6</td>
<td>24–547</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>45 ± 2</td>
<td>0.3–85</td>
</tr>
<tr>
<td>RRIHF (ms(^2))</td>
<td>4,146 ± 1,124</td>
<td>41–114,683</td>
</tr>
<tr>
<td>RRILF (ms(^2))</td>
<td>4,870 ± 2,203</td>
<td>89–233,970</td>
</tr>
<tr>
<td>( VO_{2 \text{max}} ) (mL O(_2) · kg BW(^{-1}) · min(^{-1}))</td>
<td>46 ± 1</td>
<td>25–75</td>
</tr>
<tr>
<td>TBW (L)</td>
<td>35 ± 1</td>
<td>23–60</td>
</tr>
<tr>
<td>USG</td>
<td>1.02 ± 0.0</td>
<td>1.0–1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (\( N = 104 \)). \( RRI_{\text{avg}} \), average RRI; RRISD, R-R interval standard deviation; pNN50, the percentage of adjacent RRI that varies by at least 50 ms; RRIHF and RRILF, integrated areas of \( RRI_{\text{avg}} \) spectral power at the high (0.15–0.4 Hz) and low (0.04–0.15 Hz) frequencies; \( VO_{2 \text{max}} \), maximal aerobic capacity assessed from the highest \( VO_2 \) value recorded during an incremental treadmill test; TBW, total body water; USG, urine specific gravity.

### FIGURE 2.

Maximal oxygen consumption (\( VO_{2 \text{max}} \)) during incremental treadmill exercise is plotted as a function of resting, supine R-R interval average (\( RRI_{\text{avg}} \); \( N = 104 \)).

\( r = 0.49 \), \( p < 0.0001 \)
Heart Rate Variability to Assess Combat Readiness

The ANS is fundamentally responsible for defending arterial pressure through modulation of heart rate and peripheral vascular resistance. Reductions of central blood volume, as occurs with dehydration associated with FPE, trigger vagal withdrawal and sympathetic activation demonstrated by lower HRV. Conversely, aerobic capacity is related directly to increased central blood volume.\textsuperscript{19} Individuals with high aerobic capacities tend to have greater plasma volume and ventricular compliance, allowing the hypothalamic/medullary cardiovascular control centers to maintain resting cardiac output with a greater stroke volume but a lower heart rate (i.e., increased R-R interval) and greater vagal activity at rest.\textsuperscript{19,25} Our results are consistent with these predictions. RRI\textsubscript{avg} correlated directly with VO\textsubscript{2} max and TBW, and inversely (but weakly) with USG (Figs. 2 and 3). Because changes of RRI are related directly to changes of vagal-cardiac control,\textsuperscript{26,27} our data suggest that high aerobic capacities and TBW are associated with high relative parasympathetic tone at rest and can be identified with simple interbeat interval analysis as opposed to HRV metrics. It is important to note that our subjects were well rested, fed, hydrated, and by no means fatigued. However, individual variation observed in the nonfatigued, resting state highlights the need to establish baseline relationships between physiological parameters before deployment of a field-based HRV monitor.

In this study, frequency-domain analyses failed to provide insight into associations among hydration status, aerobic capacity, and ANS control. This is likely due to statistical limitations related to extreme frequency-domain variability in our subjects (see Table II). Nevertheless, Cooke et al.\textsuperscript{28} have shown recently that standard time-domain measures of HRV (which reflect overall ANS-cardiac influences) track reductions of blood volume as induced with lower body negative pressure as well as Fourier-derived variables. Grogon et al.\textsuperscript{29} have also concluded that simple time-domain statistics are useful for monitoring patient status, and therefore it is reasonable to propose the use of interbeat interval statistics for fast, convenient assessment of ANS function in the field without the necessity of including complicated Fourier-based algorithms in future monitoring devices.

In conclusion, results of this study highlight the impact of individual differences in aerobic fitness and hydration status on resting HRV. Identification of altered ANS activity using field-based HRV will likely prove effective in preventing and diagnosing FPE during accelerated training or missions involving sleep deprivation, inadequate food and fluid intake, and physical or mental challenges. Considering such individual differences would be imperative when developing a field-based HRV monitor of combat readiness. It is important to note that correlations derived in this study resulted from using a relatively homogenous group with respect to age, fitness level, and hydration status. The application of the relationship between HRV and individual characteristics while controlling for the confounding effects of acute and chronic physical activity, dehydration, stress, and fatigue warrants further investigation.

DISCUSSION

The results of this study are encouraging with respect to development of a novel, field-based tool for real-time monitoring of human ANS function during any number of perturbations, be it exercise, acute stress, or FPE. Our subjects demonstrated above average age group predictions for VO\textsubscript{2} max\textsuperscript{23} while subjects’ TBW (≥60% and ≥50% body weight for males and females, respectively) and mean USG reflected adequate hydration (≤1.020).\textsuperscript{24} Despite the normality of these parameters, the absolute ranges of individual values were considerable. This individual variation allowed us to demonstrate correlations between HRV and TBW and VO\textsubscript{2} max. These results demonstrate how fitness level and hydration status may affect cardiac function via changes in vagal activity.

RRI\textsubscript{avg} correlated directly with TBW and inversely with USG. These data are shown in Figure 3.

RRI spectral power at the high and low frequencies correlated poorly with VO\textsubscript{2} max, TBW, and USG (all p > 0.05). No significant correlations were observed for plasma NE data and time- or frequency-domain heart rate variables.

FIGURE 3. Total body water (TBW) and urine specific gravity (USG) are plotted as functions of supine, resting R-R interval average (RRI\textsubscript{avg}); N = 104.


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study. However, the benefits of an inexpensive and noninvasive monitor of ANS function in military, athletic, and civilian (first responders, airline pilots, etc.) applications are lucid.

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