TIME SERIES ANALYSIS OF CARDIOMETABOLIC PARAMETERS: RELIABILITY AND ENERGY DRINK RESPONSE

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ABSTRACT

Cardiometabolic data is currently analyzed primarily by the use of averages. While this method can provide some data, further analysis by time series (variability) methods can provide more physiologic insights. Historically, time series analysis has been performed primarily using heart rate data in the form of heart rate variability (HRV) analysis. This was done to determine the status of the autonomic nervous system via changes in parasympathetic and sympathetic output. Researchers have used different methods of analysis, but a lack of reproducibility studies raises questions about the validity of these methods when applied to heart rate (HR) data. Currently in the literature, these methods have not applied to metabolic data such as the respiratory exchange ratio (RER). This dissertation will investigate the reliability of time series assessments of cardiometabolic parameters. We hypothesize that in healthy individuals, HRV analysis performed on the same RR intervals but by two different measurement systems, are indeed interchangeable. We further hypothesize that the time series analysis of metabolic data such as the RER will be stable and repeatable over two trials conducted under the same conditions. Lastly, we hypothesize that under conditions of physical stress (e.g. ride time-to-exhaustion) and biochemical stress (e.g. energy drink), resting HR and HR variability preexercise will be altered and the ride time-to-exhaustion will be increased after subjects consume an energy drink (standardized to 2.0mg/kg caffeine) compared to a taste-matched placebo. The results of this dissertation will provide further insight into the repeatability of these time series analyses, which could be utilized for future research to determine metabolic flexibility.
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CHAPTER 1. INTRODUCTION
Many ways currently exist to observe and quantify various physiologic processes related to cardiometabolic data, with the use of averages being the most common method. While averages do provide some baseline data, many physiologic processes are nonlinear with inherent variability. This variability provides information (Pincus, 2000). For example, resting heart rate (HR) can be obtained with ease and provides useful information on the current state of the cardiovascular system. Heart rate can be broken down further into each successive RR intervals with a time series (e.g. variability) analysis to determine the status of the autonomic nervous system, specifically the balance of parasympathetic to sympathetic tone (Task Force, 1996). Heart rate variability (HRV) analysis has been used extensively to assess autonomic control of the heart under various physiologic conditions (Aubert, Seps, & Beckers, 2003; Bornas, Llabres, Noguera, & Pez, 2006; Kaufman, Kaiser, Steinberger, Kelly, & Dengel, 2007; Niewiadomski, Gasiorowska, Krauss, Mroz, & Cybulski, 2007; Platisa & Gal, 2006; Sesay, Tauzin-Fin, Gosse, Ballanger, & Maurette, 2008). It has been demonstrated that a loss of intrinsic variability is a risk factor for the development of cardiovascular disease, obesity, and type 2 diabetes (Kaufman et al., 2007; Martini et al., 2001; Nagai, Matsumoto, Kita, & Moritani, 2003; Rabbia et al., 2003; Riva et al., 2001; Rohner-Jeanrenaud, 1999).

Currently, the best methods to analyze cardiometabolic data are unclear. One category that has emerged as a potential solution is time series analysis. This dissertation will concentrate on the issues of repeatability, stability, and application of time series analyses to cardiometabolic data. Specifically, we will address the hypotheses that: 1) in healthy individuals, HRV analysis performed on the same RR intervals, but by two
different measurement systems, are interchangeable; 2) time series analysis of metabolic data such as the respiratory exchange ratio will be stable and repeatable over two trials conducted under the same conditions; 3) under conditions of physical stress (e.g. ride time-to-exhaustion) and biochemical stress (e.g. energy drink), resting HR and HR variability preexercise will be altered. In addition, the ride time-to-exhaustion will be increased after subjects consume the energy drink (standardized to 2.0 mg per kilogram of body mass of caffeine) compared to a taste-matched placebo.

The second chapter of this dissertation examines the current literature concerning the repeatability and stability of common time series analyses. This review offers detailed background information on time series analyses, methods to determine repeatability, stability of these methods, and the relationship between linear and nonlinear methods as it pertains to cardiometabolic data.

Chapter three of this dissertation is a manuscript examining a study designed to compare HRV spectral analysis by two different programs in twenty-two healthy subjects. Specifically, we investigated the same RR interval data analyzed by two different HRV analysis programs: 1) SphygmoCor® 2000 (version 7.0; Atcor, Sydney, Australia) and 2) Kubios Heart Rate Variability (version 2.0 beta 3; Biosignal Analysis and Medical Imaging Group, Kuopio, Finland).
The fourth chapter is a manuscript examining the repeatability of time series analysis of metabolic data in healthy subjects. We used a controlled, repeated measures study design to investigate the effects of two intensities of cycle ergometry after an overnight fast on two different occasions. To date, no published study has examined the repeatability of time series analysis by sample entropy of respiratory exchange ratio (RER) data. We hypothesize that the same time series analysis used in HRV calculations can be applied to fuel usage (RER). Thus, this pilot study provides novel information regarding the potential application of a new time series analysis to metabolic data in a noninvasive way.

Chapter five of this dissertation is a manuscript examining the cardiometabolic effects of a commercially available energy drink. We utilized a randomized, double-blind, placebo controlled, crossover design. Subjects were randomized for preexercise intake with an energy drink or a taste-matched noncaffeinated placebo, and received the opposite treatment a minimum of 7 days later. The energy drink (regular version) was standardized at 2.0 mg caffeine per kg of body mass. Preexercise resting HR and HRV were determined by Kubios software via time series analysis. Peak HR, rating of perceived exertion, and expired gases were collected during exercise and ride time-to-exhaustion (TTE) was recorded at the completion of each test. All data was compared between the supplement and placebo conditions. This investigation provides novel information on time series analysis of resting HR and RER associated with performance and cardiovascular safety after consumption of an energy drink. No other study has examined this relationship in this population before.
The sixth chapter provides a brief discussion and conclusions on the data presented in the previous chapters. It also provides the opportunity to examine potential areas where information on the topics presented needs to be elaborated with further research.
References


CHAPTER 2. REVIEW OF LITERATURE
Introduction

Since metabolic and cardiovascular physiology is not a linear process, new methods of analysis beyond linear statistical methods must be employed (Galgani, Heilbronn, Azuma, Kelley, Albu, Pi-Sunyer, Smith, Ravussin, & Look AHEAD Adipose Research Group, 2008a; Kuusela, Jartti, Tahvanainen, & Kaila, 2002; Lewis & Short, 2007; S. M. Pincus, 2000a; Platisa & Gal, 2006b; Porta et al., 2007; Richman & Moorman, 2000; Seely & Macklem, 2004). A reduced variability in physiological systems is indicative of abnormal physiology and disease conditions (Kaplan et al., 1991). Variability in physiological data is more than error around a mean, but contains valuable information that is beyond the scope of traditional parametric statistics (mean, standard deviation). In order to more fully understand the information that is contained in variability, nonlinear analysis of time series data must be performed (West, 2006).

While research exists on cardiometabolic data where standard statistics are performed, much less exists that has been analyzed via time series methods (Henriques et al., 2013). Most research in this area is on heart rate as heart rate variability (HRV), but these time series methods have not currently been published when applied to metabolic data. Another factor is the sparse data on time series analysis of cardiometabolic effects of both exercise and a chemical based stimulant (energy drink). As with any new methods, they must first be repeatable. Without a repeatable measure, further research is not warranted.
The following review of the literature will establish a need for between-equipment and test-retest repeatability of time series measures with heart rate and metabolic data. Heart rate time series analysis methods will be compared between two common systems using the same raw data. Metabolic time series data in the form of respiratory exchange ratio (RER) values will be compared between two different days under the same conditions to assess repeatability. Finally, alterations in heart rate, fuel use (from RER), perceived exertion, and exercise capacity after the consumption of a supplement (energy drink) will be investigated.

**Variation and Complexity of Human Physiology**

Normal physiologic variation has historically been considered “noise.” Recently, Macfarlane & Wu (2013) found that even when measurements were taken by two identical metabolic systems during the same theoretical “steady-state exercise,” some small biological variation was measurable above the between-system technological variation. Every physiologic system has the property of complexity as information within a signal, which can be quantified via nonlinear techniques (Henriques et al., 2013; Rambihar VS., 2010).

Many physiologic systems exhibit within-subject variation (Bardet et al., 1989; Chen, Chen, Chang, & Cheah, 1996; Goedecke et al., 2000; King, Hambidge, Westcott, Kern, & Marshall, 1994; Klug, Nielsen, & Bisgaard, 2000; Marshall, 1994; McQuilkin, Nierenberg, & Bresnick, 1995; Pereira et al., 2004). For example, heart rate is known to
vary in relation to sympathetic and parasympathetic response governed by central command centers in the brain (Aubert, Seps, & Beckers, 2003), but control is also affected by baroreceptors, chemoreceptors, muscle afferent nerves, local tissue metabolism, and circulating hormones (Marshall, 1994) for beat-to-beat adjustment of hemodynamic parameters. Respiratory exchange ratio values also change on a moment by moment basis which is dependent upon energy metabolism (Brooks, Fahey, & Baldwin, 2005; Wilmore & Costill, 2007). They can also be altered by exercise (Kang, Mangine, Ratamess, Faigenbaum, & Hoffman, 2007). Goedecke and coauthors determined several factors that affect fuel use (as measured by RER) during exercise and rest such as: muscle glycogen, training volume, plasma free fatty acid concentration, and percentage of fat in dietary intake (Goedecke et al., 2000). Toubro et al. (1998) found that variations between subjects RER could be attributed to age, gender, dietary substrate intake, insulin, and plasma free fatty acids. Goedecke et al. (2000) also found a very large interindividual variability in resting RER that ranged from 0.718 up to 0.927 that even persisted during exercise of increasing intensity. This large interindividual variability in RER from 0.83 to 0.95 was also demonstrated by Helge et al. (1999) during low-intensity steady-state exercise. In summary, many factors contribute to within-subject variation in measurements of physiological systems (Bardet et al., 1989; Chen et al., 1996; Goedecke et al., 2000; King et al., 1994; Klug et al., 2000; Marshall, 1994; McQuilkin et al., 1995; Pereira et al., 2004).
Standard Statistical vs. Variability Methods

To tease out the variation due to normal physiology and potential erroneous data can be difficult. Many times this normal variation is considered “noise” and explained away as error. Now as newer and more sophisticated techniques become available, combined with newer mathematic algorithms, it may become possible to more accurately quantify this physiological “noise.” A common noninvasive measure of a dynamic system is HRV analysis which has been used extensively to assess autonomic control of the heart under various physiologic conditions (Aubert et al., 2003; Bornas et al., 2006; Bornas, Llabres, Noguera, & Pez, 2006; Kaufman, Kaiser, Steinberger, Kelly, & Dengel, 2007; Niewiadomski, Gasiorowska, Krauss, Mroz, & Cybulski, 2007; Platisa & Gal, 2006a; Platisa & Gal, 2006b; Sesay, Tauzin-Fin, Gosse, Ballanger, & Maurette, 2008). Currently, there are many methods used to perform time series analyses. They can be divided broadly into linear and nonlinear methods. Within the linear methods, time and frequency domain are the most common.

Linear Analysis

Time domain measures of this variability are easier to calculate, but tend to provide less detailed information than the frequency domain approaches (Billman, 2011). Statistical or geometric based approaches are common time domain methods. Once the data has been obtained, the normal-to-normal (NN) interval (the interval between adjacent normal QRS complexes) is determined via simple descriptive measures such as the mean NN interval, mean heart rate, and the range (longest NN minus the shortest NN) for a given time interval can be calculated (Task Force, 1996)). The most common time
domain measure is the standard deviation of the NN interval (SDNN), which measures the total variability from both periodic and random sources (Billman, 2011).

The two main methods used for spectral analysis in the frequency domain are the fast Fourier transform analysis (FFT) technique (Berger, Akselrod, Gordon, & Cohen, 1986) and autoregressive (AR) modeling (Task Force, 1996). The main difference between the FFT and AR approaches is the way in which the data are viewed (Mendonca, Fernhall, Heffernan, & Pereira, 2009). The FFT analysis assumes that the time series contains only deterministic components, whereas the AR method treats data as a composite of deterministic and stochastic components (Bigger et al., 1992). Most programs will employ Welch’s periodogram method to reduce noise in the estimated power spectra where the HRV sample is divided into overlapping segments, and the spectrum is then obtained by averaging the spectra of all the segments.

Three frequency bands (very-low frequency [VLF 0.0–0.04 Hz], low frequency [LF 0.04–0.15 Hz], and high frequency [HF 0.15–0.4 Hz]) are normally obtained from frequency domain analysis programs. LF power reflects modulation by the vagal and sympathetic systems, while HF power primarily reflects modulation of parasympathetic activity (Akselrod et al., 1985; Koh, Brown, Beightol, Ha, & Eckberg, 1994). The LF/HF can be calculated as a measure of relative sympathovagal balance (Eckberg, 1997; Montano et al., 1994). Studies have shown that time domain values have reasonable homogeneity, but frequency domain measurements (spectral measures) are heterogeneous.
(Berntson et al., 1997; Cipryan & Litschmannova, 2013; Eckberg, 1997; Kuss, Schumann, Kluttig, Greiser, & Haerting, 2008; Nunan, Sandercock, & Brodie, 2010; Piccirillo et al., 2004; Pinna et al., 2007; Sacknoff, Gleim, Stachenfeld, & Coplan, 1994; Sandercock, Bromley, & Brodie, 2005b; Scott et al., 2004), even when paced breathing is performed to reduce the potential confounding effects of respiratory variation on HRV measures (Katona & Jih, 1975). Few studies have compared different analytical systems with the same electrocardiogram (ECG) data.

**Nonlinear Analysis**

Entropy, in the original context of thermodynamics was a measure of system disorder and randomness. Pincus & Kalman (1997) first coined the use of approximate entropy as a way to quantify the dynamic control of a system (such as HR control) and possibly analyze many other “random” sequences. The promise of approximate entropy was that it could classify complex systems with only 100 data values in a diverse setting that include both deterministic chaotic and stochastic processes (S. Pincus & Kalman, 1997). To date, approximate entropy has been used in the analysis of medical data (S. M. Pincus, Gladstone, & Ehrenkranz, 1991), cardiology (Kaplan et al., 1991; Ryan, Goldberger, Pincus, Mietus, & Lipsitz, 1994) and neurohormonal responses (Juhl et al., 2000; S. M. Pincus, 2000b; S. M. Pincus, Veldhuis, & Rogol, 2000; Veldhuis, Johnson, Veldhuis, Straume, & Pincus, 2001; Veldman, Frolich, Pincus, Veldhuis, & Roelfsema, 2000).
In 2000, the concept of sample entropy appeared to reduce the bias in approximate entropy (Lewis & Short, 2007; Yentes et al., 2012) and has been employed in the analysis of time series physiologic data (Henriques et al., 2013). Richman & Moorman (2000) defined sample entropy as “precisely the negative natural logarithm of the conditional probability that two sequences similar for \( m \) points remain similar at the next point, where self-matches are not included in calculating probability.” Therefore, a lower value of sample entropy indicates more self-similarity (and thus less variability). Sample entropy does not use a template-wise approach when estimating conditional probabilities as it is in essence an event-counting statistic (Richman & Moorman, 2000). Historically, sample entropy has been employed to conduct variability analysis of heart rate data. The main advantage is that sample entropy requires few data points and has been successfully used to calculate HRV on very short ECG recordings (10 to 60 seconds), and missing data points were well tolerated (Bornas et al., 2006; Lake, Richman, Griffin, & Moorman, 2002; Porta et al., 2007).

In conclusion, newer mathematical methods beyond standard statistical methods have been developed to analyze the inherent variability and quantify the amount of regularity in data under various conditions (Heffernan, Fahs, Shinsako, Jae, & Fernhall, 2007; Lewis & Short, 2007; S. M. Pincus et al., 1991; Platisa & Gal, 2006a). These newer methods such as sample entropy have potential application throughout dynamic physiologic systems beyond just heart rate data. Sample entropy has been shown to date to be a robust algorithm to perform nonlinear time series analysis (Biltz, Harmon,
Repeatability in Time Series Analysis

In order for a new method to be clinically valuable, it must first be shown to be repeatable. Variability analysis using sample entropy has been successfully used in HRV analysis, but may be used to measure any dynamic nonlinear system such as RER data. The RER is the ratio between the amounts of carbon dioxide (CO₂) produced and oxygen (O₂) consumed (Brooks et al., 2005). It is calculated from comparing exhaled gasses to room air at the mouth via a metabolic cart. The RER at steady state is displayed as a ratio between 0.7 to 1.0 where 0.7 corresponds to 100% fat metabolism, 0.85 corresponds to 50% fat and 50% carbohydrate metabolism and 1.0 corresponds to 100% carbohydrate metabolism (Wilmore & Costill, 2007). RER has been found to be a reproducible variable during exercise under steady state standardized conditions (Brooks et al., 2005). Laplaud and Menier (2003) in a test-retest study of male and female athletes, showed that the instant of RER = 1.00 was highly reproducible. As exercise intensity increases, the RER variability range reliably decreases (Brian J. & Wasserman, 2005; Laplaud & Menier, 2003). RER has good reproducibility and it was hypothesized that the variability analysis of RER time series data should also be reproducible.
Applications

Since RER is a measurement of substrate selection of metabolism, it was hypothesized by Biltz et al. (2007) that breath-by-breath RER variability analysis may provide a means for identifying metabolic inflexibility in obese individuals. Metabolic flexibility is the ability of the body to acutely switch its reliance between fatty acids and glucose (Kelley & Mandarino, 2000; Kelley, 2005; Storlien, Oakes, & Kelley, 2004). A loss of metabolic flexibility (metabolic inflexibility) is theorized to play a role in specific diseases such as type 2 diabetes (Galgani, Heilbronn, Azuma, Kelley, Albu, Pi-Sunyer, Smith, Ravussin, & Look AHEAD Adipose Research Group, 2008b; Goodpaster & Kelley, 2002). Using sample entropy to analyze RER time series data, Biltz et al. (2007) found a trend of lower sample entropy scores in obese adolescent subjects. Despite all the work completed historically with RER, to date the variability of breath-by-breath data has not been analyzed using time series methods.

Alterations in cardiometabolic time series data may be influenced by exercise and a chemical supplement such as an energy drink. After a multivitamin, energy drinks are the most popular dietary supplement in the young adult population (Froiland, Koszewski, Hingst, & Kopecky, 2004; Hoffman et al., 2008). Despite their popularity, sparse data exists regarding their effects upon heart rate, HRV, and fuel usage (Steinke, Lanfear, Dhanapal, & Kalus, 2009; Willoughby, 2009). Willoughby (2009) found heart rate was unaffected one hour after 50 young adults consumed one 250 ml (8 oz) can of sugar-free Red Bull (approximately 80mg of caffeine). Steinke et al. (2009) however demonstrated
that heart rate was reduced 30 minutes after subjects consumed 75 mg of caffeine. Bichler and colleagues studied a combination of caffeine and taurine, two common ingredients in energy drinks, which resulted in a significant decline in heart rate (Bichler, Swenson, & Harris, 2006). To date, only one study on HRV and energy drinks exists. Wiklund et al. (2009) showed a decreased LF/HF ratio and a tendency to increased HF power (increased vagal modulation). The dose used was high as subjects consumed 3 cans of Red Bull, which represents a dose of 3000 mg of taurine and 240 mg of caffeine after an overnight fast.

**Summary**

While significant advances have been made with the use of standard linear methods to analyze cardiometabolic data, comparatively little work has been performed using time series analysis methods. While heart rate time series data has been researched the most via HRV, reproducibility has been questioned (Nunan et al., 2010; Sandercock, Bromley, & Brodie, 2005a). Additionally, little is known about the test-retest performance of time series analysis methods when applied to metabolic data under various conditions (rest, exercise, supplement, etc). Therefore, this dissertation will focus on the repeatability of times series analysis of cardiometabolic data under various conditions.


CHAPTER 3. COMPARISON OF TWO METHODS TO DETERMINE HEART RATE VARIABILITY
Comparison of Two Methods to Determine Heart Rate Variability

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Purpose  Compare heart rate variability (HRV) analysis by two different programs.

Methods  We analyzed RR interval data by SphygmoCor® 2000 or Kubios HRV for spectral data patterns. Low frequency (LF), high frequency (HF), LF/HF ratio, normalized LF, and normalized HF were calculated.

Conclusions  The high amount of agreement by Bland-Altman analysis and significant Pearson’s correlations suggest that frequency analysis results for HRV are comparable with SphygmoCor® 2000 or Kubios HRV programs.

Keywords  cardiac function; heart rate variability; autonomic nervous system; noninvasive measurement
Introduction

Several analytical methods can be employed for heart rate variability (HRV) analysis [9]. In frequency-based analysis of HRV, fast Fourier transformation (FFT) algorithms provide a power spectral density data representation. FFT algorithms may employ different mathematical methods. Therefore, there is concern regarding the comparability of HRV results when different systems are used in different laboratories [7]. Few studies have compared different analytical systems with the same electrocardiogram (ECG) data. The aim of the present study was to compare HRV spectral analysis of the same RR interval data by two different popular HRV analysis programs: 1) SphygmoCor® 2000 (version 7.0; Atcor, Sydney, Australia) and 2) Kubios Heart Rate Variability (version 2.0 beta 3; Biosignal Analysis and Medical Imaging Group, Kuopio, Finland).

Methods

Twenty-two healthy subjects, mean age ± standard deviation (17 men, 29.6 ± 11.2 years; 5 women, 24.2 ± 6.7 years) were recruited from the University of Minnesota campus. All participants gave their written informed consent, and the study protocol was reviewed and approved by the University of Minnesota’s Institutional Review Board. Participants arrived at the General Clinical Research Center following a 12-hour overnight fast and were tested between 7:00 and 10:00 AM. Height and weight were measured using a wall-mounted stadiometer (Ayrton Stadiometer, Model S100, Prior Lake, MN) and digital weight scale (Model 5002, Scale-Tronix Inc., Wheaton, IL),
respectively. Following an initial 15-minute supine relaxation period, participants remained resting for an additional 15 minutes to record resting ECG, with the last 5 minutes used for calculation of all HRV variables. During all resting and testing periods, participants were asked to pace their breathing at 0.25 Hz (approximately 15 breaths per minute) using a computer metronome (Crystal Metronome 1.4.4, MIL software & Matthew Lloyd). The ECG waveforms were digitized continuously using the SphygmoCor® 2000 using an onboard analogue-to-digital converter with a sampling frequency of 1024 Hz.

HRV Analysis

Following the recording of resting ECG, the R peaks and RR intervals were determined by software from the SphygmoCor® 2000. The raw RR intervals were also extracted from the SphygmoCor® system in a text file and then imported for analysis to the Kubios software offline. The same RR interval data were analyzed for low frequency (LF), high frequency (HF), LF/HF ratio, normalized LF, and normalized HF, and compared using both programs. Each 5-minute segment was reviewed manually for ectopic beats or arrhythmias. Segments containing such alterations of normal electrophysiological function were excluded from the analysis.

Three frequency bands (very-low frequency [VLF 0.0–0.04 Hz], LF [0.04–0.15 Hz], and HF [0.15–0.4 Hz]) were obtained from both HRV analysis programs. LF power reflects modulation by the vagal and sympathetic systems, while HF power primarily
reflects modulation of parasympathetic activity [9]. Normalized units of LF and HF components (normalized unit = LF or HF/total power – VLF) were reported. The LF/HF ratio was calculated as a measure of relative sympathovagal balance [9].

All data were tested for normality prior to statistical analysis. Means, standard errors, and coefficients of variance were calculated for all descriptive variables. The Student’s paired two tailed t-test with equal variance was used for comparisons. Significance was considered at p < 0.05. Agreement between the two computerized heart rate variability systems was assessed according to the technique of Bland and Altman [1] and Pearson’s correlation. Statistical analyses were performed using Statistical Analysis Software version 9.1 for Windows (SAS Institute, Inc., Cary, NC) and Prism® Graphpad Software version 5 (Graphpad Software, Inc., San Diego, CA).

**Results**

The mean ± standard error values for LF, HF, the LF/HF ratio, normalized LF, and normalized HF for Kubios and SphygmoCor® programs are shown in Table 1. No significant differences were found in LF, HF, LF/HF ratio, normalized LF, and normalized HF between the two systems. Pearson’s correlation for relationships among frequency parameters showed a significant correlation between the two programs for LF (r=0.59, p=0.0041), HF (r=0.69, p=0.0004), LF/HF ratio (r=0.74, p=0.0001), normalized LF (r=0.71, p=0.0002), and normalized HF (r=0.71, p=0.0002). The highest coefficients of variance were observed for the LF/HF ratio, with 96% for the Kubios HRV program.
and 81\% for the SphygmoCor®. The coefficients of variance were generally similar for the Kubios HRV program and SphygmoCor®, except that LF was more than double (94\%) with the Kubios HRV software than SphygmoCor® (45\%). However the normalized LF coefficients of variance were 44\% and 43\% and for normalized HF were 35\% and 35\%, for Kubios HRV and SphygmoCor®, respectively.

The Bland-Altman plots in figures 1 and 2 illustrate a measure of agreement between two measurements over a range of values. The 95\% confidence intervals (CIs) represent the difference in each parameter (LF, HF, the LF/HF ratio, normalized LF, and normalized HF) from the Kubios – SphygmoCor® calculations. The difference in LF values between Kubios and SphygmoCor® for the values from 0 to 0.25 Hz are shown in Figure 1A. These data did not increase with frequency and were in agreement.

This difference between the two programs for HF values from 0.15 to 0.40 Hz is shown in Figure 1B. These data were in agreement because they were centered around the mean difference of zero, with the exception of two points that fell outside the 95\% CI near the frequencies of 0.27 and 0.33 Hz. Both analysis systems showed agreement around 0.16 Hz, with a mean difference of approximately zero (Figure 1B).

Figure 2A and B shows a tight cluster around the mean difference of zero for the normalized HF and LF values, indicating agreement, with the exception of one data point.
However, the LF/HF ratio (Figure 2C) contained a skewed distribution (determined by the Shapiro–Wilk test) and were log transformed (Figure 2 D).

**Discussion**

The increasing popularity of HRV analysis in clinical and research settings has resulted in the development of a number of different programs for analyzing frequency parameters. This study compared Kubios HRV software program to the SphygmoCor® 2000 HRV software program. There was no significant difference between LF, HF, LF/HF ratio, normalized LF, and normalized HF values calculated by either program. Pearson’s correlation tests and Bland-Altman plots showed significant correlations when comparing values of each program. Coefficients of variation ranged from 35% to 96%, with the largest being observed for the LF/HF ratio. Our comparison did not yield any significant differences in HRV calculations.

For the Kubios HRV software, Welch’s periodogram method was used to reduce noise in the estimated power spectra. The HRV sample was divided into overlapping segments, and the spectrum was then obtained by averaging the spectra of these segments. The sampling rate of the interpolation was the default value of 4 Hz. In Welch’s periodogram method, the window width and window overlap can be adjusted, but in the current study, both were left at the default setting (window width set at 256 seconds with an overlap of 50%, corresponding to 128 seconds). The sampling rate of interpolation was fixed at 1 Hz vs. 4 Hz for the Kubios HRV system. The SphygmoCor®
system uses a Hamming spectral window instead of a modified periodogram. The window size is fixed, and its width related to spectrum size is 256 seconds with zero overlap.

Studies have shown that time domain values have reasonable homogeneity, but frequency domain measurements (spectral measures) are heterogeneous [2,4,5,6,8], even when paced breathing is performed to reduce the potential confounding effects of respiratory variation on HRV measures [3]. Individual differences and methodological inconsistencies are known to alter frequency analysis, as shown by a large range of up to 260,000% in spectral measures [4]. For example, the 1996 Task Force report [9] stated that a normal value for HF is 975 ± 203 ms$^2$, whereas Sacknoff et al. [6] reported HF values much higher at 5839 ± 1839 ms$^2$. Pinna et al. [5] found frequency domain values of 1047 ± 1909 ms$^2$ and 944 ± 1429 ms$^2$ in two tests taken 1 day apart from each other. On the low end of the spectrum closer to the Task Force values, Scott et al. [8] reported the lowest HF values of 3.4 ± 1.8 ms$^2$, and Cipryan et al. [2] showed similar HF measures ranging from 186 to 211 ms$^2$ across three resting trials. It should be noted that Cipryan et al. [2] did not employ paced breathing, which was used in the other studies.

Nunan et al. [4] reviewed short-term HRV studies and found that approximately 85% of the studies analyzed demonstrated values within 1.5 standard deviation of the mean publication value for one or more spectral HRV measures. The largest variation was again observed for HF, with a coefficient of variation of 118% and a range in values
across studies of 3548 ms² [4]. These data demonstrate that frequency domain measurements can vary, ranging from as low as 82 ± 104 ms² [8] up to 5839 ± 1839 ms² [6], despite the best efforts at reducing the potential confounding effects. Our values for HF at 55.4 ± 19.8 ms² for Kubios HRV and 54.4 ± 19.4 ms² for SphygmoCor® were statistically the same and on the low end for the reported range of spectral measures. This wide range of spectral values underscores the importance of studies on the repeatability of this parameter.

Conclusion

Given the high amount of agreement by Bland-Altman analysis, similar coefficients of variation, and significant Pearson’s correlations, frequency analysis results are comparable between laboratories using either Kubios HRV or SphygmoCor® for LF, HF, LF/HF ratio, normalized LF, and normalized HF values when the same RR intervals are analyzed. Further studies are needed to verify these findings by other HRV analysis programs.

Conflict of interest

None
References


36
Table 1 Comparison (mean ± standard error) of the Kubios HRV and SphygmoCor®.

<table>
<thead>
<tr>
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<th>Kubios</th>
<th>SphygmoCor®</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td><strong>LF (ms²)</strong></td>
<td>0.07 ± 0.01</td>
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<td>0.0041</td>
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<tr>
<td><strong>HF (ms²)</strong></td>
<td>0.21 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.0004</td>
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<tr>
<td><strong>LF/HF ratio</strong></td>
<td>1.14 ± 0.23</td>
<td>1.13 ± 0.20</td>
<td>0.0001</td>
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<tr>
<td><strong>LFnu (%)</strong></td>
<td>44.6 ± 4.22</td>
<td>45.6 ± 4.13</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>HFnu (%)</strong></td>
<td>55.4 ± 4.22</td>
<td>54.4 ± 4.13</td>
<td>0.0002</td>
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</tbody>
</table>

Data are presented as mean ± standard error. *P*-value provided is testing differences between changes in a given measure under the same conditions. *LF* low frequency, *HF* high frequency, *LF/HF ratio* low frequency to high frequency ratio, *LFnu* low frequency normalized units, *HFnu* high frequency normalized units.
Figure Legends

Figure 1 - Bland-Altman plots for inter-software differences between Kubios HRV and SphygmoCor®, and averaged frequencies for peak low frequency (LF) (panel A) and high frequency (HF) (panel B). Mean differences are indicated by solid lines, and 95% limits of agreement are indicated by dashed lines. The 95% confidence intervals for LF were –0.18 to 0.17 and those for HF were –0.11 to 0.11.

Figure 2 - Bland-Altman plots for inter-software differences between Kubios HRV and SphygmoCor®, and averaged frequency for normalized low frequency (LF) (panel A) and normalized high frequency (HF) (panel B), the low frequency/high frequency (LF/HF) ratio (panel C), natural log (Ln) transformed LF/HF ratio (panel D). Mean differences are indicated by solid lines, and 95% limits of agreement are indicated by dashed lines. The 95% confidence intervals for normalized LF were –30.4 to 28.5, those for normalized HF were –28.5 to 30.4, those for the LF/HF ratio were –1.5 to 1.6, and those for the Ln of the LF/HF ratio were –1.6 to 1.5.
Figures

Figure 1

Panel A

Panel B
Figure 2

Panel A

Panel B
CHAPTER 4. REPEATABILITY OF RESPIRATORY EXCHANGE RATIO
TIME SERIES ANALYSIS
Repeatability of Respiratory Exchange Ratio Time Series Analysis

Michael T. Nelson¹, George R. Biltz¹, and Donald R. Dengel¹,²

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Currently, there are few studies on the repeatability of a time series analysis of respiratory exchange ratio (RER) under the same conditions. This repeated measures study compared two trials completed under the same conditions. After an 8-hr fast, 12 (7 male and 5 female) subjects (mean ± SD): 27.3 ± 3.7 years of age, with weights of 71.8 ± 8.4 kg, percent body fat of 16.4 ± 8.1%, and peak oxygen uptakes of 46.0 ± 5.3 ml · kg\(^{-1}\) · min\(^{-1}\) completed a peak aerobic capacity test followed 7 days later by a cycle ergometer test at 30% of ventilatory threshold (VT) and 60% of VT for 15 minutes each. The same test was repeated again 7 days later. Paired t-tests revealed no significant differences between the tests for mean RER or sample entropy (SampEn) score at both intensities. The coefficients of variance were generally similar for the mean and SampEn of the RER. The intraclass correlation coefficients (ICC) values for the mean RER at 30% of VT was 1.00 and at 60% of VT it was 0.92. The ICC for the SampEn RER at 30% of VT was 0.81 and at 60% of VT it was the lowest at 0.25. Bland-Altman plots demonstrated a measure of agreement between both methods. We demonstrated that RER measurements at 30% and 60% of VT are repeatable during steady-state cycle ergometry. Future work should determine if this finding is consistent with a larger sample size and different exercise intensities.

Keywords: respiratory exchange ratio, variability, repeatability, metabolism
INTRODUCTION

Various methods have been used to analyze time series data (variability) and have been successful applied to heart rate (heart rate variability) to assess autonomic control of the heart under various physiologic conditions (15, 19). A loss of intrinsic variability has been shown to be a risk factor for the development of cardiovascular disease, obesity, and type 2 diabetes (12, 14, 18).

Other physiological data also demonstrates variability especially during exercise (7). The development of breath-by-breath metabolic gas analysis systems now allows one to collect cardiorespiratory data at each breath. This continuous cardiorespiratory time series data, even during steady-state exercise, exhibits variability from one measurement (breath) to the next. We propose that the variability in the respiratory exchange ratio (RER) from one breath to the next contains valuable data about the subject’s metabolic status. However, for RER variability to be of value it must be reproducible. To date no information has been published on the reproducibility of RER variability during exercise. The purpose of this study was to investigate the repeatability of RER measurements at 30% and 60% of ventilator threshold (VT) during cycle ergometry in recreationally active young adults. We hypothesized that RER time series analysis by sample entropy, a nonlinear method, would be repeatable over both exercise intensities on different days.
METHODS

Experimental Approach to the Problem

The present study was designed to investigate the test-retest reliability of breath-by-breath RER time series and average measurements. The data from each day was analyzed via sample entropy and standard averaging methods to assess the test-retest reliability. To examine the reliability of calculations performed on more than 1 day, another trial was repeated 7 days apart under the same conditions.

Subjects

A sample of twelve (7 male and 5 female) recreationally active subjects were recruited with the following characteristics (mean ± SD): 27.3 ± 3.7 years of age (men 29.1 ± 2.7 yrs, women 24.8 ± 3.6 yrs), weighed an average of 71.8 ± 8.4 kg (men 76.4 ± 7.2 kg, women 65.4 ± 5.1 kg), had an average body mass index of 23.1 ± 1.8 kg/m$^2$ (men 23.8 ± 1.9 kg/m$^2$, women 22.0 ± 1.3 kg/m$^2$), with an average percent body fat of 16.4 ± 8.1% (men 10.3 ± 2.9%, women 24.8 ± 3.6%), and had an average peak oxygen uptake of 46.0 ± 5.3 ml · kg$^{-1}$ · min$^{-1}$ (men 46.7 ± 5.0 ml · kg$^{-1}$ · min$^{-1}$, women 45.0 ± 6.1 ml · kg$^{-1}$ · min$^{-1}$), and a VT of 31.9 ± 6.0 ml · kg$^{-1}$ · min$^{-1}$ (men 31.5 ± 6.4 ml · kg$^{-1}$ · min$^{-1}$, women 32.6 ± 5.9 ml · kg$^{-1}$ · min$^{-1}$). Prior to testing, all participants were informed of the study details and procedures including all the potential risks. Participants completed the Physical Activity Readiness Questionnaire which assess their health history (1) and were excluded if they had any significant injury or illness in the previous two weeks. The protocol, informed consent, and related documentation were reviewed by the University of Minnesota Institutional Review Board for approval before the study started and conducted in accordance with their requirements.
Preliminary testing

Subjects were asked to make three visits to the Laboratory of Integrative Human Physiology (LIHP) on non-consecutive days. The three trips consisted of an initial peak aerobic capacity test and two ride tests at 30% of VT and 60% of VT for 15 minutes each, all performed on a stationary electronically braked cycle ergometer (Lode Corival, Groningen, The Netherlands). Subjects were instructed to fast for a minimum of 8 hours previous to all exercise tests, to avoid any caffeine for 48 hours prior, and to not participate in exercise during the previous 24 hours. The 48 hour withdrawal of caffeine was considered adequate given the half-life of caffeine is about 4-6 hours (8). An overnight fast was done to minimize any effect of the previous meal on RER (4, 13). Subjects were instructed not to change their diet or exercise during the study.

Prior to the first exercise assessment, height and weight were measured using a wall-mounted stadiometer (Ayrton Stadiometer, Model S100, Prior Lake, MN) and digital weight scale (Model 5002, Scale-Tronix Inc., Wheaton, IL). Each measurement was done three times and the mean recorded. Body Mass Index was calculated as the body weight (kg) divided by height squared (m²). Air displacement plethysmography (Bod Pod® Life Measurement Inc., Concord, CA) was used to obtain initial visit body fat percentages. Subjects were instructed to sit still and breathe normally while the body volume measurement was conducted. Thoracic gas volume was estimated according to
the methods described by Dempster and Aitkens (6). Body fat percentage was calculated by computer software using the Siri equation and the collected data (21).

Next subjects were fitted with headgear and mouthpiece for collection of expired air by a calibrated open-circuit spirometry metabolic cart (CPX-D, MedGraphics Corporation, St. Paul, MN). Seat and handlebar height were recorded and were replicated for subsequent experimental trials. After a warm-up on the bicycle ergometer for 5 minutes at 25 Watts, subjects were asked to complete a progressive resistance exercise test. Subjects rode at a cadence of 60-90 rpm against an increasing resistance of 50 Watts every 2 minutes until volitional exhaustion. Rating of perceived exertion (RPE) was obtained at the end of each stage using the 10-point Borg category scale (3). All subjects met at least two of the following criteria of maximal effort: 1) increase in VO$_2$ between the last 2 stages of less than half the expected increase, 2) RER $\geq$ 1.10, 3) or RPE $\geq$ 9 on the Borg 1-10 scale. Constant verbal encouragement was given to the subjects during each trial to elicit a maximal effort. Analyzed gas samples were used to determine peak aerobic capacity (VO$_2$ peak) and the ventilatory threshold by the $D_{\text{max}}$ method (5).

**Experimental protocol**

This study used a repeated measures design. After a minimum of 7 days from preliminary testing, subjects returned to LIHP for their initial test. They observed the same pre-testing criteria with respect to fasting, caffeine, and exercise. All testing was performed in a climate controlled environment between 6:00 to 8:00 am. They were fitted with headgear and mouthpiece for collection of ventilation, VO$_2$, CO$_2$ production,
and RER on a breath-by-breath basis. After a 5 minute warm up on a bicycle ergometer at 25 Watts, subjects pedaled at a workload corresponding to 30% of their pre-determined VT for 15 minutes, then pedaled at a workload corresponding to 60% of their VT for an additional 15 minutes. The second test was conducted with the same procedure and done a minimum of 7 days afterward.

Data analyses
The RER measurements were taken as breath-by-breath samples and stored for latter off-line analysis (Kubios Heart Rate Variability software version 2.0 beta 3; Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). The time points from 5 to 15 minutes for each stage were utilized for the calculations. Each RER time series was manually reviewed for any non-physiologic values below 0.65 and above 1.20 and were excluded from analysis.

Statistical analyses
All data were tested for normality prior to statistical analysis. Means, standard errors, and coefficients of variance were calculated for all descriptive variables. Breath-by-breath data was analyzed using Kubios software to obtain the mean RER and sample entropy score. They were compared via a two sample Student’s t test. Agreement between the two tests was assessed according to the technique of Bland and Altman (2) and Pearson’s correlation. Test-retest reliability was determined by intraclass correlation coefficients (ICC) for the tests (20). Differences were considered significant at p< 0.05.
Data were analyzed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL) and Prism® Graphpad Software version 6.0 (Graphpad Software, Inc., San Diego, CA).

RESULTS
The mean ± standard error values for the mean RER and sample entropy scores for test 1 and test 2 are shown in Table 1. No significant differences between the tests were found for mean RER or the sample entropy score at 30% of VT or 60% of VT. Pearson’s correlation for relationships between the subject’s characteristics (height, weight, and body fat) and the sample entropy of the RER parameters showed a significant correlation only between percent body fat and the sample entropy of the RER at 60% of VT ($r=-0.59$, $p=0.046$). The highest coefficients of variance were observed for the sample entropy of the RER at 30% of VT, with 23.9% for test 1 and 22.0% for test 2. The coefficients of variance for the sample entropy of the RER at 60% of VT were similar at 24.0% for test 1 and 20.1% for test 2. The coefficients of variance were lower for the mean RER at both intensities at 4.8% and 3.3% at 30% of VT and 4.2% and 3.1% at 60% VT for test 1 and test 2, respectively.

The ICC was based on the results of a repeated measures analysis of variance, which compared the test-retest trials for each of the exercises tests. ICCs were lower for the sample entropy RER scores than for the mean RER values. The ICC for the sample entropy RER at 30% of VT was 0.81 and at 60% of VT it was the lowest at 0.25. The ICC values for the mean RER at 30% of VT was 1.00 and at 60% of VT it was 0.92.
The Bland-Altman plots in figures 1 and 2 illustrate a measure of agreement between two measurements over a range of values. The 95% confidence intervals (CIs) represent the difference at each intensity (30% or 60% of VT). The difference in mean RER values between test 1 and test 2 at 30% VT and 60% of VT are shown in figure 1A and 1B respectively. These data did not increase with frequency and were in agreement. The mean RER values were centered around the mean difference of zero, with the exception of one point that fell close to the 95% CI near the RER of 0.89 in both cases. Figure 2A and 2B shows good agreement with most points within the CI for the sample entropy RER scores at both 30% and 60% of VT, indicating agreement, with the exception of one data point. All data were normally distributed as determined by the Shapiro–Wilk test.

**DISCUSSION**

To the best of our knowledge this pilot study is the first to investigate the repeatability of time series analysis of RER data by sample entropy. Respiratory, cardiovascular, and metabolic systems have been shown to exhibit inherent complex variability, even at steady-state (11). Therefore new methods of analysis beyond standard, linear statistical methods must be employed (16, 17). RER is defined as the ratio of the volume of CO$_2$ to O$_2$ measured at the mouth and can be measured with a modern metabolic cart (4). Steady-state RER is displayed as a ratio between 0.7 to 1.0 where 0.7 corresponds to 100% fat metabolism, 0.85 corresponds to 50% fat and 50% carbohydrate metabolism, and 1.0 corresponds to 100% carbohydrate metabolism (23). The respiratory exchange ratio values can change moment by moment based on energy metabolism (4, 23). They can also be altered by exercise (10). Goedecke et al. (7) determined several factors that
affected fuel use (as measured by RER) during exercise and rest such as muscle glycogen, training volume, plasma free fatty acid concentration, and percentage of fat in dietary intake all influenced RER at various levels of exercise and at rest. Toubro et al. (22) found that variations between subjects RER could be attributed to age, gender, dietary substrate intake, insulin, and plasma free fatty acids.

The respiratory exchange ratio may also serve as an efficiency marker of exercise since it contains information about which fuels (carbohydrates and fat) are being used in the body. Goedecke et al. (7) showed a very large interindividual variability in resting RER from 0.72 up to 0.93 that even persisted during exercise of increasing intensity. This corresponded to a relative rate of fat oxidation that ranged from 23 to 93%. This large interindividual variability in RER from 0.83 to 0.95 was also demonstrated by Helge et al. (9) during low-intensity steady-state exercise. Therefore, RER has the capacity to vary over a large range as an indicator of the fuel being used and may be associated with exercise capacity.

Despite all the work completed historically with RER, to date, the variability of breath-by-breath data has not been analyzed using time series methods. This study was designed as a repeated measures format to determine the test-retest performance of this new application. In this study, the coefficients of variance were generally low for the mean RER at both intensities (3.1% to 4.8%), but were higher for sample entropy RER (which ranged from 20.1% to 24.0%). Our test–retest reliability results using the Bland and
Altman bias ± 95% limits of agreement were very similar for the mean RER and SampEn RER at both intensities of 30% and 60% of VT. These results are consistent with the similar data from the one way ANOVA results for both the mean and SampEn of the RER values where they were not statistically different. Test-retest analysis represents an important aspect of exercise performance testing when evaluating potential new methods. A test with poor reliability is not useful. Our results demonstrated an acceptable level of test-retest performance.

PRACTICAL APPLICATIONS
Time series analysis has become increasingly more popular as a valid method to analyze current data to discover new insights. Unfortunately little data exists to support the repeatability of these new measurement methods. This pilot study was the first to provide repeatability data on RER time series measurements collected under the same conditions on different days. We demonstrated that RER measurements at 30% and 60% of VT are repeatable during steady-state cycle ergometry. Future work should determine if this finding is consistent with a larger sample size and different exercise intensities.
REFERENCES


Tables

Table 1 Comparison (mean ± standard error) of test 1 and test 2.

<table>
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<th>Test 1</th>
<th>Test 2</th>
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<tr>
<td><strong>Mean RER at 30% VT</strong></td>
<td>0.92 ± 0.01</td>
<td>0.92 ± 0.01</td>
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<td><strong>Mean RER at 60% VT</strong></td>
<td>0.95 ± 0.01</td>
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<td><strong>SampEn RER at 30% VT</strong></td>
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<td><strong>SampEn RER at 60% VT</strong></td>
<td>0.84 ± 0.06</td>
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Data are presented as mean ± standard error. P-value provided is testing differences between test 1 and test 2. RER - respiratory exchange ratio (no units), SampEn - sample entropy (no units), VT - ventilatory threshold
Figure Legends

Figure 1 - Bland-Altman plots for the mean RER (respiratory exchange ratio) differences between test 1 and test 2 at 30% of VT (ventilatory threshold) (panel A) and 60% of VT (panel B). Mean differences are indicated by solid lines, and 95% limits of agreement are indicated by dashed lines. The 95% confidence intervals for 30% of VT were –0.90 to 0.91 and for 60% of VT were –0.91 to 0.81.

Figure 2 - Bland-Altman plots for the SampEn (sample entropy) RER (respiratory exchange ratio) differences between test 1 and test 2 at 30% of VT (ventilator threshold) (panel A) and 60% of VT (panel B). Mean differences are indicated by solid lines, and 95% limits of agreement are indicated by dashed lines. The 95% confidence intervals for 30% of VT were –0.48 to 0.50 and for 60% of VT were –0.25 to 0.35.
Figure 1

Panel A

Panel B
Figure 2

Panel A

Panel B
CHAPTER 5. CARDIOVASCULAR AND RIDE TIME-TO-EXHAUSTION EFFECTS OF AN ENERGY DRINK
Cardiovascular and Ride Time-to-Exhaustion Effects of an Energy Drink

Michael T, Nelson ¹, George R. Biltz¹, and Donald R. Dengel ¹,²

¹School of Kinesiology, University of Minnesota, Minneapolis, MN 55455, ²Department of Pediatrics, University of Minnesota Medical School, Minneapolis, MN 55455
Background: Currently, there are few studies on the cardiovascular and fatigue effects of commercially available energy drinks. This study investigated the effects of Monster energy drink (Monster Beverage Corporation, Corona, California), on resting heart rate (HR), heart rate variability (HRV), ride time-to-exhaustion, peak exercise HR, respiratory exchange ratio (RER), and peak rating of perceived exertion (RPE).

Methods: The study used a double-blind, randomized, placebo controlled, crossover design. After an 8-hr fast, 15 subjects consumed Monster Energy Drink (ED standardized to 2.0 mg·kg$^{-1}$·caffeine) or a flavor-matched placebo preexercise. Resting HR and HRV were determined. After an initial submaximal workload for 30 minutes, subjects completed 10 min at 80% ventilatory threshold (VT) and rode until volitional fatigue at 100% VT.

Results: Resting HR was significantly different (ED: 65±10 bpm vs. placebo: 58±8 bpm, p=0.02), but resting HRV was not different between the energy drink and placebo trials. Ride time-to-exhaustion was not significantly different between trials (ED: 45.5± 9.8 vs. placebo: 43.8±9.3 min, p=0.62). No difference in peak RPE (ED: 9.1 ± 0.5 vs. placebo: 9.0 ± 0.8, p=1.00) nor peak HR (ED: 177 ± 11 vs. placebo: 175 ± 12, p=0.73) was seen. The RER at 30% of VT was significantly different (ED: 0.94 ± 0.06 vs. placebo: 0.91 ± 0.05, p=0.046), but no difference between the two conditions were seen at the other intensities.

Conclusion: Although preexercise ingestion of the energy drink does increase resting HR there was no alteration in HRV parameters. Ride time-to-exhaustion was not enhanced.

Keywords: aerobic endurance, heart rate variability, glucose, caffeine, energy drink
Background

After a multivitamin, energy drinks (ED) are the most popular dietary supplement in the young adult population [1, 2]. Despite their popularity, sparse data exists to support the efficacy and cardiovascular effects, especially in younger adults, which is the target audience [3]. In a small meta-analysis, Shah et al. [4] found that subjects had a 10 mm Hg increase in systolic blood pressure. The main ingredients in most commercially available energy drinks are carbohydrates, B vitamins, caffeine, taurine, herbs, and flavorings.

Caffeine and carbohydrates taken separately have been previously shown to increase exercise duration and capacity [5-9]. A limited number of published studies on preexercise ingestion of energy drinks, however have produced mixed results [10-15]. Some studies showed positive effects such as increased cycling time-trial performance [10], increased bench-press muscle endurance [11], decreased sprint times [13], and increased exercise time at 65-75% of maximum heart rate (HR) on a cycle ergometer [12]. Other studies though [11, 14, 15], have failed to show any beneficial effect.

Currently there are little data on the cardiovascular effects of energy drinks [16, 17]. In addition to caffeine the amino acid taurine, a common energy drink ingredient, is theorized to have potential cardiac effects [18, 19]. Bichler and colleagues [20] investigated the combination of caffeine and taurine vs. a placebo and found it actually caused a significant decline in heart rate.
The purpose of this study was to investigate a preexercise ingestion of Monster energy drink (Monster Beverage Corporation, Corona, California) on resting HR and HR variability in addition to ride time-to-exhaustion (TTE) in recreationally active young adults. We hypothesize that resting HR and HR variability preexercise will be altered and the ride TTE will be increased after the subjects consume the energy drink (ED standardized to 2.0 mg per kilogram of body mass of caffeine) compared to a taste-matched placebo.

Methods

Participants

There were 15 recreationally active subjects (8 male and 7 female). They averaged (mean ± SD) 25.5 ± 4.1 years of age (men 24.1 ± 2.7, women 27.1 ± 5.0), weighed an average of 77.9 ± 18.4 kg (men 86.7 ± 17.6, women 67.9 ± 4.4), had an average body mass index of 25.1 ± 4.0 kg/m² (men 26.6 ± 3.6, women 23.4 ± 3.8), with an average percent body fat of 22.3 ± 8.4% (men 18.0 ± 7.4, women 27.3 ± 6.7), and had an average peak oxygen uptake of 39.5 ± 7.0 ml • kg⁻¹ • min⁻¹ (men 41.3 ± 3.0, women 37.6 ± 9.7).

Prior to testing, all participants were informed of the study details and procedures including all the potential risks. Participants completed the Physical Activity Readiness Questionnaire which assess their health history [21] and were excluded if they had any significant injury or illness in the previous two weeks. The protocol, informed consent, and related documentation were reviewed by the University of Minnesota Institutional Review Board for approval before the study started and conducted in accordance with their requirements.
Preliminary testing

Subjects were asked to make three visits to the Laboratory of Integrative Human Physiology (LIHP) on non-consecutive days. The three trips consisted of an initial peak aerobic capacity test and two ride time-to-exhaustion tests, all performed on a stationary electronically braked cycle ergometer (Lode Corival, Groningen, The Netherlands). Subjects were instructed to fast for a minimum of 8 hours previous to all exercise tests, to avoid any caffeine for 48 hours prior, and to not participate in exercise during the previous 24 hours. The 48 hour withdrawal of caffeine was considered adequate given the half-life of caffeine is about 4-6 hours [22]. An overnight fast was done to minimize any effect of the previous meal on respiratory exchange ratio (RER) [23-25]. Subjects were instructed to not change their diet or exercise during the study.

Prior to the first exercise assessment, height and weight were measured using a wall-mounted stadiometer (Ayrton Stadiometer, Model S100, Prior Lake, MN) and digital weight scale (Model 5002, Scale-Tronix Inc., Wheaton, IL). Each measurement was done three times and the mean recorded. Body mass index was calculated as the body weight (kg) divided by height squared (m\(^2\)). Air displacement plethysmography (Bod Pod® Life Measurement Inc., Concord, CA) was used to obtain initial visit body fat percentages. Subjects were instructed to sit still and breathe normally while the body volume measurement was conducted. Thoracic gas volume was estimated according to the methods described by Dempster and Aitkens [26]. Body fat percentage was calculated by computer software using the Siri equation and the collected data [27].
Heart rate was collected prior to exercise to further characterize resting cardiovascular parameters via heart rate variability (HRV) analysis. Participants were prepped for electrode placement for measurement of HR via a 3-lead electrocardiograph (ECG). The ECG (Lead II) was continuously recorded via an automated tonometer (Colin Pilot 7000; Colin Medical Instruments Corp., San Antonio, TX). Participants were asked to pace their breathing at 0.25 Hz (approximately 15 breaths per min) using a computer metronome (Crystal Metronome 1.4.4, MIL software & Matthew Lloyd) cadence. Participants were instructed to lay flat on their backs on a cushioned bed for 10 minutes to ensure that a resting state was attained. After the initial rest period, participants continued to lie relaxed for an additional 10 minutes to record resting ECG measures.

Following resting measures of HR, subjects were fitted with headgear and mouthpiece for collection of expired air by a calibrated open-circuit spirometry metabolic cart (CPX-D, MedGraphics Corporation, St. Paul, MN). Then subjects were fitted with a HR monitor (Polar, Polar Electro Oy, Finland) placed around their chest at the level of the xiphoid process to ensure a quality heart rate signal. Seat and handlebar height were recorded and were replicated for subsequent experimental trials. After warm-up on the bicycle ergometer for 5 minutes at 25 Watts, subjects were asked to complete a progressive resistance exercise test. Subjects rode at a cadence of 60-90 rpm against an increasing resistance of 50 Watts every 2 minutes until volitional exhaustion. Rating of perceived exertion (RPE) was obtained at the end of each stage using the 10-point Borg category scale [28]. All subjects met at least two of the following criteria to be considered a maximal test: 1) increase in VO₂ between the last 2 stages of less than half the expected
increase, 2) RER $\geq 1.10$, or 3) RPE $\geq 9$ on the Borg 1-10 scale. Analyzed gas samples were used to determine peak aerobic capacity ($\text{VO}_2\text{peak}$) and the ventilatory threshold (VT) by the $D_{\text{max}}$ method [29].

**Experimental design**

This study used a randomized, double-blind, placebo controlled, crossover design. Subjects were randomized for preexercise intake with the ED or placebo and received the opposite treatment a minimum of 7 days later (see Table 1 for ingredients). Regular version Monster ED was standardized at 2.0 mg per kilogram of body mass (mg·kgBM$^{-1}$) of caffeine and the placebo was prepared from noncaffeinated diet Mountain Dew and lemon juice by a lab staff member. Both drinks were served in a dark, opaque container and consumed 60 minutes before testing started. The beverage was consumed within a 10-minute period from the time it was received. The mean total beverage volume was 467 ± 109 mL (about one 16 oz can). Resting HR data were obtained as explained above followed by exercise. After a minimum of 7 days from preliminary testing, subjects returned to LIHP for their initial energy drink trial. They observed the same pre-testing criteria with respect to fasting, caffeine, and exercise. All testing was performed in a climate controlled environment between 6:00 to 8:00 am at a minimum of 1 week apart. Participants were informed that they would receive either an energy drink or a taste-matched placebo before experimental testing and a small amount of water (75mL total) at the 15 minute and 30 minute mark during exercise. Participants were instructed to not discuss the characteristics of the beverages with other participants and were asked at the end of the experimental trial which beverage they received.
Experimental protocol

After a minimum of 7 days from preliminary testing, subjects returned to LIHP for their initial energy drink trial. They were fitted with headgear and mouthpiece for collection of ventilation, oxygen consumption (VO$_2$), carbon dioxide production (VCO$_2$), and RER on a breath-by-breath basis. They were also fitted with a HR monitor as described above. After a 5 minute warm up on a bicycle ergometer at 25 Watts, subjects pedaled at a workload corresponding to 30% of their pre-determined VT for 15 minutes, then pedaled at a workload corresponding to 60% of their VT for an additional 15 minutes.

For the ride TTE portion, subjects continued to pedal at 80% of their VT for 10 minutes and then an additional 10 minutes at a workload equal to 100% of VT until volitional fatigue. The total time ride TTE was recorded. Heart rate and RPE were recorded every 2 minutes during exercise. Constant verbal encouragement by the same tester was given to the subjects during each trial to elicit a maximal effort.

The second drink trial was conducted a minimum of 7 days afterwards. Subjects received the opposite assigned preexercise drink from their first exercise trial. The cycle ergometer test protocol and data collection methods remained the same.

Heart rate variability data analyses

Lead II ECG data for HRV preexercise was collected as described above and were digitally recorded continuously using a desktop computer with WinDaq Pro data collection software (DATAQ Instruments Inc., Akron,OH). The signal was sampled at
500 Hz throughout all testing. The WinDaq Pro software allowed for instantaneous analog to digital conversion of the ECG signal with recordings stored for latter off-line analysis (Kubios Heart Rate Variability software version 2.0 beta 3; Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). Standard time domain parameters [the root mean square of successive differences (RMSSD), the standard deviation of all NN (normal RR) intervals (SDNN) and the percentage of successive NN intervals differing >50 ms (pNN50)] and frequency domain parameters [low frequency power (LF, (0.04–0.15 Hz)), high frequency power (HF, (0.15–0.4 Hz)) and the ratio of LF/HF] in addition to mean resting HR were calculated. All analysis was performed according to the standards set by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [30]. The time points from 2 to 8 minutes of the last 10 minute resting period were utilized for calculation of all resting HRV variables. Each 5-minute segment was manually reviewed for ectopic beats or arrhythmias. Segments containing such alterations of normal electrophysiological function were excluded from analysis.

The power spectral density of the RR interval data was calculated using a fast-Fourier transform for the frequency domain parameters. This was based on Welch’s periodogram method to reduce noise in the estimated power spectra with a sampling rate of 4 Hz, and a window width of 256 seconds with an overlap of 50 %, corresponding to 128 seconds. Paced breathing was performed to reduce the potential confounding effects of respiratory variation on HRV measures [31].
Statistical analyses

Beat-by-beat resting HR data was analyzed using Kubios Heart Rate Variability software to obtain the mean HR, time domain, frequency domain, and sample entropy scores for both the supplement and placebo trial. They were compared via a two sample Student’s \( t \) test. Exercise ride TTE, HR during exercise, and RPE were also analyzed using a two sample Student’s \( t \) test. Differences were considered significant at \( p < 0.05 \). Data are expressed as mean ± SD and were analyzed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL) and Prism® Graphpad Software version 6.0 (Graphpad Software, Inc., San Diego, CA).

Results

Preliminary testing

A total of 16 participants completed the study, but one was excluded from the analysis due to heavy exercise prior to testing. Resting HR was significantly higher following the ED than the placebo (ED: 65 ± 10 bpm vs. placebo: 58 ± 8 bpm, \( p = 0.02 \)). Heart rate variability as calculated via RMSSD, SDNN, pNN50, HF power, LF power, LF/HF ratio, and sample entropy however were not significantly different (see Table 2).

Experimental testing

Exercise TTE between the ED and the placebo condition was not statistically different between trials (ED: 45.5 ± 9.8 vs. placebo: 43.8 ± 9.3 min \( p = 0.62 \)). There was no significant difference in peak RPE (ED: 9.1 ± 0.5 vs. placebo: 9.0 ± 0.8, \( p = 1.00 \)) or peak HR (ED: 177 ± 11 bpm vs. placebo: 175 ± 12 bpm, \( p = 0.73 \)) during exercise in either the
supplement or placebo condition. The RER at 60% VT (ED: 0.99 ± 0.05 vs. placebo: 0.98 ± 0.05, p =0.60), 80% of VT (ED: 1.02 ± 0.07 vs. placebo: 1.03 ± 0.07, p=0.51), and 100% of VT (ED: 1.04 ± 0.09 vs. placebo: 1.04 ± 0.08, p=0.62) were not significantly different between the two conditions (Figure 1). The RER at 30% of VT however was significantly higher following the ingestion of ED vs. the placebo (0.94 ± 0.06 vs. 0.91 ± 0.05, p =0.046). There were no side effects reported from the exercise testing, ED, or placebo. Only one subject dropped out after the initial baseline. At the completion of the experimental trial, six subjects correctly identified the order of ED vs. placebo, four did not, and five were not sure.

Discussion

This was the first study to investigate preexercise ingestion of the ED Monster in relation to ride TTE and cardiovascular parameters. Cardiovascular parameters at rest did show an increase in HR after consuming the ED, but there were no changes in any HRV parameters. Ride TTE during cycle ergometry testing, peak RPE, and peak HR during exercise were not different between the two conditions. The RER measurements during each intensity were not different between the two conditions, except for the RER at 30% of VT where the placebo condition was lower.

Exercise effects

The main finding in this study is consistent with data by Candow et al. [14] who conducted a high-intensity run TTE study in young adults (VO2max of 45.5 ± 6.3 ml • kg⁻¹ • min⁻¹) using a double-blind, crossover, repeated-measures method. They showed no increase in run time or change in RPE with the energy drink Red Bull given preexercise.
However, Ivy et al. [10] did see an improvement with preexercise Red Bull. Their study also used a double-blind, randomized, crossover design, but was conducted in athletes with a higher $\text{VO}_2\text{max}$ ($54.9 \pm 2.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and employed a time trial format. Kazemi et al. [32] demonstrated that Phantom and Dragon energy drinks also significantly increased TTE vs. placebo by 9.3% and 6.5% respectively during a Bruce treadmill test.

**Caffeine**

One reason for the lack of increased ride time was possibly the lower dose of caffeine standardized at $2 \text{ mg} \cdot \text{kgBM}^{-1}$. The recent International Society of Sports Nutrition (ISSN) position stand on energy drinks [33] concluded that although they contain a number of nutrients, the primary ergogenic nutrients appear to be carbohydrate and/or caffeine. The exact mechanism of how caffeine works is still debated, but it is believed to primarily function by acting as an adenosine receptor antagonist, increasing release of free fatty acids, and increasing calcium release and uptake [34]. The track record of positive effects of caffeine is quite good and most studies showed an improvement in exercise capacity in the range of $3-13 \text{ mg} \cdot \text{kgBM}^{-1}$ [9, 33, 35-40], although Cox et al. [41] did show a decreased time during a time trial performance undertaken at the end of a prolonged cycling bout with a low dose at approximately $1.5 \text{ mg} \cdot \text{kgBM}^{-1}$. Denadai, et al. [39] used a dose of around $3 \text{ mg} \cdot \text{kgBM}^{-1}$ and showed that in untrained subjects who exercised below their anaerobic threshold, caffeine increased ride TTE and reduced perceived exertion. Further enhancement in performance does not result when caffeine is consumed in higher dosages ($\geq 9 \text{ mg} \cdot \text{kgBM}^{-1}$).
This study used a standardized dose of 2.0 mg·kgBM$^{-1}$, which is on the lower end for a dose to increase ride TTE. Subjects had to consume the entire ED amount prior to testing, therefore a higher amount may have resulted in gastrointestinal issues due to the increased level of fluid. Subjects were fasted and asked to abstain from caffeine for 48 hours prior to testing, but no other diet controls were applied to make it as applicable to free living subjects as possible.

**Rating of perceived exertion**

In the current study, there was no significant difference between peak RPEs when supplementing with an ED or placebo. A meta-analysis in 2005 [42] on caffeine found that it reduced RPE during exercise by 5.6%. Our results are in agreement with Candow et al. [14] and Ivy et al. [10] who did not show any difference in RPE during a high-intensity run time-to-exhaustion and a simulated cycling time trial, respectively.

**Heart rate**

Surprisingly, there are little data on the effects of energy drinks on heart rate. No difference was found for peak HR during exercise in this study, but resting HR was higher under the ED condition. Willoughby et al. [16] found HR was unaffected one hour after 50 young adults consumed one 250 ml (8 oz) can of sugar-free Red Bull (approximately 80mg of caffeine). Steinke et al. [17] however demonstrated that HR was reduced 30 minutes after subjects consumed 75 mg of caffeine. Bichler and colleagues [20] studied a combination of caffeine and taurine, two common ingredients in energy drinks, which resulted in a significant decline in HR.
Heart rate variability

Heart rate variability may serve as a method to further investigate the cardiac effects of these drinks as it allows quantification of sympathovagal balance [43, 44]. Some subjects may be more sensitive to energy drinks resulting in a more sympathetic response, thus altered HRV. In this study, we did not find any difference in time domain, frequency domain, or sample entropy HRV analysis. Since their inception, energy drinks have been suspected of leading to an increased risk of cardiac issues [45]. A recent review on energy drinks [46] regarding safety concluded that there is not enough data currently to allow a definitive dietary recommendation to be made regarding safe levels of ED consumption, and recommended caution. The ISSN Position Stand [33] stated that indiscriminant use of energy drinks, especially if more than one serving per day, may lead to adverse events and harmful side effects.

The only other study on HRV and energy drinks done by Wiklund et al. [47] showed a decreased LF/HF ratio and a tendency to increased HF power (increased vagal modulation). The dose used was high as subjects consumed 3 cans of Red Bull, which represents a dose of 3000 mg of taurine and 240 mg of caffeine after an overnight fast. They also measured RR intervals for the HRV analysis at 30 minutes after the intake of the ED compared to the 60 minute timeframe here. These differences may account for the variance in the results obtained.
As mentioned, the two ingredients in energy drinks that could affect HRV are taurine and caffeine. Taurine has been shown to moderate the flow of cations, especially calcium, across the cell membranes, thus protecting the heart muscle from both high and low concentrations [18, 19]. Caffeine is known to increase vagal autonomic nerve activity in resting subjects [48, 49]. Ingestion of caffeine preexercise has also been associated with exaggerated vagal withdrawal during post-exercise recovery because of higher baseline level of vagal activity before exercise [49]. However, Rauh et al. [50] did not find any significant differences in respective HRV parameters (HR, RMSSD, SDNN, pNN50, LF, HF and LF/HF) conducted at rest 30, 60, and 90 minutes after 100 and 200 mg caffeine doses were taken and compared to a placebo. They concluded that caffeine at a dose up to 200 mg does not influence HRV [50].

**Conclusion**

In conclusion, the results of this present study indicate that consuming Monster ED increases resting HR, but does not increase ride time-to-exhaustion. The ED did not have an impact on parasympathetic and sympathetic balance at rest via HRV analysis. RER was higher after the ED demonstrating a greater reliance on glucose during exercise, but this was only seen at the lowest intensity. The ED did not change the perception of exercise intensity as measured by peak RPE. Future research should compare the effects of regular energy drinks at various caffeine dosages during a ride time-to-exhaustion and a time trial format.
Abbreviations

ED, energy drink; BM, Body mass; TTE, Time-to-exhaustion; RPE, Rating of perceived exertion; RER, Respiratory exchange ratio; VO$_2^{\text{max}}$, Maximal oxygen consumption; HR, Heart rate; LIHP, Laboratory of integrative human physiology; VO$_2$, oxygen consumption; CO$_2$, carbon dioxide; HRV, Heart rate variability; ECG, Electrocardiograph; VO$_2$peak, Peak aerobic capacity; VT, Ventilatory threshold; SD, standard deviation; RMSSD, Root mean square of successive differences; SDNN, Standard deviation of all NN (normal RR) intervals; pNN50, Percentage of successive NN intervals differing >50 ms; LF, Low frequency power; HF, High frequency power; LF/HF, Ratio of low frequency to high frequency; ISSN, International society of sports nutrition.

Competing interest

No conflict of interest was reported by the authors of this paper.

Authors’ contributions

MN developed the study design, collected data, conducted statistical analysis, and drafted and submitted the manuscript. DD and GB assisted in the study design, interpretation of data, and critically reviewed the manuscript. All authors read and approved the final manuscript.
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Tables

Table 1. Monster energy drink ingredients.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (per kg body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>0.65 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Caffeine</td>
<td>2 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Taurine</td>
<td>25 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Panax-ginseng</td>
<td>5 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.5 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.04 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.50 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>0.05 mg·kgBM⁻¹</td>
</tr>
<tr>
<td>Vitamin B 12</td>
<td>0.15 mcg·kgBM⁻¹</td>
</tr>
</tbody>
</table>
Table 2: Comparison of resting heart rate variability parameters under energy drink and placebo conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Energy Drink</th>
<th>Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSSD (ms)</td>
<td>76.1 (46.0)</td>
<td>83.7 (54.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>94.1 (34.3)</td>
<td>102.0 (51.9)</td>
<td>0.28</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>38.8 (24.7)</td>
<td>38.8 (21.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>LF (ms$^2$)</td>
<td>1319 (756)</td>
<td>2295 (2593)</td>
<td>0.12</td>
</tr>
<tr>
<td>HF (ms$^2$)</td>
<td>4047 (4569)</td>
<td>4235 (5317)</td>
<td>0.79</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>0.93 (1.15)</td>
<td>0.91 (0.93)</td>
<td>0.90</td>
</tr>
<tr>
<td>SampEn</td>
<td>1.33 (0.37)</td>
<td>1.44 (0.37)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation). RMSSD - root-mean square differences of successive R-R intervals, SDNN- standard deviation of normal-to-normal intervals, pNN50 percentage of successive NN intervals differing $>50$ ms, LF - low frequency, HF - high frequency, LF/HF ratio low frequency to high frequency ratio (no units), SampEn - Sample Entropy (no units).
Figures Legend

Figure 1. Respiratory exchange ratio vs. exercise intensity as a percentage of ventilatory threshold (% of VT) for energy drink and placebo conditions.

Values are mean ± standard deviation. Only 30% of VT intensity was different from experimental vs. placebo (p < 0.046).
Figure 1
CHAPTER 6. CONCLUSION
Overall Research Implications

The most common methods to analyze cardiometabolic data currently are based on the calculation of averages. While these methods can provide some basic information, times series analysis may offer more insights (Pincus, 2000; Richman & Moorman, 2000; Seely & Macklem, 2004). However, there are currently many methods to perform time series analysis and questions remain about the reliability of each method (Pinna 2007; Sandercock, Bromley, & Brodie, 2005a). In order for a new method to be clinically useful though, it has to be shown to be repeatable first.

We have presented data here to show that frequency analysis results are comparable between laboratories using either Kubios HRV or SphygmoCor® for LF, HF, LF/HF ratio, normalized LF, and normalized HF values when the same RR intervals are analyzed. When the time series analysis method of sample entropy was applied to RER data obtained under the same conditions, it was shown to be repeatable. Heart rate variability analysis as performed by time series at rest after a biochemical stressor (e.g. energy drink) was shown to be unchanged, but resting HR was increased. The RER during the energy drink condition was increased at the lowest intensity, but no enhancement of ride time-to-exhaustion was observed.
Summary of Study Results

Chapter three described the repeatability of two HRV analysis systems. Both systems provided similar analysis from the same raw RR data as shown by the high amount of agreement by Bland-Altman analysis, similar coefficients of variation, and significant Pearson’s correlations. This suggests that frequency analysis results are comparable between laboratories using either Kubios HRV or SphygmoCor® systems for LF, HF, LF/HF ratio, normalized LF, and normalized HF values when the same RR intervals are analyzed.

Chapter four examined the repeatability of a time series analysis of respiratory exchange ratio via sample entropy. A repeated measures design was used to compare two trials completed under the same conditions. It was demonstrated that RER measurements at 30% and 60% of VT were repeatable during steady-state cycle ergometry. This will allow researchers to investigate future applications and perhaps a noninvasive way to measure metabolic flexibility.

The comparison between Monster energy drink and a taste-matched caffeine-free placebo for resting HR, HRV, ride time-to-exhaustion, exercise HR, RER and RPE were made in chapter five. Resting HR was increased, but no change in resting HRV was seen. This indicates that the parasympathetic to sympathetic balance was not altered
(Task Force, 1996). RER was higher after the consumption of the energy drink, demonstrating a greater reliance on glucose during exercise, but this was only seen at the lowest exercise intensity. No change was seen in any other parameter.

**Clinical Significance of Presented Research**

The research presented in this dissertation is a reliability assessment of time series analysis of cardiometabolic data. Between-equipment comparisons were similar for HRV analysis. This allows researchers to use either system for valid comparisons between different labs. The test-retest repeatability of RER time series data demonstrated similar results when performance on two different days under the same conditions. This new analysis method of RER variability may allow for future applications such as a noninvasive method to determine metabolic flexibility.

Heart rate variability is associated with the risk of developing cardiovascular disease, obesity, and type 2 diabetes (Kaufman et al., 2007; Martini et al., 2001; Nagai, Matsumoto, Kita, & Moritani, 2003;). Variability in the respiratory exchange ratio between breaths may also give valuable information about metabolic status and exercise capacity, but such data must be reproducible to be useful. To our knowledge, no previous studies have reported on the reproducibility of RER variability during exercise.
Finally, changes in heart rate, fuel use (from RER), perceived exertion, and exercise capacity under a chemical stressor (consumption of an energy drink) were discussed. HRV measurements can be conducted after supplement consumption to determine any changes in the autonomic nervous system status. A shift towards sympathetic stimulation may enhance exercise capacity or may indicate a potential safety concern. This information may be useful in investigation of supplements that enhance exercise capacity without added cardiovascular stress.

**Suggestions for Future Research**

The repeatability of both HRV and RER parameters by time series methods allows for comparisons in healthy populations. As the obesity rate continues to increase along with other metabolic diseases such as type 2 diabetes, noninvasive measurements that can determine early metabolic disturbances would be very beneficial. Applications to other populations such as adolescents and athletes should be considered. These methods could be used to monitor disease progression or even regression after an intervention in much earlier stages. Athletes are always looking for new ways to advance performance. Time series analysis of RER may serve as useful noninvasive marker of exercise efficiency and performance.

Further research is also needed to better understand the exact underlying mechanisms of the breath-by-breath variability under different conditions (e.g. post prandial, exercise modes, higher exercise intensity, etc); in addition to a larger sample size. Accurate and
reliable time series measurements of cardiometabolic data may provide greater insights into metabolically inflexible disease states.
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doi:10.1331/JAPhA.2008.07055


