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 caridiometaoblic 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 cardiometaoblic 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

Aubert, A. E., Seps, B., & Beckers, F. (2003). Heart rate variability in athletes. *Sports Medicine (Auckland, N.Z.), 33*(12), 889-919.

Bornas, X., Llabres, J., Noguera, M., & Pez, A. (2006). Sample entropy of ECG time series of fearful flyers: Preliminary results. *Nonlinear Dynamics, Psychology, and Life Sciences, 10*(3), 301-318.

Kaufman, C. L., Kaiser, D. R., Steinberger, J., Kelly, A. S., & Dengel, D. R. (2007). Relationships of cardiac autonomic function with metabolic abnormalities in childhood obesity. *Obesity (Silver Spring, Md.), 15*(5), 1164-1171.

Martini, G., Riva, P., Rabbia, F., Molini, V., Ferrero, G. B., Cerutti, F., . . . Veglio, F. (2001). Heart rate variability in childhood obesity. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society, 11*(2), 87-91.

Nagai, N., Matsumoto, T., Kita, H., & Moritani, T. (2003). Autonomic nervous system activity and the state and development of obesity in japanese school children. *Obesity Research, 11*(1), 25-32.

Niewiadomski, W., Gasiorowska, A., Krauss, B., Mroz, A., & Cybulski, G. (2007). Suppression of heart rate variability after supramaximal exertion. *Clinical Physiology and Functional Imaging, 27*(5), 309-319. doi:10.1111/j.1475-097X.2007.00753.x

Pincus, S. M. (2000). Irregularity and asynchrony in biologic network signals. *Methods in Enzymology, 321*, 149-182.

Platisa, M. M., & Gal, V. (2006). Dependence of heart rate variability on heart period in disease and aging. *Physiological Measurement, 27*(10), 989-998. doi:10.1088/0967-3334/27/10/005

Rabbia, F., Silke, B., Conterno, A., Grosso, T., De Vito, B., Rabbone, I., . . . Veglio, F. (2003). Assessment of cardiac autonomic modulation during adolescent obesity. *Obesity Research, 11*(4), 541-548. doi:10.1038/oby.2003.76

Riva, P., Martini, G., Rabbia, F., Milan, A., Paglieri, C., Chiandussi, L., & Veglio, F. (2001). Obesity and autonomic function in adolescence. *Clinical and Experimental Hypertension (New York, N.Y.: 1993), 23*(1-2), 57-67.

Rohner-Jeanrenaud, F. (1999). Neuroendocrine regulation of nutrient partitioning. *Annals of the New York Academy of Sciences, 892*, 261-271.

Sesay, M., Tauzin-Fin, P., Gosse, P., Ballanger, P., & Maurette, P. (2008). Real-time heart rate variability and its correlation with plasma catecholamines during laparoscopic adrenal pheochromocytoma surgery. *Anesthesia and Analgesia, 106*(1), 164-70, table of contents. doi:10.1213/01.ane.0000289531.18937.0a

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation, 93*(5), 1043-1065.

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,

Dengel, Unnithan, & Witten, 2007; M. T. Nelson et al., 2008a; M. T. Nelson et al., 2008b).

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 (CO_2) produced and oxygen (O_2) 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.

References

Akselrod, S., Gordon, D., Madwed, J. B., Snidman, N. C., Shannon, D. C., & Cohen, R. J. (1985). Hemodynamic regulation: Investigation by spectral analysis. *The American Journal of Physiology, 249*(4 Pt 2), H867-75.

Aubert, A. E., Seps, B., & Beckers, F. (2003). Heart rate variability in athletes. *Sports Medicine (Auckland, N.Z.), 33*(12), 889-919.

Bardet, S., Pasqual, C., Maugendre, D., Remy, J. P., Charbonnel, B., & Sai, P. (1989). Inter and intra individual variability of acute insulin response during intravenous glucose tolerance tests. *Diabete & Metabolisme, 15*(5), 224-232.

Berger, R. D., Akselrod, S., Gordon, D., & Cohen, R. J. (1986). An efficient algorithm for spectral analysis of heart rate variability. *IEEE Transactions on Bio-Medical Engineering, 33*(9), 900-904. doi:10.1109/TBME.1986.325789

Berntson, G. G., Bigger, J. T.,Jr, Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., . . . van der Molen, M. W. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology, 34*(6), 623-648.

Bichler, A., Swenson, A., & Harris, M. A. (2006). A combination of caffeine and taurine has no effect on short term memory but induces changes in heart rate and mean arterial blood pressure. *Amino Acids, 31*(4), 471-476. doi:10.1007/s00726-005-0302-x

Bigger, J. T.,Jr, Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., & Rottman, J. N. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation, 85*(1), 164-171.

Billman, G. E. (2011). Heart rate variability – A historical perspective *Front Physiol., 2*(86)

Biltz, G. R., Harmon, J. H., Dengel, D. R., Unnithan, V. B., & Witten, G. (2007). RER variability analysis by sample entropy: A preliminary comparison of obese and lean children. [Abstract]. *Acta Kinesiologiae Universitatis Tartuensis, 12* 53. 24th Pediatric Work Physiology Meeting Children and Exercise

Bornas, X., Llabres, J., Noguera, M., & Pez, A. (2006). Sample entropy of ECG time series of fearful flyers: Preliminary results. *Nonlinear Dynamics, Psychology, and Life Sciences, 10*(3), 301-318.

Brian J., P. W., & Wasserman, K. (2005). *Principles of exercise testing and interpretation: Including pathophysiology and clinical applications* Philadelphia : Lippincott Williams & Wilkins, c2005.

Brooks, G. H., Fahey, T. D., & Baldwin, K. D. S. (2005). *Exercise physiology: Human bioenergetics and its applications* Boston : McGraw-Hill, c2005.

Chen, C. C., Chen, R. H., Chang, C. T., & Cheah, N. (1996). Reproducibility of the first-phase insulin release in the intravenous glucose tolerance test. *Journal of the Formosan Medical Association = Taiwan Yi Zhi, 95*(8), 639-641.

Cipryan, L., & Litschmannova, M. (2013). Intra-day and inter-day reliability of heart rate variability measurement. *Journal of Sports Sciences, 31*(2), 150-158. doi:10.1080/02640414.2012.721931; 10.1080/02640414.2012.721931

Eckberg, D. L. (1997). Sympathovagal balance: A critical appraisal. *Circulation, 96*(9), 3224-3232.

Froiland, K., Koszewski, W., Hingst, J., & Kopecky, L. (2004). Nutritional supplement use among college athletes and their sources of information. *International Journal of Sport Nutrition and Exercise Metabolism, 14*(1), 104-120.

Galgani, J. E., Heilbronn, L. K., Azuma, K., Kelley, D. E., Albu, J. B., Pi-Sunyer, X., . . . Look AHEAD Adipose Research Group. (2008a). Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes, 57*(4), 841-845. doi:10.2337/db08-0043

Galgani, J. E., Heilbronn, L. K., Azuma, K., Kelley, D. E., Albu, J. B., Pi-Sunyer, X., . . . Look AHEAD Adipose Research Group. (2008b). Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes, 57*(4), 841-845. doi:10.2337/db08-0043

Goedecke, J. H., St Clair Gibson, A., Grobler, L., Collins, M., Noakes, T. D., & Lambert, E. V. (2000). Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *American Journal of Physiology.Endocrinology and Metabolism, 279*(6), E1325-34.

Goodpaster, B. H., & Kelley, D. E. (2002). Skeletal muscle triglyceride: Marker or mediator of obesity-induced insulin resistance in type 2 diabetes mellitus? *Current Diabetes Reports, 2*(3), 216-222.

Heffernan, K. S., Fahs, C. A., Shinsako, K. K., Jae, S. Y., & Fernhall, B. (2007). Heart rate recovery and heart rate complexity following resistance exercise training and detraining in young men. *American Journal of Physiology.Heart and Circulatory Physiology, 293*(5), H3180-6. doi:10.1152/ajpheart.00648.2007

Helge, J. W., Fraser, A. M., Kriketos, A. D., Jenkins, A. B., Calvert, G. D., Ayre, K. J., & Storlien, L. H. (1999). Interrelationships between muscle fibre type, substrate oxidation and body fat. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 23*(9), 986-991.

Henriques, T., Goncalves, H., Antunes, L., Matias, M., Bernardes, J., & Costa-Santos, C. (2013). Entropy and compression: Two measures of complexity. *Journal of Evaluation in Clinical Practice,* doi:10.1111/jep.12068; 10.1111/jep.12068

Hoffman, J. R., Faigenbaum, A. D., Ratamess, N. A., Ross, R., Kang, J., & Tenenbaum, G. (2008). Nutritional supplementation and anabolic steroid use in adolescents. *Medicine and Science in Sports and Exercise, 40*(1), 15-24. doi:10.1249/mss.0b013e31815a5181

Juhl, C. B., Schmitz, O., Pincus, S., Holst, J. J., Veldhuis, J., & Porksen, N. (2000). Short-term treatment with GLP-1 increases pulsatile insulin secretion in type II diabetes with no effect on orderliness. *Diabetologia, 43*(5), 583-588.

Kang, J., Mangine, G. T., Ratamess, N. A., Faigenbaum, A. D., & Hoffman, J. R. (2007). Influence of intensity fluctuation on exercise metabolism. *European Journal of Applied Physiology, 100*(3), 253-260. doi:10.1007/s00421-007-0424-9

Kaplan, D. T., Furman, M. I., Pincus, S. M., Ryan, S. M., Lipsitz, L. A., & Goldberger, A. L. (1991). Aging and the complexity of cardiovascular dynamics. *Biophysical Journal, 59*(4), 945-949.

Katona, P. G., & Jih, F. (1975). Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic cardiac control. *Journal of Applied Physiology, 39*(5), 801-805.

Kaufman, C. L., Kaiser, D. R., Steinberger, J., Kelly, A. S., & Dengel, D. R. (2007). Relationships of cardiac autonomic function with metabolic abnormalities in childhood obesity. *Obesity (Silver Spring, Md.), 15*(5), 1164-1171.

Kelley, D. E. (2005). Skeletal muscle fat oxidation: Timing and flexibility are everything. *The Journal of Clinical Investigation, 115*(7), 1699-1702. doi:10.1172/JCI25758

Kelley, D. E., & Mandarino, L. J. (2000). Fuel selection in human skeletal muscle in insulin resistance: A reexamination. *Diabetes, 49*(5), 677-683.

King, J. C., Hambidge, K. M., Westcott, J. L., Kern, D. L., & Marshall, G. (1994). Daily variation in plasma zinc concentrations in women fed meals at six-hour intervals. *The Journal of Nutrition, 124*(4), 508-516.

Klug, B., Nielsen, K. G., & Bisgaard, H. (2000). Observer variability of lung function measurements in 2-6-yr-old children. *The European Respiratory Journal : Official Journal of the European Society for Clinical Respiratory Physiology, 16*(3), 472-475.

Koh, J., Brown, T. E., Beightol, L. A., Ha, C. Y., & Eckberg, D. L. (1994). Human autonomic rhythms: Vagal cardiac mechanisms in tetraplegic subjects. *The Journal of Physiology, 474*(3), 483-495.

Kuss, O., Schumann, B., Kluttig, A., Greiser, K. H., & Haerting, J. (2008). Time domain parameters can be estimated with less statistical error than frequency domain parameters in the analysis of heart rate variability. *Journal of Electrocardiology, 41*(4), 287-291. doi:10.1016/j.jelectrocard.2008.02.014; 10.1016/j.jelectrocard.2008.02.014

Kuusela, T. A., Jartti, T. T., Tahvanainen, K. U., & Kaila, T. J. (2002). Nonlinear methods of biosignal analysis in assessing terbutaline-induced heart rate and blood pressure changes. *American Journal of Physiology.Heart and Circulatory Physiology, 282*(2), H773-83. doi:10.1152/ajpheart.00559.2001

Lake, D. E., Richman, J. S., Griffin, M. P., & Moorman, J. R. (2002). Sample entropy analysis of neonatal heart rate variability. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 283*(3), R789-97. doi:10.1152/ajpregu.00069.2002

Laplaud, D., & Menier, R. (2003). Reproducibility of the instant of equality of pulmonary gas exchange and its physiological significance. *The Journal of Sports Medicine and Physical Fitness, 43*(4), 437-443.

Lewis, M. J., & Short, A. L. (2007). Sample entropy of electrocardiographic RR and QT time-series data during rest and exercise. *Physiological Measurement, 28*(6), 731-744. doi:10.1088/0967-3334/28/6/011

Macfarlane, D. J., & Wu, H. L. (2013). Inter-unit variability in two ParvoMedics TrueOne 2400 automated metabolic gas analysis systems. *European Journal of Applied Physiology, 113*(3), 753-762. doi:10.1007/s00421-012-2483-9; 10.1007/s00421-012-2483-9

Marshall, J. M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiological Reviews, 74*(3), 543-594.

McQuilkin, S. H., Nierenberg, D. W., & Bresnick, E. (1995). Analysis of within-subject variation of caffeine metabolism when used to determine cytochrome P4501A2 and N-acetyltransferase-2 activities. *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology, 4*(2), 139-146.

Mendonca, G. V., Fernhall, B., Heffernan, K. S., & Pereira, F. D. (2009). Spectral methods of heart rate variability analysis during dynamic exercise. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society, 19*(4), 237-245. doi:10.1007/s10286-009-0018-1

Montano, N., Ruscone, T. G., Porta, A., Lombardi, F., Pagani, M., & Malliani, A. (1994). Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation, 90*(4), 1826-1831.

Nelson, M. T., Biltz, G. R., Winsley, R. J., Armstrong, N., Fawkner, S. G., Bywater, K., & Dengel, D. R. (2008a). Reliability of heart rate variability by sample entropy at rest and during light exercise in children [Abstract]. *ACSM Annual Meeting,*

Nelson, M. T., Biltz, G. R., Winsley, R. J., Roberts, A. C., Armstrong, N., Stoedefalke, K., & Dengel, D. R. (2008b). Sample entropy assessment of short term training on heart rate variability *North American Society of Pediatric Exercise Medicine Annual Meeting,* Colorado Springs, CO.

Niewiadomski, W., Gasiorowska, A., Krauss, B., Mroz, A., & Cybulski, G. (2007). Suppression of heart rate variability after supramaximal exertion. *Clinical Physiology and Functional Imaging, 27*(5), 309-319. doi:10.1111/j.1475-097X.2007.00753.x

Nunan, D., Sandercock, G. R., & Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and Clinical Electrophysiology : PACE, 33*(11), 1407-1417. doi:10.1111/j.1540-8159.2010.02841.x; 10.1111/j.1540-8159.2010.02841.x

Pereira, M. A., Weggemans, R. M., Jacobs, D. R.,Jr, Hannan, P. J., Zock, P. L., Ordovas, J. M., & Katan, M. B. (2004). Within-person variation in serum lipids: Implications for clinical trials. *International Journal of Epidemiology, 33*(3), 534-541. doi:10.1093/ije/dyh057

Piccirillo, G., Magri, D., Naso, C., di Carlo, S., MoisE, A., De Laurentis, T., . . . Nocco, M. (2004). Factors influencing heart rate variability power spectral analysis during

controlled breathing in patients with chronic heart failure or hypertension and in healthy normotensive subjects. *Clinical Science (London, England : 1979), 107*(2), 183-190. doi:10.1042/CS20030401

Pincus, S., & Kalman, R. E. (1997). Not all (possibly) "random" sequences are created equal. *Proceedings of the National Academy of Sciences of the United States of America, 94*(8), 3513-3518.

Pincus, S. M. (1991). Approximate entropy as a measure of system complexity. *Proceedings of the National Academy of Sciences of the United States of America, 88*(6), 2297-2301.

Pincus, S. M. (2000a). Irregularity and asynchrony in biologic network signals. *Methods in Enzymology, 321*, 149-182.

Pincus, S. M. (2000b). Orderliness of hormone release. *Novartis Foundation Symposium, 227*, 82-96; discussion 96-104.

Pincus, S. M., Gladstone, I. M., & Ehrenkranz, R. A. (1991). A regularity statistic for medical data analysis. *Journal of Clinical Monitoring, 7*(4), 335-345.

Pincus, S. M., Veldhuis, J. D., & Rogol, A. D. (2000). Longitudinal changes in growth hormone secretory process irregularity assessed transpubertally in healthy boys. *American Journal of Physiology.Endocrinology and Metabolism, 279*(2), E417-24.

Pinna, G. D., Maestri, R., Torunski, A., Danilowicz-Szymanowicz, L., Szwoch, M., La Rovere, M. T., & Raczak, G. (2007). Heart rate variability measures: A fresh look at reliability. *Clinical Science (London, England), 113*(3), 131-140. doi:10.1042/CS20070055

Platisa, M. M., & Gal, V. (2006a). Dependence of heart rate variability on heart period in disease and aging. *Physiological Measurement, 27*(10), 989-998. doi:10.1088/0967-3334/27/10/005

Platisa, M. M., & Gal, V. (2006b). Reflection of heart rate regulation on linear and nonlinear heart rate variability measures. *Physiological Measurement, 27*(2), 145-154. doi:10.1088/0967-3334/27/2/005

Porta, A., Gnecchi-Ruscone, T., Tobaldini, E., Guzzetti, S., Furlan, R., & Montano, N. (2007). Progressive decrease of heart period variability entropy-based complexity during graded head-up tilt. *Journal of Applied Physiology (Bethesda, Md.: 1985), 103*(4), 1143-1149. doi:10.1152/japplphysiol.00293.2007

Rambihar VS. (2010). Complexity: The science for medicine and the human story. *Lancet, Apr 3*(375(9721)), 1162.

Richman, J. S., & Moorman, J. R. (2000). Physiological time-series analysis using approximate entropy and sample entropy. *American Journal of Physiology.Heart and Circulatory Physiology, 278*(6), H2039-49.

Ryan, S. M., Goldberger, A. L., Pincus, S. M., Mietus, J., & Lipsitz, L. A. (1994). Gender- and age-related differences in heart rate dynamics: Are women more complex than men? *Journal of the American College of Cardiology, 24*(7), 1700-1707.

Sacknoff, D. M., Gleim, G. W., Stachenfeld, N., & Coplan, N. L. (1994). Effect of athletic training on heart rate variability. *American Heart Journal, 127*(5), 1275-1278.

Sandercock, G. R., Bromley, P. D., & Brodie, D. A. (2005a). The reliability of short-term measurements of heart rate variability. *International Journal of Cardiology, 103*(3), 238-247. doi:10.1016/j.ijcard.2004.09.013

Sandercock, G. R., Bromley, P. D., & Brodie, D. A. (2005b). The reliability of short-term measurements of heart rate variability. *International Journal of Cardiology, 103*(3), 238-247. doi:10.1016/j.ijcard.2004.09.013

Scott, A. S., Eberhard, A., Ofir, D., Benchetrit, G., Dinh, T. P., Calabrese, P., . . . Perrault, H. (2004). Enhanced cardiac vagal efferent activity does not explain training-induced bradycardia. *Autonomic Neuroscience : Basic & Clinical, 112*(1-2), 60-68. doi:10.1016/j.autneu.2004.04.006

Seely, A. J., & Macklem, P. T. (2004). Complex systems and the technology of variability analysis. *Critical Care (London, England), 8*(6), R367-84. doi:10.1186/cc2948

Sesay, M., Tauzin-Fin, P., Gosse, P., Ballanger, P., & Maurette, P. (2008). Real-time heart rate variability and its correlation with plasma catecholamines during laparoscopic adrenal pheochromocytoma surgery. *Anesthesia and Analgesia, 106*(1), 164-70, table of contents. doi:10.1213/01.ane.0000289531.18937.0a

Steinke, L., Lanfear, D. E., Dhanapal, V., & Kalus, J. S. (2009). Effect of "energy drink" consumption on hemodynamic and electrocardiographic parameters in healthy young adults. *The Annals of Pharmacotherapy, 43*(4), 596-602. doi:10.1345/aph.1L614

Storlien, L., Oakes, N. D., & Kelley, D. E. (2004). Metabolic flexibility. *The Proceedings of the Nutrition Society, 63*(2), 363-368. doi:10.1079/PNS2004349

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation, 93*(5), 1043-1065.

Toubro, S., Sorensen, T. I., Hindsberger, C., Christensen, N. J., & Astrup, A. (1998). Twenty-four-hour respiratory quotient: The role of diet and familial resemblance. *The Journal of Clinical Endocrinology and Metabolism, 83*(8), 2758-2764.

Veldhuis, J. D., Johnson, M. L., Veldhuis, O. L., Straume, M., & Pincus, S. M. (2001). Impact of pulsatility on the ensemble orderliness (approximate entropy) of neurohormone secretion. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 281*(6), R1975-85.

Veldman, R. G., Frolich, M., Pincus, S. M., Veldhuis, J. D., & Roelfsema, F. (2000). Growth hormone and prolactin are secreted more irregularly in patients with cushing's disease. *Clinical Endocrinology, 52*(5), 625-632.

West, B. J. (2006). *Where medicine went wrong: Rediscovering the path to complexity (studies of nonlinear phenomena in life science)* (1st ed.). NJ: World Scientific Publishing Company.

Wiklund, U., Karlsson, M., Ostrom, M., & Messner, T. (2009). Influence of energy drinks and alcohol on post-exercise heart rate recovery and heart rate variability. *Clinical Physiology and Functional Imaging, 29*(1), 74-80. doi:10.1111/j.1475-097X.2008.00837.x; 10.1111/j.1475-097X.2008.00837.x

Willoughby, S. R. (2009). Energy drink effects platelet aggregation and endothelial function: A possible link to increased cardiovascular risk *Heart Lung and Circulation, 18*, S265. doi:10.1016/j.hlc.2009.05.653

Wilmore, J. H., & Costill, D. L. (2007). *Physiology of sport and exercise, fourth edition* Human Kinetics Publishers.

Yentes, J. M., Hunt, N., Schmid, K. K., Kaipust, J. P., McGrath, D., & Stergiou, N. (2012). The appropriate use of approximate entropy and sample entropy with short data sets. *Annals of Biomedical Engineering,* doi:10.1007/s10439-012-0668-3

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.

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

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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 ms^2 [4]. These data demonstrate that frequency domain measurements can vary, ranging from as low as 82 ± 104 ms^2 [8] up to 5839 ± 1839 ms^2 [6], despite the best efforts at reducing the potential confounding effects. Our values for HF at 55.4 ± 19.8 ms^2 for Kubios HRV and 54.4 ± 19.4 ms^2 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

1. Bland, JM, & Altman, DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet, 1*(8476), 307-310

2. Cipryan, L, & Litschmannova, M (2013) Intra-day and inter-day reliability of heart rate variability measurement. *Journal of Sports Sciences, 31*(2), 150-158. doi:10.1080/02640414.2012.721931; 10.1080/02640414.2012.721931

3. Katona, PG, & Jih, F (1975) Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic cardiac control. *Journal of Applied Physiology, 39*(5), 801-805

4. Nunan, D, Sandercock, GR, & Brodie, DA (2010) A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and Clinical Electrophysiology : PACE, 33*(11), 1407-1417. doi:10.1111/j.1540-8159.2010.02841.x; 10.1111/j.1540-8159.2010.02841.x

5. Pinna, G D, Maestri, R, Torunski, A, Danilowicz-Szymanowicz, L, Szwoch, M, La Rovere, MT, & Raczak, G (2007) Heart rate variability measures: A fresh look at reliability. *Clinical Science (London, England), 113*(3), 131-140. doi:10.1042/CS20070055

6. Sacknoff, DM, Gleim, GW, Stachenfeld, N, & Coplan, NL (1994) Effect of athletic training on heart rate variability. *American Heart Journal, 127*(5), 1275-1278

7. Sandercock, GR, Bromley, PD, & Brodie, DA (2005) The reliability of short-term measurements of heart rate variability. *International Journal of Cardiology, 103*(3), 238-247. doi:10.1016/j.ijcard.2004.09.013

8. Scott, AS, Eberhard, A, Ofir, D, Benchetrit, G, Dinh, TP, Calabrese, P, et al, Perrault, H (2004) Enhanced cardiac vagal efferent activity does not explain training-induced bradycardia. *Autonomic Neuroscience : Basic & Clinical, 112*(1-2), 60-68. doi:10.1016/j.autneu.2004.04.006

9. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation, 93*(5), 1043-1065

Tables

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

	Kubios	**SphygmoCor®**	**p-value**
LF (ms^2)	0.07 ± 0.01	0.08 ± 0.01	0.0041
HF (ms^2)	0.21 ± 0.02	0.21 ± 0.02	0.0004
LF/HF ratio	1.14 ± 0.23	1.13 ± 0.20	0.0001
LFnu (%)	44.6 ± 4.22	45.6 ± 4.13	0.0002
HFnu (%)	55.4 ± 4.22	54.4 ± 4.13	0.0002

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
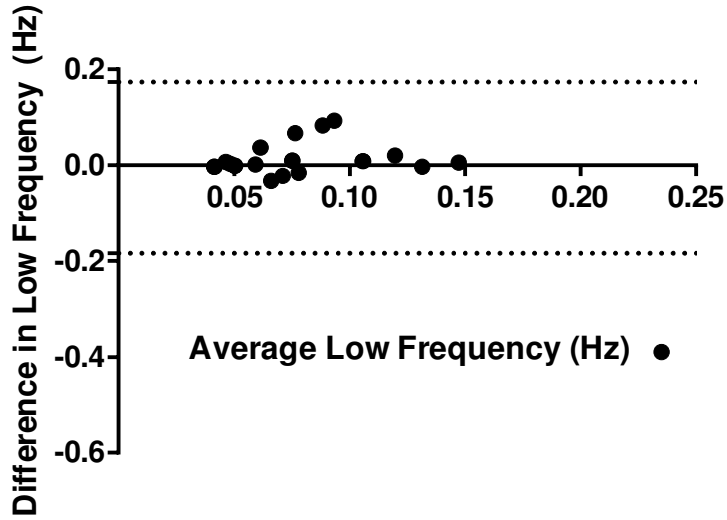
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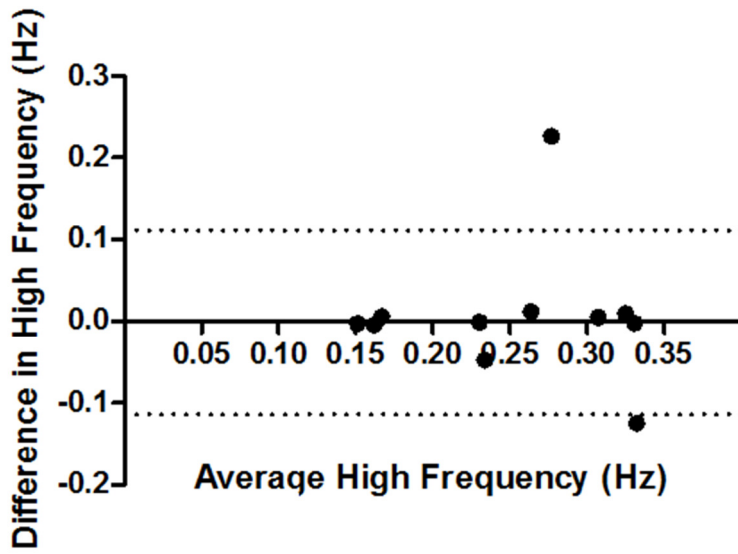
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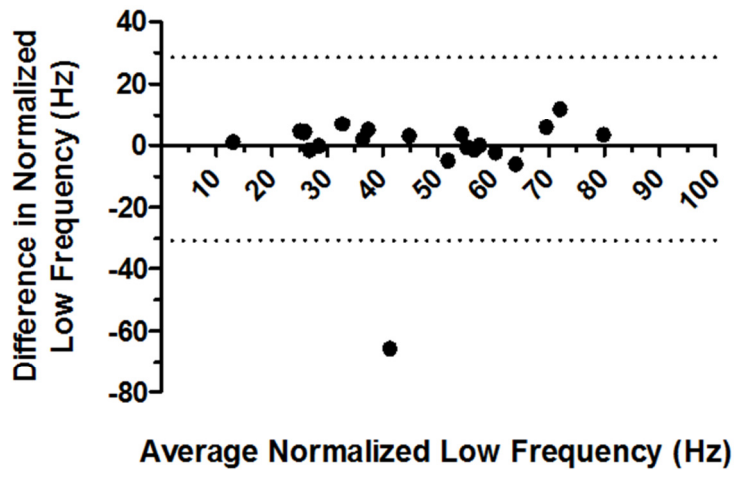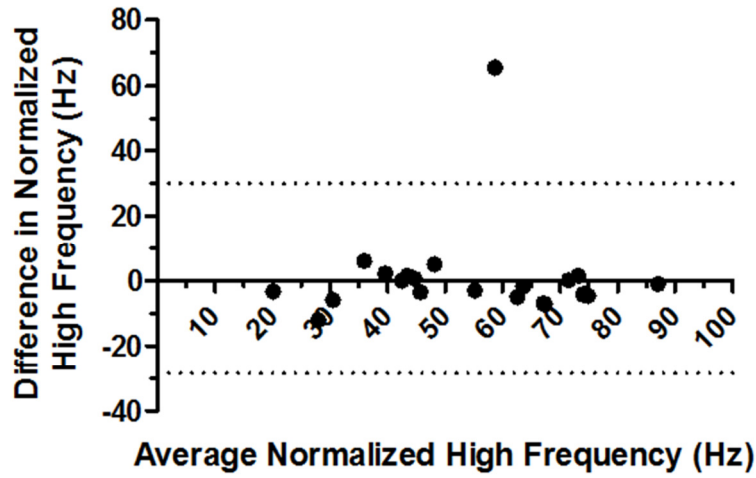
Panel A

Panel B

Panel C
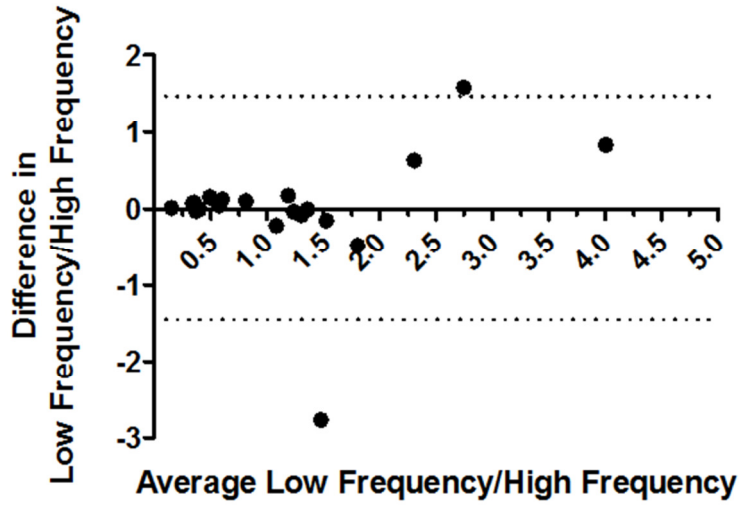
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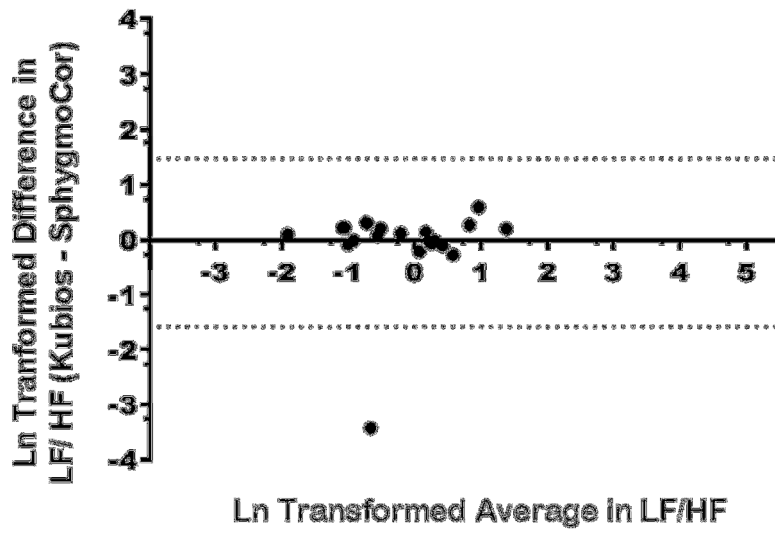
CHAPTER 4. REPEATABILITY OF RESPIRATORY EXCHANGE RATIO
TIME SERIES ANALYSIS

Repeatability of Respiratory Exchange Ratio Time Series Analysis

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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 ergometery. 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 ergometery 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 (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 (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_{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-restest 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 ergometery. Future work should determine if this finding is consistent with a larger sample size and different exercise intensities.

REFERENCES

1. Adams, R. Revised Physical Activity Readiness Questionnaire *Can. Fam. Physician* 45: 992, 995, 1004-5, 1999.

2. Bland, JM, and Altman, DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307-310, 1986.

3. Borg, GA. Psychophysical bases of perceived exertion. *Med. Sci. Sports Exerc.* 14: 377-381, 1982.

4. Brooks, GH, Fahey, TD, and Baldwin, KDS. Exercise Physiology: Human Bioenergetics and its Applications. Boston : McGraw-Hill, c2005., 2005.

5. Cheng, B, Kuipers, H, Snyder, AC, Keizer, HA, Jeukendrup, A, and Hesselink, M. A new approach for the determination of ventilatory and lactate thresholds. *Int. J. Sports Med.* 13: 518-522, 1992.

6. Dempster, P, and Aitkens, S. A new air displacement method for the determination of human body composition. *Med. Sci. Sports Exerc.* 27: 1692-1697, 1995.

7. Goedecke, JH, St Clair Gibson, A, Grobler, L, Collins, M, Noakes, TD, and Lambert, EV. Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *Am. J. Physiol. Endocrinol. Metab.* 279: E1325-34, 2000.

8. Graham, TE. Caffeine and exercise: metabolism, endurance and performance. *Sports Med.* 31: 785-807, 2001.

9. Helge, JW, Fraser, AM, Kriketos, AD, Jenkins, AB, Calvert, GD, Ayre, KJ, and Storlien, LH. Interrelationships between muscle fibre type, substrate oxidation and body fat. *Int. J. Obes. Relat. Metab. Disord.* 23: 986-991, 1999.

10. Kang, J, Mangine, GT, Ratamess, NA, Faigenbaum, AD, and Hoffman, JR. Influence of intensity fluctuation on exercise metabolism. *Eur. J. Appl. Physiol.* 100: 253-260, 2007.

11. Macfarlane, DJ, and Wu, HL. Inter-unit variability in two ParvoMedics TrueOne 2400 automated metabolic gas analysis systems. *Eur. J. Appl. Physiol.* 113: 753-762, 2013.

12. Martini, G, Riva, P, Rabbia, F, Molini, V, Ferrero, GB, Cerutti, F, Carra, R, and Veglio, F. Heart rate variability in childhood obesity. *Clin. Auton. Res.* 11: 87-91, 2001.

13. Miller, SL, Maresh, CM, Armstrong, LE, Ebbeling, CB, Lennon, S, and Rodriguez, NR. Metabolic response to provision of mixed protein-carbohydrate supplementation during endurance exercise. *Int. J. Sport Nutr. Exerc. Metab.* 12: 384-397, 2002.

14. Nagai, N, Matsumoto, T, Kita, H, and Moritani, T. Autonomic nervous system activity and the state and development of obesity in Japanese school children. *Obes. Res.* 11: 25-32, 2003.

15. Niewiadomski, W, Gasiorowska, A, Krauss, B, Mroz, A, and Cybulski, G. Suppression of heart rate variability after supramaximal exertion. *Clin. Physiol. Funct. Imaging* 27: 309-319, 2007.

16. Porta, A, Gnecchi-Ruscone, T, Tobaldini, E, Guzzetti, S, Furlan, R, and Montano, N. Progressive decrease of heart period variability entropy-based complexity during graded head-up tilt. *J. Appl. Physiol.* 103: 1143-1149, 2007.

17. Richman, JS, and Moorman, JR. Physiological time-series analysis using approximate entropy and sample entropy. *Am. J. Physiol. Heart Circ. Physiol.* 278: H2039-49, 2000.

18. Rohner-Jeanrenaud, F. Neuroendocrine regulation of nutrient partitioning. *Ann. N. Y. Acad. Sci.* 892: 261-271, 1999.

19. Sesay, M, Tauzin-Fin, P, Gosse, P, Ballanger, P, and Maurette, P. Real-time heart rate variability and its correlation with plasma catecholamines during laparoscopic adrenal pheochromocytoma surgery. *Anesth. Analg.* 106: 164-70, table of contents, 2008.

20. Shrout, PE, and Fleiss, JL. Intraclass correlations: uses in assessing rater reliability. *Psychol. Bull.* 86: 420-428, 1979.

21. Siri, WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* 9: 480-91; discussion 480, 492, 1993.

22. Toubro, S, Sorensen, TI, Hindsberger, C, Christensen, NJ, and Astrup, A. Twenty-four-hour respiratory quotient: the role of diet and familial resemblance. *J. Clin. Endocrinol. Metab.* 83: 2758-2764, 1998.

23. Wilmore, JH, and Costill, DL. Physiology of Sport and Exercise, Fourth Edition. Human Kinetics Publishers, 2007.

Tables

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

	Test 1	Test 2	p-value
Mean RER at 30% VT	0.92 ± 0.01	0.92 ± 0.01	1.00
Mean RER at 60% VT	0.95 ± 0.01	0.95 ± 0.01	0.84
SampEn RER at 30% VT	0.82 ± 0.06	0.80 ± 0.05	0.75
SampEn RER at 60% VT	0.84 ± 0.06	0.79 ± 0.05	0.45

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
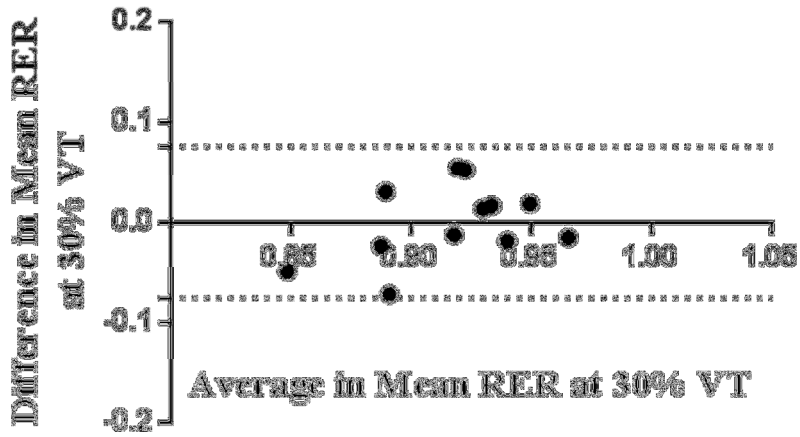
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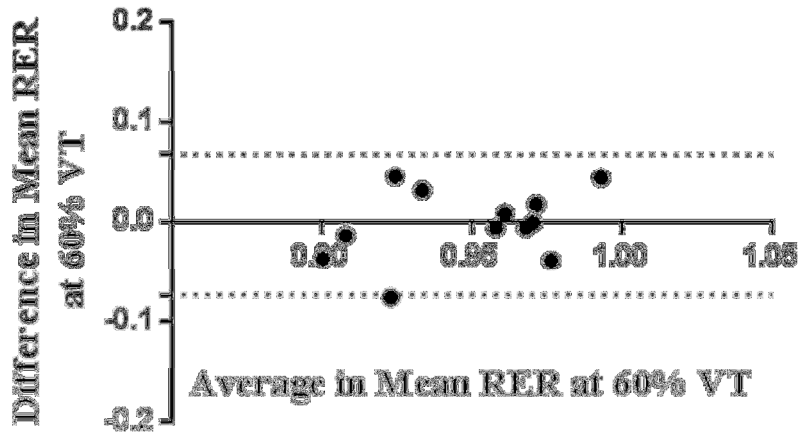
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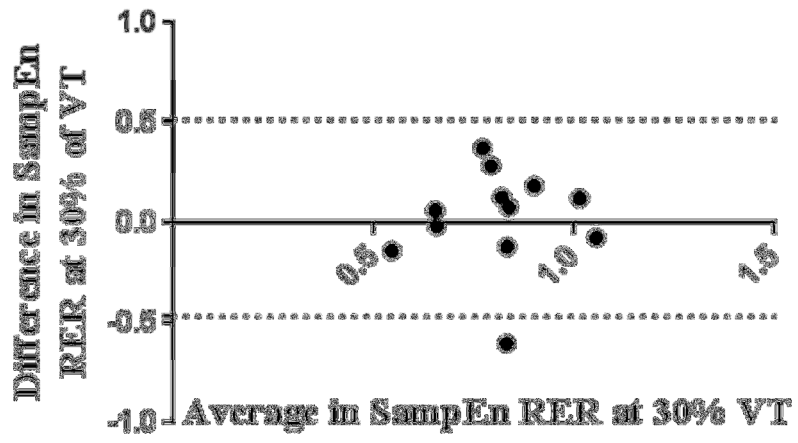
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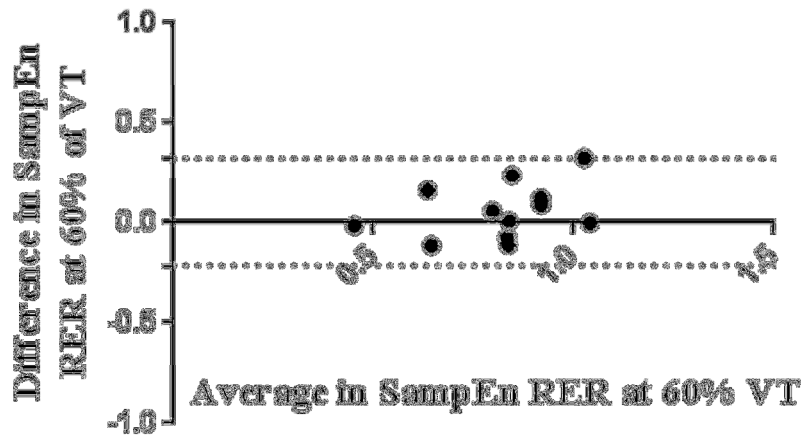
CHAPTER 5. CARDIOVASCULAR AND RIDE TIME-TO-EXHAUSTION EFFECTS OF AN ENERGY DRINK

Cardiovascular and Ride Time-to-Exhaustion Effects of an Energy Drink

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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$\cdot$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/m^2 (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 VO_2 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 ($VO_{2\ peak}$) and the ventilatory threshold (VT) by the D_{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 ($mg \cdot kgBM^{-1}$) 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 ergometery 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 (VO$_{2max}$ 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 VO_{2max} (54.9 ± 2.3 ml • kg^{-1} • 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 mg·$kgBM^{-1}$. 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 mg·$kgBM^{-1}$ [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 mg·$kgBM^{-1}$. Denadai, et al. [39] used a dose of around 3 mg·$kgBM^{-1}$ 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 (≥ 9 mg·$kgBM^{-1}$).

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_{2max}, Maximal oxygen consumption; HR, Heart rate; LIHP, Laboratory of integrative human physiology; VO_2, oxygen consumption; CO_2, carbon dioxide; HRV, Heart rate variably; 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.

References

1. Hoffman JR, Faigenbaum AD, Ratamess NA, Ross R, Kang J, Tenenbaum G: **Nutritional supplementation and anabolic steroid use in adolescents.** *Med Sci Sports Exerc* 2008, **40**(1):15-24.

2. Froiland K, Koszewski W, Hingst J, Kopecky L: **Nutritional supplement use among college athletes and their sources of information.** *Int J Sport Nutr Exerc Metab* 2004, **14**(1):104-120.

3. Clauson KA, Shields KM, McQueen CE, Persad N: **Safety issues associated with commercially available energy drinks.** *J Am Pharm Assoc* (2003) 2008, **48**(3):e55-63; quiz e64-7.

4. Shah S, Lacey C, Riddock I,: **Impact of energy drinks on electrocardiographic and blood pressure parameters: A meta-analysis of clinical studies.[abstract].** *EPI\NPAM 2013* 2013; **Abstract P324.:**.

5. Noakes TD, Lambert EV, Lambert MI, McArthur PS, Myburgh KH, Benade AJ: **Carbohydrate ingestion and muscle glycogen depletion during marathon and ultramarathon racing.** *Eur J Appl Physiol Occup Physiol* 1988, **57**(4):482-489.

6. Jeukendrup AE: **Carbohydrate intake during exercise and performance.** *Nutrition* 2004, **20**(7-8):669-677.

7. Kang J, Robertson RJ, Goss FL, DaSilva SG, Visich P, Suminski RR, Utter AC, Denys BC: **Effect of carbohydrate substrate availability on ratings of perceived exertion during prolonged exercise of moderate intensity.** *Percept Mot Skills* 1996, **82**(2):495-506.

8. Costill DL, Dalsky GP, Fink WJ: **Effects of caffeine ingestion on metabolism and exercise performance.** *Med Sci Sports* 1978, **10**(3):155-158.

9. Graham TE, Spriet LL: **Performance and metabolic responses to a high caffeine dose during prolonged exercise.** *J Appl Physiol* 1991, **71**(6):2292-2298.

10. Ivy JL, Kammer L, Ding Z, Wang B, Bernard JR, Liao YH, Hwang J: **Improved cycling time-trial performance after ingestion of a caffeine energy drink.** *Int J Sport Nutr Exerc Metab* 2009, **19**(1):61-78.

11. Forbes SC, Candow DG, Little JP, Magnus C, Chilibeck PD: **Effect of Red Bull energy drink on repeated Wingate cycle performance and bench-press muscle endurance.** *Int J Sport Nutr Exerc Metab* 2007, **17**(5):433-444.

12. Alford C, Cox H, Wescott R: **The effects of red bull energy drink on human performance and mood.** *Amino Acids* 2001, **21**(2):139-150.

13. Del Coso J, Portillo J, Munoz G, Abian-Vicen J, Gonzalez-Millan C, Munoz-Guerra J: **Caffeine-containing energy drink improves sprint performance during an international rugby sevens competition.** *Amino Acids* 2013, **44**(6):1511-9.

14. Candow DG, Kleisinger AK, Grenier S, Dorsch KD: **Effect of sugar-free Red Bull energy drink on high-intensity run time-to-exhaustion in young adults.** *J Strength Cond Res 2009*, **23**(4):1271-1275.

15. Astorino TA, Matera AJ, Basinger J, Evans M, Schurman T, Marquez R: **Effects of red bull energy drink on repeated sprint performance in women athletes.** *Amino Acids* 2011, **42**(5):1803-8.

16. Willoughby SR: **Energy drink effects platelet aggregation and endothelial function: A possible link to increased cardiovascular risk.** *Heart Lung and Circulation* 2009, **18**:S265.

17. Steinke L, Lanfear DE, Dhanapal V, Kalus JS: **Effect of "Energy Drink" Consumption on Hemodynamic and Electrocardiographic Parameters in Healthy Young Adults.** *Ann Pharmacother* 2009, **43**(4):596-602.

18. Huxtable RJ: **Physiological actions of taurine.** *Physiol Rev* 1992, **72**(1):101-163.

19. Schaffer SW, Azuma J: **Review: myocardial physiological effects of taurine and their significance.** *Adv Exp Med Biol* 1992, **315**:105-120.

20. Bichler A, Swenson A, Harris MA: **A combination of caffeine and taurine has no effect on short term memory but induces changes in heart rate and mean arterial blood pressure.** *Amino Acids* 2006, **31**(4):471-476.

21. Adams R: **Revised Physical Activity Readiness Questionnaire.** *Can Fam Physician* 1999, **45**:992, 995, 1004-5.

22. Graham TE: **Caffeine and exercise: metabolism, endurance and performance.** *Sports Med* 2001, **31**(11):785-807.

23. Brooks GH, Fahey TD, Baldwin KDS: *Exercise physiology: human bioenergetics and its applications:* Boston : McGraw-Hill, c2005.; 2005.

24. Wilmore JH, Costill DL: *Physiology of Sport and Exercise, Fourth Edition:* Human Kinetics Publishers; 2007.

25. Miller SL, Maresh CM, Armstrong LE, Ebbeling CB, Lennon S, Rodriguez NR: **Metabolic response to provision of mixed protein-carbohydrate supplementation during endurance exercise.** *Int J Sport Nutr Exerc Metab* 2002, **12**(4):384-397.

26. Dempster, P., & Aitkens, S. **A new air displacement method for the determination of human body composition.** *Med Sci Sports Exerc* 1995, **27**(12):1692-1697.

27. Siri WE: **Body composition from fluid spaces and density: analysis of methods. 1961.** *Nutrition* 1993, **9**(5):480-91.

28. Borg GA: **Psychophysical bases of perceived exertion.** *Med Sci Sports Exerc* 1982, **14**(5):377-381.

29. Cheng B, Kuipers H, Snyder AC, Keizer HA, Jeukendrup A, Hesselink M: **A new approach for the determination of ventilatory and lactate thresholds.** *Int J Sports Med* 1992, **13**(7):518-522.

30. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: **Heart rate variability: standards of measurement, physiological interpretation and clinical use.** *Circulation* 1996, **93**(5):1043-1065.

31. Katona PG, Jih F: **Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control.** *J Appl Physiol* 1975, **39**(5):801-805.

32. Kazemi F, Gaeini A, Kordi M, Rahnama N: **The acute effects of two energy drinks on endurance performance in female athlete students.** *Sport Sciences for Health* 2009, **5**(2).

33. Campbell B, Wilborn C, La Bounty P, Taylor L, Nelson MT, Greenwood M, Ziegenfuss TN, Lopez HL, Hoffman JR, Stout JR, Schmitz S, Collins R, Kalman DS, Antonio J, Kreider RB: **International Society of Sports Nutrition position stand: energy drinks.** *J Int Soc Sports Nutr* 2013, **10**(1):1-2783-10-1.

34. Davis JK, Green JM: **Caffeine and anaerobic performance: ergogenic value and mechanisms of action.** *Sports Med* 2009, **39**(10):813-832.

35. Kovacs EM, Stegen JHCH, Brouns F: **Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance.** *J Appl Physiol* 1998, **85**(2):709-715.

36. Spriet LL, MacLean DA, Dyck DJ, Hultman E, Cederblad G, Graham TE: **Caffeine ingestion and muscle metabolism during prolonged exercise in humans.** *Am J Physiol* 1992, **262**(6 Pt 1):E891-8.

37. Graham TE, Spriet LL: **Metabolic, catecholamine, and exercise performance responses to various doses of caffeine.** *J Appl Physiol* 1995, **78**(3):867-874.

38. Pasman WJ, van Baak MA, Jeukendrup AE, de Haan A: **The effect of different dosages of caffeine on endurance performance time.** *Int J Sports Med* 1995, **16**(4):225-230.

39. Denadai BS, Denadai ML: **Effects of caffeine on time to exhaustion in exercise performed below and above the anaerobic threshold.** *Braz J Med Biol Res* 1998, **31**(4):581-585.

40. Burke LM: **Caffeine and spo_frts performance.** *Appl Physiol Nutr Metab* 2008, **33**(6):1319-1334.

41. Cox GR, Desbrow B, Montgomery PG, Anderson ME, Bruce CR, Macrides TA, Martin DT, Moquin A, Roberts A, Hawley JA, Burke LM: **Effect of different protocols of caffeine intake on metabolism and endurance performance.** *J Appl Physiol* 2002, **93**(3):990-999.

42. Doherty M, Smith PM: **Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis.** *Scand J Med Sci Sports* 2005, **15**(2):69-78.

43. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A: **Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt.** *Circulation* 1994, **90**(4):1826-1831.

44. Eckberg DL: **Sympathovagal balance: a critical appraisal.** *Circulation* 1997, **96**(9):3224-3232.

45. Finnegan D: **The health effects of stimulant drinks.** *Br Nutr Found Nutr Bull* 2003, **28**:147–155.

46. Burrows T, Pursey K, Neve M, Stanwell P: **What are the health implications associated with the consumption of energy drinks? A systematic review.** Nutr Rev 2013, **71**(3):135-148.

47. Wiklund U, Karlsson M, Ostrom M, Messner T: **Influence of energy drinks and alcohol on post-exercise heart rate recovery and heart rate variability.** *Clin Physiol Funct Imaging* 2009, **29**(1):74-80.

48. Hibino G, Moritani T, Kawada T, Fushiki T: **Caffeine enhances modulation of parasympathetic nerve activity in humans: quantification using power spectral analysis.** *J Nutr* 1997, **127**(7):1422-1427.

49. Yeragani VK, Krishnan S, Engels HJ, Gretebeck R: **Effects of caffeine on linear and nonlinear measures of heart rate variability before and after exercise.** *Depress Anxiety* 2005, **21**(3):130-134.

50. Rauh R, Burkert M, Siepmann M, Mueck-Weymann M: **Acute effects of caffeine on heart rate variability in habitual caffeine consumers.** *Clin Physiol Funct Imaging* 2006, **26**(3):163-166.

Tables

Table 1. Monster energy drink ingredients.

Ingredient	Amount (per kg body mass)
Carbohydrate	0.65 mg·kgBM^{-1}
Caffeine	2 mg·kgBM^{-1}
Taurine	25 mg·kgBM^{-1}
Panax-ginseng	5 mg·kgBM^{-1}
Vitamin C	1.5 mg·kgBM^{-1}
Riboflavin	0.04 mg·kgBM^{-1}
Niacin	0.50 mg·kgBM^{-1}
Vitamin B6	0.05 mg·kgBM^{-1}
Vitamin B 12	0.15 mcg·kgBM^{-1}

Table 2: Comparison of resting heart rate variability parameters under energy drink and placebo conditions.

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

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
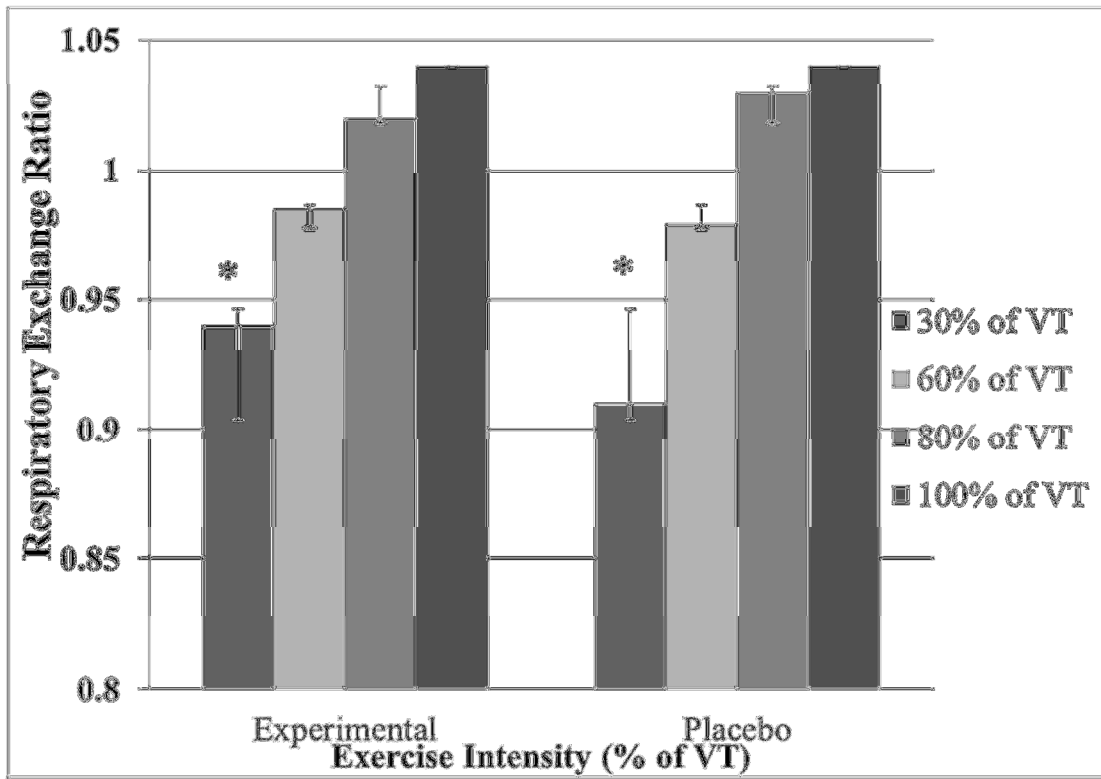
CHAPTER 6. CONCLUSION

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 variaibilty 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 ergometery. 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.

References

Kaufman, C. L., Kaiser, D. R., Steinberger, J., Kelly, A. S., & Dengel, D. R. (2007). Relationships of cardiac autonomic function with metabolic abnormalities in childhood obesity. *Obesity (Silver Spring, Md.), 15*(5), 1164-1171.

Martini, G., Riva, P., Rabbia, F., Molini, V., Ferrero, G. B., Cerutti, F., . . . Veglio, F. (2001). Heart rate variability in childhood obesity. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society, 11*(2), 87-91.

Nagai, N., Matsumoto, T., Kita, H., & Moritani, T. (2003). Autonomic nervous system activity and the state and development of obesity in japanese school children. *Obesity Research, 11*(1), 25-32.

Pincus, S. M. (2000). Irregularity and asynchrony in biologic network signals. *Methods in Enzymology, 321*, 149-182.

Pinna, G. D., Maestri, R., Torunski, A., Danilowicz-Szymanowicz, L., Szwoch, M., La Rovere, M. T., & Raczak, G. (2007). Heart rate variability measures: A fresh look at reliability. *Clinical Science (London, England), 113*(3), 131-140. doi:10.1042/CS20070055

Richman, J. S., & Moorman, J. R. (2000). Physiological time-series analysis using approximate entropy and sample entropy. *American Journal of Physiology.Heart and Circulatory Physiology, 278*(6), H2039-49.

Sandercock, G. R., Bromley, P. D., & Brodie, D. A. (2005a). The reliability of short-term measurements of heart rate variability. *International Journal of Cardiology, 103*(3), 238-247. doi:10.1016/j.ijcard.2004.09.013

Seely, A. J., & Macklem, P. T. (2004). Complex systems and the technology of variability analysis. *Critical Care (London, England), 8*(6), R367-84. doi:10.1186/cc2948

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation, 93*(5), 1043-1065.

Bibliography

Adams, R. (1999). Revised physical activity readiness questionnaire *Canadian Family Physician Medecin De Famille Canadien, 45*, 992, 995, 1004-5.

Akselrod, S., Gordon, D., Madwed, J. B., Snidman, N. C., Shannon, D. C., & Cohen, R. J. (1985). Hemodynamic regulation: Investigation by spectral analysis. *The American Journal of Physiology, 249*(4 Pt 2), H867-75.

Alford, C., Cox, H., & Wescott, R. (2001). The effects of red bull energy drink on human performance and mood. *Amino Acids, 21*(2), 139-150.

Astorino, T. A., Matera, A. J., Basinger, J., Evans, M., Schurman, T., & Marquez, R. (2011). Effects of red bull energy drink on repeated sprint performance in women athletes. *Amino Acids, 42*(5), 1803-8. doi:10.1007/s00726-011-0900-8

Aubert, A. E., Seps, B., & Beckers, F. (2003). Heart rate variability in athletes. *Sports Medicine (Auckland, N.Z.), 33*(12), 889-919.

Bardet, S., Pasqual, C., Maugendre, D., Remy, J. P., Charbonnel, B., & Sai, P. (1989). Inter and intra individual variability of acute insulin response during intravenous glucose tolerance tests. *Diabete & Metabolisme, 15*(5), 224-232.

Berger, R. D., Akselrod, S., Gordon, D., & Cohen, R. J. (1986). An efficient algorithm for spectral analysis of heart rate variability. *IEEE Transactions on Bio-Medical Engineering, 33*(9), 900-904. doi:10.1109/TBME.1986.325789

Berntson, G. G., Bigger, J. T.,Jr, Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., . . . van der Molen, M. W. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology, 34*(6), 623-648.

Bichler, A., Swenson, A., & Harris, M. A. (2006). A combination of caffeine and taurine has no effect on short term memory but induces changes in heart rate and mean arterial blood pressure. *Amino Acids, 31*(4), 471-476. doi:10.1007/s00726-005-0302-x

Bigger, J. T.,Jr, Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., & Rottman, J. N. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation, 85*(1), 164-171.

Billman, G. E. (2011). Heart rate variability – A historical perspective *Front Physiol., 2*(86)

Biltz, G. R., Harmon, J. H., Dengel, D. R., Unnithan, V. B., & Witten, G. (2007). RER variability analysis by sample entropy: A preliminary comparison of obese and lean children. [Abstract]. *Acta Kinesiologiae Universitatis Tartuensis, 12* 53. 24th Pediatric Work Physiology Meeting Children and Exercise

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet, 1*(8476), 307-310.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise, 14*(5), 377-381.

Bornas, X., Llabres, J., Noguera, M., & Pez, A. (2006). Sample entropy of ECG time series of fearful flyers: Preliminary results. *Nonlinear Dynamics, Psychology, and Life Sciences, 10*(3), 301-318.

Brian J., P. W., & Wasserman, K. (2005). *Principles of exercise testing and interpretation: Including pathophysiology and clinical applications* Philadelphia : Lippincott Williams & Wilkins, c2005.

Brooks, G. H., Fahey, T. D., & Baldwin, K. D. S. (2005). *Exercise physiology: Human bioenergetics and its applications* Boston : McGraw-Hill, c2005.

Burke, L. M. (2008). Caffeine and sports performance. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme, 33*(6), 1319-1334. doi:10.1139/H08-130; 10.1139/H08-130

Burrows, T., Pursey, K., Neve, M., & Stanwell, P. (2013). What are the health implications associated with the consumption of energy drinks? A systematic review. *Nutrition Reviews, 71*(3), 135-148. doi:10.1111/nure.12005; 10.1111/nure.12005

Campbell, B., Wilborn, C., La Bounty, P., Taylor, L., Nelson, M. T., Greenwood, M., . . . Kreider, R. B. (2013). International society of sports nutrition position stand: Energy drinks. *Journal of the International Society of Sports Nutrition, 10*(1), 1-2783-10-1. doi:10.1186/1550-2783-10-1; 10.1186/1550-2783-10-1

Candow, D. G., Kleisinger, A. K., Grenier, S., & Dorsch, K. D. (2009). Effect of sugar-free red bull energy drink on high-intensity run time-to-exhaustion in young adults. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 23*(4), 1271-1275. doi:10.1519/JSC.0b013e3181a026c2

Chen, C. C., Chen, R. H., Chang, C. T., & Cheah, N. (1996). Reproducibility of the first-phase insulin release in the intravenous glucose tolerance test. *Journal of the Formosan Medical Association = Taiwan Yi Zhi, 95*(8), 639-641.

Cheng, B., Kuipers, H., Snyder, A. C., Keizer, H. A., Jeukendrup, A., & Hesselink, M. (1992). A new approach for the determination of ventilatory and lactate thresholds. *International Journal of Sports Medicine, 13*(7), 518-522.

Cipryan, L., & Litschmannova, M. (2013). Intra-day and inter-day reliability of heart rate variability measurement. *Journal of Sports Sciences, 31*(2), 150-158. doi:10.1080/02640414.2012.721931; 10.1080/02640414.2012.721931

Clauson, K. A., Shields, K. M., McQueen, C. E., & Persad, N. (2008). Safety issues associated with commercially available energy drinks. *Journal of the American*

Pharmacists Association : JAPhA, 48(3), e55-63; quiz e64-7. doi:10.1331/JAPhA.2008.07055

Costill, D. L., Dalsky, G. P., & Fink, W. J. (1978). Effects of caffeine ingestion on metabolism and exercise performance. *Medicine and Science in Sports, 10*(3), 155-158.

Cox, G. R., Desbrow, B., Montgomery, P. G., Anderson, M. E., Bruce, C. R., Macrides, T. A., . . . Burke, L. M. (2002). Effect of different protocols of caffeine intake on metabolism and endurance performance. *Journal of Applied Physiology (Bethesda, Md.: 1985), 93*(3), 990-999. doi:10.1152/japplphysiol.00249.2002

Davis, J. K., & Green, J. M. (2009). Caffeine and anaerobic performance: Ergogenic value and mechanisms of action. *Sports Medicine (Auckland, N.Z.), 39*(10), 813-832. doi:10.2165/11317770-000000000-00000

Del Coso, J., Portillo, J., Munoz, G., Abian-Vicen, J., Gonzalez-Millan, C., & Munoz-Guerra, J. (2013). Caffeine-containing energy drink improves sprint performance during an international rugby sevens competition. *Amino Acids, 44*(6), 1511-9. doi:10.1007/s00726-013-1473-5

Dempster, P., & Aitkens, S. (1995). A new air displacement method for the determination of human body composition. *Medicine and Science in Sports and Exercise, 27*(12), 1692-1697.

Denadai, B. S., & Denadai, M. L. (1998). Effects of caffeine on time to exhaustion in exercise performed below and above the anaerobic threshold. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas e Biologicas / Sociedade Brasileira De Biofisica ...[Et Al.], 31*(4), 581-585.

Doherty, M., & Smith, P. M. (2005). Effects of caffeine ingestion on rating of perceived exertion during and after exercise: A meta-analysis. *Scandinavian Journal of Medicine & Science in Sports, 15*(2), 69-78. doi:10.1111/j.1600-0838.2005.00445.x

Eckberg, D. L. (1997). Sympathovagal balance: A critical appraisal. *Circulation, 96*(9), 3224-3232.

Finnegan, D. (2003). The health effects of stimulant drinks. *Br Nutr found Nutr Bull, 28*, 147–155.

Forbes, S. C., Candow, D. G., Little, J. P., Magnus, C., & Chilibeck, P. D. (2007). Effect of red bull energy drink on repeated wingate cycle performance and bench-press muscle endurance. *International Journal of Sport Nutrition and Exercise Metabolism, 17*(5), 433-444.

Froiland, K., Koszewski, W., Hingst, J., & Kopecky, L. (2004). Nutritional supplement use among college athletes and their sources of information. *International Journal of Sport Nutrition and Exercise Metabolism, 14*(1), 104-120.

Galgani, J. E., Heilbronn, L. K., Azuma, K., Kelley, D. E., Albu, J. B., Pi-Sunyer, X., . . . Look AHEAD Adipose Research Group. (2008a). Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes, 57*(4), 841-845. doi:10.2337/db08-0043

Galgani, J. E., Heilbronn, L. K., Azuma, K., Kelley, D. E., Albu, J. B., Pi-Sunyer, X., . . . Look AHEAD Adipose Research Group. (2008b). Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes, 57*(4), 841-845. doi:10.2337/db08-0043

Goedecke, J. H., St Clair Gibson, A., Grobler, L., Collins, M., Noakes, T. D., & Lambert, E. V. (2000). Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *American Journal of Physiology.Endocrinology and Metabolism, 279*(6), E1325-34.

Goodpaster, B. H., & Kelley, D. E. (2002). Skeletal muscle triglyceride: Marker or mediator of obesity-induced insulin resistance in type 2 diabetes mellitus? *Current Diabetes Reports, 2*(3), 216-222.

Graham, T. E. (2001). Caffeine and exercise: Metabolism, endurance and performance. *Sports Medicine (Auckland, N.Z.), 31*(11), 785-807.

Graham, T. E., & Spriet, L. L. (1991). Performance and metabolic responses to a high caffeine dose during prolonged exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 71*(6), 2292-2298.

Graham, T. E., & Spriet, L. L. (1995). Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *Journal of Applied Physiology (Bethesda, Md.: 1985), 78*(3), 867-874.

Heffernan, K. S., Fahs, C. A., Shinsako, K. K., Jae, S. Y., & Fernhall, B. (2007). Heart rate recovery and heart rate complexity following resistance exercise training and detraining in young men. *American Journal of Physiology.Heart and Circulatory Physiology, 293*(5), H3180-6. doi:10.1152/ajpheart.00648.2007

Helge, J. W., Fraser, A. M., Kriketos, A. D., Jenkins, A. B., Calvert, G. D., Ayre, K. J., & Storlien, L. H. (1999). Interrelationships between muscle fibre type, substrate oxidation and body fat. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 23*(9), 986-991.

Henriques, T., Goncalves, H., Antunes, L., Matias, M., Bernardes, J., & Costa-Santos, C. (2013). Entropy and compression: Two measures of complexity. *Journal of Evaluation in Clinical Practice,* doi:10.1111/jep.12068; 10.1111/jep.12068

Hibino, G., Moritani, T., Kawada, T., & Fushiki, T. (1997). Caffeine enhances modulation of parasympathetic nerve activity in humans: Quantification using power spectral analysis. *The Journal of Nutrition, 127*(7), 1422-1427.

Hoffman, J. R., Faigenbaum, A. D., Ratamess, N. A., Ross, R., Kang, J., & Tenenbaum, G. (2008). Nutritional supplementation and anabolic steroid use in adolescents. *Medicine and Science in Sports and Exercise, 40*(1), 15-24. doi:10.1249/mss.0b013e31815a5181

Huxtable, R. J. (1992). Physiological actions of taurine. *Physiological Reviews, 72*(1), 101-163.

Ivy, J. L., Kammer, L., Ding, Z., Wang, B., Bernard, J. R., Liao, Y. H., & Hwang, J. (2009). Improved cycling time-trial performance after ingestion of a caffeine energy drink. *International Journal of Sport Nutrition and Exercise Metabolism, 19*(1), 61-78.

Jeanrenaud, B., & Rohner-Jeanrenaud, F. (2001). Effects of neuropeptides and leptin on nutrient partitioning: Dysregulations in obesity. *Annual Review of Medicine, 52*, 339-351. doi:10.1146/annurev.med.52.1.339

Jeukendrup, A. E. (2004). Carbohydrate intake during exercise and performance. *Nutrition (Burbank, Los Angeles County, Calif.), 20*(7-8), 669-677. doi:10.1016/j.nut.2004.04.017

Juhl, C. B., Schmitz, O., Pincus, S., Holst, J. J., Veldhuis, J., & Porksen, N. (2000). Short-term treatment with GLP-1 increases pulsatile insulin secretion in type II diabetes with no effect on orderliness. *Diabetologia, 43*(5), 583-588.

Kang, J., Mangine, G. T., Ratamess, N. A., Faigenbaum, A. D., & Hoffman, J. R. (2007). Influence of intensity fluctuation on exercise metabolism. *European Journal of Applied Physiology, 100*(3), 253-260. doi:10.1007/s00421-007-0424-9

Kang, J., Robertson, R. J., Goss, F. L., DaSilva, S. G., Visich, P., Suminski, R. R., . . . Denys, B. C. (1996). Effect of carbohydrate substrate availability on ratings of perceived exertion during prolonged exercise of moderate intensity. *Perceptual and Motor Skills, 82*(2), 495-506.

Kaplan, D. T., Furman, M. I., Pincus, S. M., Ryan, S. M., Lipsitz, L. A., & Goldberger, A. L. (1991). Aging and the complexity of cardiovascular dynamics. *Biophysical Journal, 59*(4), 945-949.

Katona, P. G., & Jih, F. (1975). Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic cardiac control. *Journal of Applied Physiology, 39*(5), 801-805.

Kaufman, C. L., Kaiser, D. R., Steinberger, J., Kelly, A. S., & Dengel, D. R. (2007). Relationships of cardiac autonomic function with metabolic abnormalities in childhood obesity. *Obesity (Silver Spring, Md.), 15*(5), 1164-1171.

Kazemi, F., Gaeini, A., Kordi, M., & Rahnama, N. (2009). The acute effects of two energy drinks on endurance performance in female athlete students. *Sport Sciences for Health, 5*(2)

Kelley, D. E. (2005). Skeletal muscle fat oxidation: Timing and flexibility are everything. *The Journal of Clinical Investigation, 115*(7), 1699-1702. doi:10.1172/JCI25758

Kelley, D. E., & Mandarino, L. J. (2000). Fuel selection in human skeletal muscle in insulin resistance: A reexamination. *Diabetes, 49*(5), 677-683.

King, J. C., Hambidge, K. M., Westcott, J. L., Kern, D. L., & Marshall, G. (1994). Daily variation in plasma zinc concentrations in women fed meals at six-hour intervals. *The Journal of Nutrition, 124*(4), 508-516.

Klug, B., Nielsen, K. G., & Bisgaard, H. (2000). Observer variability of lung function measurements in 2-6-yr-old children. *The European Respiratory Journal : Official Journal of the European Society for Clinical Respiratory Physiology, 16*(3), 472-475.

Koh, J., Brown, T. E., Beightol, L. A., Ha, C. Y., & Eckberg, D. L. (1994). Human autonomic rhythms: Vagal cardiac mechanisms in tetraplegic subjects. *The Journal of Physiology, 474*(3), 483-495.

Kovacs, E. M., Stegen, J. H. C. H., & Brouns, F. (1998). Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *Journal of Applied Physiology (Bethesda, Md.: 1985), 85*(2), 709-715.

Kuss, O., Schumann, B., Kluttig, A., Greiser, K. H., & Haerting, J. (2008). Time domain parameters can be estimated with less statistical error than frequency domain parameters in the analysis of heart rate variability. *Journal of Electrocardiology, 41*(4), 287-291. doi:10.1016/j.jelectrocard.2008.02.014; 10.1016/j.jelectrocard.2008.02.014

Kuusela, T. A., Jartti, T. T., Tahvanainen, K. U., & Kaila, T. J. (2002). Nonlinear methods of biosignal analysis in assessing terbutaline-induced heart rate and blood pressure changes. *American Journal of Physiology.Heart and Circulatory Physiology, 282*(2), H773-83. doi:10.1152/ajpheart.00559.2001

Lake, D. E., Richman, J. S., Griffin, M. P., & Moorman, J. R. (2002). Sample entropy analysis of neonatal heart rate variability. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 283*(3), R789-97. doi:10.1152/ajpregu.00069.2002

Laplaud, D., & Menier, R. (2003). Reproducibility of the instant of equality of pulmonary gas exchange and its physiological significance. *The Journal of Sports Medicine and Physical Fitness, 43*(4), 437-443.

Lewis, M. J., & Short, A. L. (2007). Sample entropy of electrocardiographic RR and QT time-series data during rest and exercise. *Physiological Measurement, 28*(6), 731-744. doi:10.1088/0967-3334/28/6/011

Macfarlane, D. J., & Wu, H. L. (2013). Inter-unit variability in two ParvoMedics TrueOne 2400 automated metabolic gas analysis systems. *European Journal of*

Applied Physiology, 113(3), 753-762. doi:10.1007/s00421-012-2483-9; 10.1007/s00421-012-2483-9

Marshall, J. M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiological Reviews, 74*(3), 543-594.

Martini, G., Riva, P., Rabbia, F., Molini, V., Ferrero, G. B., Cerutti, F., . . . Veglio, F. (2001). Heart rate variability in childhood obesity. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society, 11*(2), 87-91.

McQuilkin, S. H., Nierenberg, D. W., & Bresnick, E. (1995). Analysis of within-subject variation of caffeine metabolism when used to determine cytochrome P4501A2 and N-acetyltransferase-2 activities. *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology, 4*(2), 139-146.

Mendonca, G. V., Fernhall, B., Heffernan, K. S., & Pereira, F. D. (2009). Spectral methods of heart rate variability analysis during dynamic exercise. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society, 19*(4), 237-245. doi:10.1007/s10286-009-0018-1

Miller, S. L., Maresh, C. M., Armstrong, L. E., Ebbeling, C. B., Lennon, S., & Rodriguez, N. R. (2002). Metabolic response to provision of mixed protein-carbohydrate supplementation during endurance exercise. *International Journal of Sport Nutrition and Exercise Metabolism, 12*(4), 384-397.

Montano, N., Ruscone, T. G., Porta, A., Lombardi, F., Pagani, M., & Malliani, A. (1994). Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation, 90*(4), 1826-1831.

Nagai, N., Matsumoto, T., Kita, H., & Moritani, T. (2003). Autonomic nervous system activity and the state and development of obesity in japanese school children. *Obesity Research, 11*(1), 25-32.

Nelson, M. T., Biltz, G. R., Winsley, R. J., Armstrong, N., Fawkner, S. G., Bywater, K., & Dengel, D. R. (2008a). Reliability of heart rate variability by sample entropy at rest and during light exercise in children [Abstract]. *ACSM Annual Meeting,*

Nelson, M. T., Biltz, G. R., Winsley, R. J., Roberts, A. C., Armstrong, N., Stoedefalke, K., & Dengel, D. R. (2008b). Sample entropy assessment of short term training on heart rate variability *North American Society of Pediatric Exercise Medicine Annual Meeting,* Colorado Springs, CO.

Niewiadomski, W., Gasiorowska, A., Krauss, B., Mroz, A., & Cybulski, G. (2007). Suppression of heart rate variability after supramaximal exertion. *Clinical Physiology and Functional Imaging, 27*(5), 309-319. doi:10.1111/j.1475-097X.2007.00753.x

Noakes, T. D., Lambert, E. V., Lambert, M. I., McArthur, P. S., Myburgh, K. H., & Benade, A. J. (1988). Carbohydrate ingestion and muscle glycogen depletion during marathon and ultramarathon racing. *European Journal of Applied Physiology and Occupational Physiology, 57*(4), 482-489.

Nunan, D., Sandercock, G. R., & Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and Clinical Electrophysiology : PACE, 33*(11), 1407-1417. doi:10.1111/j.1540-8159.2010.02841.x; 10.1111/j.1540-8159.2010.02841.x

Pasman, W. J., van Baak, M. A., Jeukendrup, A. E., & de Haan, A. (1995). The effect of different dosages of caffeine on endurance performance time. *International Journal of Sports Medicine, 16*(4), 225-230.

Pereira, M. A., Weggemans, R. M., Jacobs, D. R.,Jr, Hannan, P. J., Zock, P. L., Ordovas, J. M., & Katan, M. B. (2004). Within-person variation in serum lipids: Implications for clinical trials. *International Journal of Epidemiology, 33*(3), 534-541. doi:10.1093/ije/dyh057

Piccirillo, G., Magri, D., Naso, C., di Carlo, S., MoisE, A., De Laurentis, T., . . . Nocco, M. (2004). Factors influencing heart rate variability power spectral analysis during controlled breathing in patients with chronic heart failure or hypertension and in healthy normotensive subjects. *Clinical Science (London, England : 1979), 107*(2), 183-190. doi:10.1042/CS20030401

Pincus, S., & Kalman, R. E. (1997). Not all (possibly) "random" sequences are created equal. *Proceedings of the National Academy of Sciences of the United States of America, 94*(8), 3513-3518.

Pincus, S. M. (1991). Approximate entropy as a measure of system complexity. *Proceedings of the National Academy of Sciences of the United States of America, 88*(6), 2297-2301.

Pincus, S. M. (2000a). Irregularity and asynchrony in biologic network signals. *Methods in Enzymology, 321*, 149-182.

Pincus, S. M. (2000b). Orderliness of hormone release. *Novartis Foundation Symposium, 227*, 82-96; discussion 96-104.

Pincus, S. M., Gladstone, I. M., & Ehrenkranz, R. A. (1991). A regularity statistic for medical data analysis. *Journal of Clinical Monitoring, 7*(4), 335-345.

Pincus, S. M., Veldhuis, J. D., & Rogol, A. D. (2000). Longitudinal changes in growth hormone secretory process irregularity assessed transpubertally in healthy boys. *American Journal of Physiology.Endocrinology and Metabolism, 279*(2), E417-24.

Pinna, G. D., Maestri, R., Torunski, A., Danilowicz-Szymanowicz, L., Szwoch, M., La Rovere, M. T., & Raczak, G. (2007). Heart rate variability measures: A fresh look at

reliability. *Clinical Science (London, England : 1979), 113*(3), 131-140. doi:10.1042/CS20070055

Platisa, M. M., & Gal, V. (2006a). Dependence of heart rate variability on heart period in disease and aging. *Physiological Measurement, 27*(10), 989-998. doi:10.1088/0967-3334/27/10/005

Platisa, M. M., & Gal, V. (2006b). Reflection of heart rate regulation on linear and nonlinear heart rate variability measures. *Physiological Measurement, 27*(2), 145-154. doi:10.1088/0967-3334/27/2/005

Porta, A., Gnecchi-Ruscone, T., Tobaldini, E., Guzzetti, S., Furlan, R., & Montano, N. (2007). Progressive decrease of heart period variability entropy-based complexity during graded head-up tilt. *Journal of Applied Physiology (Bethesda, Md.: 1985), 103*(4), 1143-1149. doi:10.1152/japplphysiol.00293.2007

Rabbia, F., Silke, B., Conterno, A., Grosso, T., De Vito, B., Rabbone, I., . . . Veglio, F. (2003). Assessment of cardiac autonomic modulation during adolescent obesity. *Obesity Research, 11*(4), 541-548. doi:10.1038/oby.2003.76

Rambihar VS. (2010). Complexity: The science for medicine and the human story. *Lancet, Apr 3*(375(9721)), 1162.

Rauh, R., Burkert, M., Siepmann, M., & Mueck-Weymann, M. (2006). Acute effects of caffeine on heart rate variability in habitual caffeine consumers. *Clinical Physiology and Functional Imaging, 26*(3), 163-166. doi:10.1111/j.1475-097X.2006.00663.x

Richman, J. S., & Moorman, J. R. (2000). Physiological time-series analysis using approximate entropy and sample entropy. *American Journal of Physiology.Heart and Circulatory Physiology, 278*(6), H2039-49.

Riva, P., Martini, G., Rabbia, F., Milan, A., Paglieri, C., Chiandussi, L., & Veglio, F. (2001). Obesity and autonomic function in adolescence. *Clinical and Experimental Hypertension (New York, N.Y.: 1993), 23*(1-2), 57-67.

Rohner-Jeanrenaud, F. (1999). Neuroendocrine regulation of nutrient partitioning. *Annals of the New York Academy of Sciences, 892*, 261-271.

Ryan, S. M., Goldberger, A. L., Pincus, S. M., Mietus, J., & Lipsitz, L. A. (1994). Gender- and age-related differences in heart rate dynamics: Are women more complex than men? *Journal of the American College of Cardiology, 24*(7), 1700-1707.

Sacknoff, D. M., Gleim, G. W., Stachenfeld, N., & Coplan, N. L. (1994). Effect of athletic training on heart rate variability. *American Heart Journal, 127*(5), 1275-1278.

Sandercock, G. R., Bromley, P. D., & Brodie, D. A. (2005a). The reliability of short-term measurements of heart rate variability. *International Journal of Cardiology, 103*(3), 238-247. doi:10.1016/j.ijcard.2004.09.013

Sandercock, G. R., Bromley, P. D., & Brodie, D. A. (2005b). The reliability of short-term measurements of heart rate variability. *International Journal of Cardiology, 103*(3), 238-247. doi:10.1016/j.ijcard.2004.09.013

Schaffer, S. W., & Azuma, J. (1992). Review: Myocardial physiological effects of taurine and their significance. *Advances in Experimental Medicine and Biology, 315*, 105-120.

Scott, A. S., Eberhard, A., Ofir, D., Benchetrit, G., Dinh, T. P., Calabrese, P., . . . Perrault, H. (2004). Enhanced cardiac vagal efferent activity does not explain training-induced bradycardia. *Autonomic Neuroscience : Basic & Clinical, 112*(1-2), 60-68. doi:10.1016/j.autneu.2004.04.006

Seely, A. J., & Macklem, P. T. (2004). Complex systems and the technology of variability analysis. *Critical Care (London, England), 8*(6), R367-84. doi:10.1186/cc2948

Sesay, M., Tauzin-Fin, P., Gosse, P., Ballanger, P., & Maurette, P. (2008). Real-time heart rate variability and its correlation with plasma catecholamines during laparoscopic adrenal pheochromocytoma surgery. *Anesthesia and Analgesia, 106*(1), 164-70, table of contents. doi:10.1213/01.ane.0000289531.18937.0a

Shah , S., Lacey, C., & Riddock, I.,. (2013). Impact of energy drinks on electrocardiographic and blood pressure parameters: A meta-analysis of clinical studies. [Abstract]. *Epi\npam 2013, Abstract P324.*(New Orleans, LA, March 21, 2013)

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: Uses in assessing rater reliability. *Psychological Bulletin, 86*(2), 420-428.

Siri, W. E. (1993). Body composition from fluid spaces and density: Analysis of methods. 1961. *Nutrition (Burbank, Los Angeles County, Calif.), 9*(5), 480-91; discussion 480, 492.

Spriet, L. L., MacLean, D. A., Dyck, D. J., Hultman, E., Cederblad, G., & Graham, T. E. (1992). Caffeine ingestion and muscle metabolism during prolonged exercise in humans. *The American Journal of Physiology, 262*(6 Pt 1), E891-8.

Steinke, L., Lanfear, D. E., Dhanapal, V., & Kalus, J. S. (2009). Effect of "energy drink" consumption on hemodynamic and electrocardiographic parameters in healthy young adults. *The Annals of Pharmacotherapy, 43*(4), 596-602. doi:10.1345/aph.1L614

Storlien, L., Oakes, N. D., & Kelley, D. E. (2004). Metabolic flexibility. *The Proceedings of the Nutrition Society, 63*(2), 363-368. doi:10.1079/PNS2004349

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation, 93*(5), 1043-1065.

Toubro, S., Sorensen, T. I., Hindsberger, C., Christensen, N. J., & Astrup, A. (1998). Twenty-four-hour respiratory quotient: The role of diet and familial resemblance. *The Journal of Clinical Endocrinology and Metabolism, 83*(8), 2758-2764.

Veldhuis, J. D., Johnson, M. L., Veldhuis, O. L., Straume, M., & Pincus, S. M. (2001). Impact of pulsatility on the ensemble orderliness (approximate entropy) of neurohormone secretion. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 281*(6), R1975-85.

Veldman, R. G., Frolich, M., Pincus, S. M., Veldhuis, J. D., & Roelfsema, F. (2000). Growth hormone and prolactin are secreted more irregularly in patients with cushing's disease. *Clinical Endocrinology, 52*(5), 625-632.

West, B. J. (2006). *Where medicine went wrong: Rediscovering the path to complexity (studies of nonlinear phenomena in life science)* (1st ed.). NJ: World Scientific Publishing Company.

Wiklund, U., Karlsson, M., Ostrom, M., & Messner, T. (2009). Influence of energy drinks and alcohol on post-exercise heart rate recovery and heart rate variability. *Clinical Physiology and Functional Imaging, 29*(1), 74-80. doi:10.1111/j.1475-097X.2008.00837.x; 10.1111/j.1475-097X.2008.00837.x

Willoughby, S. R. (2009). Energy drink effects platelet aggregation and endothelial function: A possible link to increased cardiovascular risk *Heart Lung and Circulation, 18*, S265. doi:10.1016/j.hlc.2009.05.653

Wilmore, J. H., & Costill, D. L. (2007). *Physiology of sport and exercise, fourth edition* Human Kinetics Publishers.

Yentes, J. M., Hunt, N., Schmid, K. K., Kaipust, J. P., McGrath, D., & Stergiou, N. (2012). The appropriate use of approximate entropy and sample entropy with short data sets. *Annals of Biomedical Engineering,* doi:10.1007/s10439-012-0668-3

Yeragani, V. K., Krishnan, S., Engels, H. J., & Gretebeck, R. (2005). Effects of caffeine on linear and nonlinear measures of heart rate variability before and after exercise. *Depression and Anxiety, 21*(3), 130-134. doi:10.1002/da.20061