Respiratory Exchange Ratio Variability in Novice College-age Marathon Runners

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Dedication

I dedicate this to my mother Lou Ann and my best friend and partner Sarah Ott!

Abstract

The aim of this study was to assess the effects of 16 weeks of marathon training on breath-to-breath (btb) respiratory exchange ratio (RER) variability, measured by sample entropy (SampEn), in college-age, healthy novice marathon runners. Average SampEn scores for participants increased with marathon training. SampEn analysis of RER time series may detect trainability, metabolic adaptations, or provide substrate utilization recommendations with endurance training.

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Metabolism has been at the forefront of scientific research due to its importance in maintaining a healthy lifestyle. Unfortunately there has been a steep rise of individuals with metabolic disorders in world, which has resulted in an increased demand for metabolic research (Kelley, Goodpaster, Wing, & Simoneau, 1999). Metabolism and exercise are closely integrated; therefore studies assessing the impact of exercise training will remain critical in the fight to curb the growing number of metabolic disorders.

One way of assessing changes in metabolism is by investigating an individual's respiratory exchange ratio, (RER) (Goodpaster, Wolfe, & Kelley, 2002). Unfortunately, there is limited information that can be derived from looking at the respiratory exchange ratio under a certain set of circumstances (resting vs. exercising). The standard use of linear-based methods, such as calculating means and standard deviation of a person's RER to assess their metabolism is not sufficient to capture the inherent variability of metabolism within the human body. Non-linear analysis methods are quickly becoming commonly used to explore the depth and complexity of human physiology (Pincus & Huang, 1992; Richman & Moorman, 2000; West, 2006). Within the inherent variability in humans there may be valuable information that may be the key to understanding metabolism from both a clinical and sports performance perspective.

Background

RER variability as a potential marker for metabolic health was developed from the work examining substrate utilization differences between obese individuals and healthy controls (Kelley et al., 1999). In a study that compared leg respiratory quotient (RQ) in obese vs. lean individuals during a fasted and insulin stimulated state it was found that obese individuals had a higher RQ during fasting conditions and the RQ did not change under insulin-stimulated conditions (Kelley et al., 1999). In comparison, lean individuals had lower RQ during the fasted state, and were able to switch to increased carbohydrate metabolism in insulin-stimulated conditions, both indicative of a healthy metabolism. The same result of inability to switch between substrates was also found in a study comparing fuel selection during exercise for lean vs. obese subjects (Goodpaster et al., 2002). The inability to switch substrate use based on demand was termed, metabolic inflexibility. Metabolic inflexibility refers the lack of adaptability between substrate flows in accordance with changing physiological conditions and demands within the body (Corpeleijn, Saris, & Blaak, 2009). Specifically, increased fat oxidation during fasting conditions or at low exercise intensities, and carbohydrate metabolism during insulin-stimulated conditions or at high exercise intensities. Metabolic inflexibility has been shown in many studies where obese individuals are compared to non-obese individuals (Corpeleijn et al., 2009; Goodpaster et al., 2002).

There has been little exploration into RER variability using non-linear analysis methods. Variations in breath-to-breath (btb) metabolic gas data have shown both short

and long-term variability (Cadena Méndez, Rodriguez, Medel, Infante, & Escalante, 2008). These variations may be more pronounced in obese subjects. An exercise intervention study observed that obese children tend to have less RER variability, measured using a non-linear analysis technique called sample entropy (SampEn), compared to lean children, but the difference was not statistically significant (Biltz, Harmon, Dengel, Unnithan, & Witten, 2008). Biltz et al. (2011) found a statistically significant difference ($p=0.03$) in RER variability, measured by sample entropy, between trained and untrained female adolescent soccer players. Interestingly, there was also great variation between the sample entropy scores of the trained players. This supports what Goedecke et al. (2000) found when observing average RER values of trained cyclists at different exercise intensities. Both resting RER and RER at different workloads varied for the trained cyclists (Goedecke et al., 2000). This suggests that the underlying metabolism of humans is highly variable regardless of training status.

Published research is lacking specific to analyzed RER variability using non-linear methods, but the precedence for non-linear analysis of physiological data has been established. The focus of non-linear dynamics research has been heart rate variability (HRV), and the clinical applications of such methods. The research contains an extensive collection of studies on HRV and its clinical application. Recently there has been a wave of studies that have focused on the use of HRV in the sports performance domain (Bailón, Garatachea, de la Iglesia, Casajús, & Laguna, 2013; Bravi, Longtin, & Seely, 2011; Kaikkonen, Hynynen, Mann, Rusko, & Nummela, 2012; Plews, Laursen, Kilding, &

Buchheit, 2012). Two studies specifically investigated changes in HRV in marathon runners after training, and used non-linear dynamics to assess the physiology of sports performance (Lucia, Oliván, Bravo, Gonzalez-Freire, & Foster, 2008; Tulppo et al., 2003). There is a need for more research specific to the variation in metabolism along with exploration of the use of non-linear analysis methods in assessing physiological responses to exercise.

Variables and Hypothesis

With known deficiencies in research, the aim of this study was to determine the effect of marathon training on RER variability, as measured by SampEn, in healthy college-age novice marathon runners. A secondary aim is to determine if breath-to-breath (btb) RER SampEn changes correlate with changes in average RER, % body fat, 2-mile time, and VO_{2max} following marathon training. The hypothesis is RER variability, measured by SampEn, will increase from pre-marathon training intervention to post-marathon training intervention in college students enrolled in the marathon-training course at the University of Minnesota.

Significance

This study is significant because it may show that there is an analysis method that may give additional insight into individual metabolism through a non-invasive means. In the clinical realm, the study may provide health care professionals with a quick non-invasive test that would allow for quick diagnosis of alterations to an individual's metabolism. Human performance studies may be able to use these results

to assess adaptations to exercise training. From a sports performance perspective, this study may provide insight into improvements in an athlete's metabolism and the affects on sports performance. The results of this study have the potential to the lay groundwork for using non-linear analysis methods to assess the complexity of metabolism non-invasively.

CHAPTER 2: Literature Review

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2.1 Introduction

This literature review will start with an introduction to non-linear analysis methods along with applications of the methods in the literature. Special attention will be paid to the analysis method that will be used to analyze the data, sample entropy. From there, the focus will be on the history of respiratory exchange ratio along with clinical and sports performance studies, which have utilized respiratory exchange ratio as a non-invasive measure of metabolism. This section will conclude with a discussion on variability in RER among trained athletes and discuss the studies on which this research study was founded on.

2.2 Non-Linear Dynamics

Fractals, non-linear dynamics, and chaos theory have all been used in an attempt to understanding the complexity of the human body (Peng et al., 2002; Richman & Moorman, 2000; Stergiou, 2004; Weiss, Garfinkel, Spano, & Ditto, 1994; West, 2006). Pioneering researchers in this field, provided insight into a way of analyzing human physiology and anatomy using non-linear dynamics (Goldberger & West, 1987). They recognized the complexity of human anatomy and physiology and chose to apply non-linear dynamics to study the fractal (complexity beyond looking at the geometry of shape from a birds-eye view) nature of the human body. Specifically, whether or not there were inherent mechanisms that controlled the size of the bronchioles in the lung or the shape of the vasculature within the heart and muscles (Goldberger & West,

1987). They were also interested in whether or not regular physiology is truly regular or is there inherent variability in the signal (Goldberger & West, 1987). What was found is that not only does the physiology of the human body exhibit variability, but by looking at the variability within a physiologic signal there was important clinical information that could indicate a disease state of an individual (Ferrario, Signorini, Magenes, & Cerutti, 2006). This introductory research spawned 20+ years of using non-linear dynamics to analyze the human body (Seely & Macklem, 2004). Along the way, several new non-linear analysis techniques were developed (Pincus, Gladstone, & Ehrenkranz, 1991; Richman & Moorman, 2000; Voss, Schulz, Schroeder, Baumert, & Caminal, 2009), and a call for a change in the way the human body is analyzed was established (West, 2006).

2.3 Traditional Variability Analysis Methods

Time and Frequency Domain.

Time and frequency domain analysis have been applied in all areas of science for many years. The use of means and standard deviations to describe biological signals is used in seemingly every scientific study in exercise physiology. Traditional methods looking at the time component of a signal (means, standard deviations) and frequency component (box-plots, normal curve plots) are quick and easy, but fail to give insight into the biological complexity of the system being measured (Seely & Macklem, 2004). Frequency domain analysis use mathematical conversions such as Fourier transform (most commonly used) or wavelet transformations to convert time series data into a graphical representation that looks at the amplitude of the data-generating event (Seely

& Macklem, 2004). Time and frequency analysis both require stationarity of the data-generating event. This means, if the physiological conditions change during data collection, by varying the exercise intensity, the calculation will not give an accurate reflection of the consistency of the signal (Voss et al., 2009). Frequency calculations also require that there is periodicity in the data generating process (fluctuations in the amplitude of the signal), which may not be applicable to all physiological processes (Seely & Macklem, 2004). The use of time and frequency domain analysis techniques will continue, but their application is limited in scope.

Power Law.

Power law methods are used when one would like to gain insight into the fractal nature of the system being studied. The traditional way of doing power law analysis is by plotting the log of the power of the signal versus the log of the frequency of the signal where the slope of the line will be negative one (Seely & Macklem, 2004). The book "Where Medicine Went Wrong" discusses power law curves in great detail (West, 2006). The Richter scale for assessing earthquakes uses this method (Richter, 1935). One interesting application in the biological realm is looking at the movement of cells (Fabry et al., 2001). Like most of the methods discussed in this review, power law curves require stationarity of the data and large data sets (Seely & Macklem, 2004).

Detrended Fluctuation Analysis.

Detrended fluctuation analysis (DFA) is a method that was first developed to analyze the complex nature of deoxyribose nucleic acid (DNA) sequencing (Peng et al.,

1994). The strength of this method is that it can detect long-term fluctuations in data and prevents incorrectly detecting long-range correlations in the data that don't actually exist (Peng et al., 1994). This method excels over traditional power law calculations because it has the ability to remove extrinsic factors (head-tilt during heart rate recording) from intrinsic factors controlling the biological signal (Seely & Macklem, 2004). The primary advantage that DFA offers over other non-linear techniques is that it does not require stationarity of the data (Seely & Macklem, 2004). Ultimately, one can expect to gain insight in the long-term fractal nature of a signal when using DFA. No method comes without limitations though and DFA suffers from the limitation of requiring a large amount of data points (greater than 8000). This non-linear analysis method has been used in a variety of clinical applications: HRV in sleep apnea patients (Penzel, Kantelhardt, Grote, Peter, & Bunde, 2003), electroencephalograhy (EEG) changes in clinical depressed men (Leistedt, Dumont, Lanquart, Jurysta, & Linkowski, 2007), and assessing depth of anesthesia using EEG readings (Jospin et al., 2007), but its usefulness is still debated in the literature (Willson & Francis, 2003).

Poincare Plots.

Poincare plots are a graphical representation of short-term and long-term dynamics of a biological signal (Voss et al., 2009). Most commonly used in HRV studies, Poincare plots provide a visual representation of the complexity of the data by plotting the RR_n interval vs. the RR_{n+1} for HRV (Mourot et al., 2004; Tulppo, Makikallio, Takala, Seppanen, & Huikuri, 1996). From these plots, the short-term standard deviation (SD1)

and long-term standard deviation (SD2) can be calculated along with a ratio of the short to long-term standard deviations (SD1/SD2) (Voss et al., 2009). A normal and healthy beating heart displays itself with a cigar shaped Poincare plot. Poincare plots have been used in exercise studies looking at short and long-term HRV changes to assess sympathetic and parasympathetic changes to heart rate with exercise in sedentary individuals (Tulppo et al., 1996; Tulppo et al., 2003), endurance trained cyclists (Mourot, Bouhaddi, Perrey, Rouillon, & Regnard, 2004), and as a indication of overtraining (Mourot et al., 2004). There have been no stated limitations of using Poincare plots, but there does not appear to be a way to quantify the shape of the plot into a useful metric.

2.4 Non-Linear Variability Analysis Approaches

Approximate Entropy.

In 1991 Pincus developed an analysis method called approximate entropy (ApEn) (Pincus et al., 1991). Entropy measures seek to determine the degree of complexity in a system along with fluctuations in the pattern of the data and follow the second law of thermodynamics, which simply states that the world tends towards disorder. The inherent complexity of human physiology is quantified with a mathematical formula based in chaos theory (Pincus et al., 1991). By using ApEn to analyze physiological time signals one can determine how regular a signal is beyond looking at traditional statistics such as the mean and standard deviation. The method is used to determine the complexity of an ever-adapting system like physiology within the human body (Pincus et al., 1991). Entropy measures give researcher's insight into the complexity of a data-

generating event such as a heart rate or for the purposes of this study, respiratory exchange ratio (RER) (Biltz et al., 2008; Pincus & Viscarello, 1992).

Approximate entropy looks at a data series of length (N) and seeks to determine the pattern of the data within that series. This is done by looking at a defined length (m) of data points within N and determining whether or not consecutive sets of data points the same length as m are similar or not within a specific tolerance (r) (Seely & Macklem, 2004). The frequency of matching sequences is then determined along with the prevalence of the series. Prevalence is determined by taking the negative natural logarithm of the conditional probability (Pincus et al., 1991). Small ApEn values indicate greater regularity, which means that m and m+1 data points do not differ greatly from each other.

One of the main benefits of using ApEn as a non-linear analysis tool is that it only requires as few as 50 data points in order to do the analysis (Seely & Macklem, 2004). This is in stark contrast to other methods, discussed above, which require thousands of data points (Voss et al., 2009). ApEn can be used to analyze physiological systems that do not produce data points at the rate of a heartbeat. Using that advantage, researchers were able to examine fluctuations in growth hormone levels in acromegaly patients every 5 minutes for 24 hours, giving 120 data points (van den Berg, Pincus, Frolich, Veldhuis, & Roelfsema, 1998).

ApEn was the first entropy measure used in a wide range of clinical applications: heart rate variability in premature infants (Lake, Richman, Griffin, & Moorman, 2002),

fetal heart rate changes (Pincus & Viscarello, 1992), EEG changes in Alzheimer's patients (Abásolo et al., 2005), and respiratory changes in panic disorder patients (Caldirola, Bellodi, Caumo, Migliarese, & Perna, 2004). Entropy measures seek to give insight into the health of an individual by looking at how regular the data-generating event is. Typically the more regular the event (lower ApEn score) the higher chance that there is some underlying dysfunction causing the regularity.

The clinical value of being able to detect changes in the regularity of infant's heartbeats, may mean the difference between life and death (Pincus, Cummins, & Haddad, 1993). Heart rate dynamics can be monitored using ApEn and potential disorders can be identified prior to the escalation of a life threatening condition, such as atrial fibrillation (Vikman et al., 1999).

Approximate entropy has also been a popular measure in analyzing the dynamics of human movement (Stergiou, 2004). Postural stability ApEn scores have been found to decrease following a concussion in collegiate athletes (Cavanaugh et al., 2006). Patients who have had their anterior cruciate ligament (ACL) removed also exhibit lower ApEn compared to controls (Georgoulis, Moraiti, Ristanis, & Stergiou, 2006). This lowering of the variability score is indicative of a system, which is unable to respond as well to outside stressors. Approximate entropy has also been used to characterize the development of postural control in developing infants (Harbourne & Stergiou, 2003).

The benefits of using ApEn as a measure in the clinical realm are immeasurable and this will continue to be used to monitor changes in complexity for years to come.

With any measurement technique there are limitations though, luckily the limitations of ApEn have been addressed and a new entropy analysis technique was developed in response to those limitations, sample entropy SampEn (Richman & Moorman, 2000).

Sample Entropy.

Approximate entropy was used for almost ten years as the gold standard for calculating complexity in biological systems. In 2000, the ApEn calculation was revisited and it was determined that there were a few shortcomings of using this statistical method (Richman & Moorman, 2000). Particularly, ApEn is heavily dependent on the length of the recorded data, and for smaller data sets ApEn is calculated to be lower than what it actual should be (Richman & Moorman, 2000). In addition, ApEn lacked consistency when comparing different data sets (Richman & Moorman, 2000). It turns out there is inherent bias with how the ApEn score was calculated based on including self-matches (Seely & Macklem, 2004). Self-matches need to be included in the ApEn calculation to avoid a situation where the natural log of zero needs to be calculated (Seely & Macklem, 2004). By changing how the entropy score was calculated, by not counting self-matches, a new entropy statistic, sample entropy, was developed (Richman & Moorman, 2000).

Sample entropy has been used in a wide variety of clinical studies just like ApEn (Ferrario et al., 2006; Lake et al., 2002; Lewis & Short, 2007; Porta et al., 2007). The first study using SampEn looked at the heart rate variability in neonates and found that

SampEn scores fall before the clinical signs of neonatal sepsis present themselves (Lake et al., 2002).

The benefit of using entropy scores is that they are not as sensitive to noise compared to other non-linear analysis methods. This becomes important because the inherent nature of time series signals from biological systems is to be noisy. Sample entropy is also not effected by missing data points in a series of data (Lake et al., 2002). The largest benefit for using SampEn scores is that they can be obtained from relatively small data sets, 100 points or more without bias being introduced (Seely & Macklem, 2004). Many physiological processes can only generate a small amount of data points in a reasonable time frame. Having this non-linear analysis method only requiring a small amount of data points is beneficial to researchers and allows for the application of SampEn to many potential physiological processes that one would like to study.

Limitations and proposed changes to sample entropy.

The one major limitation to using SampEn is that the data-generating event requires stationarity (Voss et al., 2009). This can easily be accounted for by doing studies where stationarity is built into the study design. There has also been discussion around whether or not SampEn accurately reflects changes to the data generating process. To account for this, a time lag was introduced to the SampEn calculation, but these changes have not been supported further by the literature (Govindan, Wilson, Eswaran, Lowery, & Preibl, 2007). What has received support in the literature is the optimization of the parameters of the SampEn equation. Typically the values for the parameters are m=2

and r=0.1-0.25 times the standard deviation of the data set N (Seely & Macklem, 2004). For most situations where SampEn would be acceptable to use, m=2 and r=0.2*SD seem to be the appropriate values for analysis (Richman & Moorman, 2000).

The research suggests the use and application of non-linear analysis methods are here to stay. There are textbooks dedicated solely to the study of variability analysis in human movement (Stergiou, 2004). The natural progression of the application of these methods may be in sports performance research. There are already examples of non-linear dynamics in the realm of sports performance (Buchheit et al., 2010; Lamoth, van Lummel, & Beek, 2009; Manzi et al., 2009). Just as in the clinic, it appears that variability analysis may provide information beyond means and standard deviations about the nature of human performance.

2.5 Respiratory Exchange Ratio

History and Background

Calorimetry is a technique that has long been used to study the amount of heat given off when a particular chemical substance burns in the presence of oxygen to give off energy, water, and carbon dioxide. From this, spawned indirect calorimetry, which is a scientific technique that estimates how much of a substance was burned based on how much oxygen was consumed during the reaction. Scientists who were interested in the study of metabolism used indirect calorimetry to measure variables affecting normal metabolism (Edwards, Margaria, & Dill, 1934). Measurement of heat production directly in the working cells of a muscle is implausible, so scientists realized that using indirect

calorimetry may be a way to non-invasively determine how much fuel is being burned in the working human body. Specifically, if one were to know the amount of oxygen taken in per breath and the amount of carbon dioxide expelled per breath, knowledge about what type of fuel is being burned could be obtained. Specifically, whether not an individual is burning more fat or carbohydrate. Krough and Lindhard (1920) performed pioneering research showing that a so-called respiratory quotient (RQ) (measure of respiration at the muscle level) could be measured non-invasively by collecting gas samples from the mouth during human respiration (Krogh & Lindhard, 1920). This non-invasive technique was called the respiratory exchange ratio (RER or R). What was later found is that RQ and RER were equal to each other at workloads below one's ventilatory threshold (Jeukendrup & Wallis, 2005).

Respiratory exchange ratio is simply the ratio of carbon dioxide expelled to oxygen taken in during a breath. If a pure fat is being burned the RER for the fat will be equal to approximately 0.7, whereas if pure carbohydrate is being burned (in the form of glucose) the RER will be equal to 1 (Brooks, Fahey, & Baldwin, 2005).

Carbohydrate:

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

RER= $CO_2/\ O_2$ = 6/6 = **1** for glucose

Fat:

$C_{57}H_{104}O_6 + 80\ O_2 \rightarrow 57\ CO_2 + 52\ H_2O$

RER = 57/80 = **0.71** for this particular fat

The reason for this is that it takes more oxygen to burn fat than glucose. It is possible to obtain RERs above 1.0, but that involves the presence of carbon dioxide produced through the buffering of acid by bicarbonate (Naimark, Wasserman, & McIlroy, 1964). In order for RQ to be equal to RER the following assumptions must hold true: all ATP comes from oxidative sources, ATP and creatine phosphate stores remain constant, amino acid and protein catabolism is negligible (Brooks et al., 2005).

The measurement of RER has evolved from collecting gas samples in bags to be analyzed at a later date to the current systems which are able to measure gas samples in real-time while a subject is exercising (Naimark et al., 1964). Even though the technology involved in measuring RER has become highly technical the original principles behind the technique stay true. A wealth of research using RER as an analysis technique has been accumulated over the years (Bergman & Brooks, 1999; Goedecke et al., 2000; Krogh & Lindhard, 1920). The application and use of RER has found its way in every research setting that seeks to look at metabolism, non-invasively, from clinical studies (Goodpaster et al., 2002) to sports performance studies (Goedecke et al., 2000).

RER has been shown to provide information about one's susceptibility to weight gain (Zurlo et al., 1990), ability to spare glycogen in endurance events (Girandola & Katch, 1976), substrate utilization (Jeukendrup & Wallis, 2005), a fitness indicator (Ramos-Jiménez et al., 2008), and monitor the progress of metabolic disorders (Kelley et al., 1999). Typically RER is measured during activity and an average RER value over the activity interval or an instantaneous RER is determined. Based on the magnitude of the RER or the change in RER (if looking at an intervention) one gets a quick, non-invasive, idea of metabolic changes that may be happening. What this project hopes to accomplish is to show that the btb RER signal for humans is variable, and there is information to be discovered by analyzing the variability in the RER signal.

Clinical uses.

Respiratory exchange ratio has been used in experiments that have focused on disturbances to metabolism in a clinical realm (Kelley et al., 1999). Metabolic inflexibility is a term that was coined to describe the effects of disturbed mitochondrial function, an inability to adequately use fat for fuel, and an inability to switch fuel sources based on disturbances in homeostasis (Corpeleijn et al., 2009; Kelley et al., 1999). The inability to readily switch between fats and carbohydrates as a fuel source based on the demand in the body has been linked to obesity and insulin resistance (Corpeleijn et al., 2009). Under fasted and resting conditions a healthy metabolism would rely on primarily fat as fuel, which would be indicated by a lower RER (0.7). Metabolically inflexible individuals have shown that under resting conditions they burn more carbohydrate (CHO) than

their lean counterparts (Kelley et al., 1999). Conversely, at higher intensities of exercise (when CHO should be the primary fuel) the overweight individuals tend to burn more fat than carbohydrate (Kelley et al., 1999). A study comparing obese to lean subjects showed that obese individuals tended to utilize more fat and less glycogen during exercise, and the fat came from non-plasma sources (Goodpaster et al., 2002). This lack of flexibility between fuel sources is an indication of disturbed metabolism and is associated with health risks (Corpeleijn et al., 2009). Kelley, Goodpaster, Wing, & Simoneau (1999) showed that obese individuals had higher resting leg RQs and showed resistance to fat metabolism suppression under insulin-stimulated conditions. This dysfunction in metabolism has been associated with insulin resistance due to increased intramuscular fat stores and a reduced capacity to utilize fat as fuel (Kelley et al., 1999). Lipid derived intermediates tend to accumulate in obese individuals and it may be these intermediates (that accumulate with a lack of exercise), which cause insulin insensitivity (Taube, Eckardt, & Eckel, 2009).

Metabolic inflexibility seems to be reversible with exercise (Goodpaster, Katsiaras, & Kelley, 2003; Meex et al., 2010). In fact, it has been shown that mitochondrial function and metabolic flexibility can be restored in type II diabetes patients following a 12-week exercise program (Meex et al., 2010).

An additional study confirmed that obese individuals who participated in physical activity were able to become more flexible, metabolically, and also had less insulin resistance after an activity intervention (Goodpaster et al., 2003). This suggests that

regular physical activity that aims to lower weight will have beneficial effects that may reverse metabolic disorders. That idea supports research suggesting that leanness along with aerobic fitness is associated with a greater ability to oxidize fat (Kelley, 2005). The results of these studies indicate that even the most metabolically out-of-shape subjects are trainable in terms of restoring a healthy metabolism.

While metabolically inflexibility describes a clinical situation, the ideas around metabolic flexibility may be able to be translated into the sports performance realm, especially, when looking at athletes who may have weight issues, such as lineman in football. Having a healthy metabolism would be important for every athlete, regardless of their sport. There may even be possible performance benefits to individuals who are more metabolically flexible (able to readily switch from one fuel source to another in relatively fast time frames) such as marathon runners who rely on many types of fuel sources to power them through to the finish line. Researchers have investigated how substrate utilization is controlled during exercise (Brooks & Mercier, 1994; Coyle, 1995; Messonnier, Denis, Prieur, & Lacour, 2005).

2.6 Substrate Utilization

There are four main types of fuel sources used during exercise: muscle glycogen, plasma glucose, muscle triglyceride, and plasma free fatty acids (Coyle, 1995). The mixture and flux of these fuels is dependent on many different factors and a complete review of each individual factor is beyond the scope of this review. The relative contribution of each substrate at different exercise intensities is relatively stable. At low

intensities (<25% VO_{2max}) plasma fatty acids are the main fuel source, as intensities increase (~65% VO_{2max}) there is an increased reliance on muscle triglycerides and muscle glycogen, and at high intensities (>85% VO_{2max}) the body relies mostly on muscle glycogen even though use of muscle triglycerides also remains much higher than the amount used at low intensities (Coyle, 1995). Remarkably, the research seems to varied, with some indicating that fuel regulation is tightly regulated and consistent (Bergman & Brooks, 1999; Weber, 2011) while other research suggest a high degree of variability (Goedecke et al., 2000; Romijn et al., 1993). Fat utilization is complex and highly variable (Romijn et al., 1993).

Indirect calorimetry has long been used as a method to quantify the amount of substrate consumed during rest or exercise (Krogh & Lindhard, 1920). The principles around RER provide a clear scientific basis to designate how much fat and carbohydrate is being metabolized. Researcher's have challenged the simplicity of quantifying substrate use using RER and provided additional (more complex) models to quantify substrate utilization (Frayn, 1983). One study, was able to correlate RER with oxygen uptake kinetics during exercise in an attempt to determine fuel use patterns throughout a graded exercise test (Mole & Hoffman, 1999). The researchers were able to develop a kinetic VO_2 model to account for the dynamics of fat and CHO oxidation based on the shape of the VO_2 curve. The model can only account for the dynamics of fuel use during the first 5-10 minutes of exercise, but accounts for some of the variability in fuel use. Others have challenged that metabolism is complex and there are fuel sources other

than fat and glucose that are used during exercise, such as: ketone bodies, lactate, glycogen, and amino acids (Frayn, 1983; Jeukendrup & Wallis, 2005). There is also a suggestion that the degree of lipogenesis and gluconeogenesis that occurs may alter RER values, but this would only apply to methods that record RER over long intervals of time (Frayn, 1983). The major limitation of using RER to quantify substrate utilization is that RER cannot distinguish between different types of fats (Jeukendrup & Wallis, 2005). There are different types of fatty acids from different locations that may be utilized and RER alone cannot distinguish which type of fatty acid is being oxidized (Sidossis, Gastaldelli, Klein, & Wolfe, 1997). These models do address the complexity of metabolism, but the literature suggests that humans are consistent in fuel use patterns at particular exercise intensities and RER serves as a valid measure to quantify fuel use (Weber, 2011).

Lipid oxidation.

Lipid oxidation occurs in the mitochondria of working cells, and lipid oxidation during exercise has been the focus of an extensive amount of studies. Carbohydrate metabolism appears to be directly related to carbohydrate availability in the body (McConell, Snow, Proietto, & Hargreaves, 1999). Lipid metabolism appears to be much more complicated though and many factors may play a role in lipid metabolism (Romijn et al., 1993). There appears to be health (Zurlo et al., 1990) and performance (Horowitz & Klein, 2000) implications to being able to successfully oxidize lipids while sparing glycogen. At high intensities (80% VO_{2max}) of exercise lipid oxidation appears to be

limited to by both availability of lipid at the mitochondria and a direct inhibition of fatty chain entry into mitochondria (Sidossis et al., 1997). There was a long-standing acceptance that fatty acid oxidation inhibited glucose oxidation in working muscle, this inhibition was called the Randle Cycle (Randle, Garland, Hales, & Newsholme, 1963). Studies have shown that the exact opposite appears to be true, glucose oxidation and increases in malonyl-CoA, (product of glucose metabolism) which leads to inhibition of lipid oxidation (Horowitz & Klein, 2000; Kelley & Mandarino, 2000). Fatty acid oxidation has been shown to decrease with increasing exercise intensity (Romijn et al., 1993). Highest fat oxidation rates occurred during exercise of 25% VO_{2max} and were lowest at 85% VO_{2max} in endurance trained cyclists (Romijn et al., 1993). These results possibly confirm the notion that increased glucose oxidation at higher exercise intensities may indeed inhibit lipid oxidation.

A paradox in fat storage is that both endurance athletes and obese individuals store more fat within the muscle. For athletes, this is a healthy adaptation potentially allowing them to oxidize more lipid and spare carbohydrate. For obese individuals, excess muscle triglyceride storage has been linked to insulin resistance (Kelley & Goodpaster, 2001). The research specific to fat use during exercise suggests that fat utilization during exercise has a high degree of individual variability that cannot be accounted for by current methods (Jeukendrup & Wallis, 2005).

There appears to be an endurance training effect that causes individuals to utilize lipids in a greater proportion at a given exercise intensity level compared to

untrained persons (Hawley, 2002; Klein, Coyle, & Wolfe, 1994). Many believe endurance training provides a benefit of glycogen sparring by increased fat oxidation, but there have been studies showing that not all athletes receive that benefit (Bosch, Dennis, & Noakes, 1993; Goedecke et al., 2000; Rauch, Hawley, Woodey, Noakes, & Dennis, 1999). These subjects showed higher rates of CHO utilization, measured by RER, then other subjects with the same level of training (Goedecke et al., 2000; Rauch et al., 1999). Studies have shown that endurance training reduces RER during exercise indicating a shift towards more fat utilization (Bergman & Brooks, 1999; G. A. Brooks & Mercier, 1994; Messonnier et al., 2005; Ramos-Jiménez et al., 2008). Trained cyclists had lower average RER values during two hours of bike riding at 40% VO_{2max} (Bergman & Brooks, 1999). What was interesting about these findings is that the researchers were unable to detect any difference between fat utilization in trained vs. untrained cyclists at exercise intensities greater than 40% VO_{2max} (Bergman & Brooks, 1999). This supports the notion, previously stated, that at exercise intensities greater than 50% VO_{2max} carbohydrate predominates unless there is a shortage of CHO in the body (Goedecke et al., 2000). Bergman and Brooks (1999) made the statement that, "because most athletes train and compete at intensities greater than 40% VO_{2max}, they will not oxidize a greater proportion of lipids compared with untrained subjects, regardless of nutritional state." This contradicts the idea that athletes can train their metabolism to spare glycogen by burning a greater amount of fat at higher intensities of endurance exercise. This result was later contradicted in a study that showed that RER was lower (increased lipid

oxidation) at exercise intensities greater than 40% VO_{2max} in trained compared to untrained subjects (Messonnier et al., 2005). It has been suggested that animals, including humans, use the same fuel blend at the same relative exercise intensities (Weber, 2011), suggesting that metabolism is a fixed phenomena that is shared among different aerobic species. The research is inconclusive about whether or not one can be trained to oxidize more lipid at exercise intensities above 40% VO_{2max}.

2.7 Metabolic Adaptations to Endurance Training

Endurance training has been shown to cause muscle adaptation that increases the aerobic metabolism capacity of working muscle (Holloszy & Booth, 1976). These adaptations include; an increase in mitochondrial biogenesis (Holloszy & Coyle, 1984), increased gene expression of enzymes involved in metabolism (Hawley & Spargo, 2007), increases in the concentration of oxidative enzymes involved in glucose and fatty acid metabolism (Holloszy & Booth, 1976), increased capillary density and myoglobin concentration (Holloszy & Booth, 1976), increased blood flow (Hawley, 2002), which all lead to an increase in the ability to generate ATP. All of these adaptations, specific to endurance training, allow individuals to work at higher intensities for longer duration without the accumulation of acidic metabolic by-products (lactate), conserving CP stores, and conserving glycogen stores (Hawley & Spargo, 2007).

One of the original papers exploring specific muscle adaptation to endurance training found increases in succinate dehydrogenase (Krebs cycle enzyme) activity measured in endurance trained athletes when compared to untrained controls (Fink,

Costill, & Pollock, 1977). Others have found that not only do endurance trained individuals have increased mitochondria, but their mitochondria have a tightly regulated electron transport chain that is efficiently able to produce ATP (Holloszy & Coyle, 1984). The respiratory control of endurance trained athletes allows them to perform at high levels without concern that they will not be able to properly fuel the event they are participating in.

There is research specific to fuel use and adaptations to marathon training that comes from the study of 5'-AMP-activated protein kinase (AMPK) signaling pathway (Frøsig, Jørgensen, Hardie, Richter, & Wojtaszewski, 2004; Langfort, Viese, Ploug, & Dela, 2003). AMPK has been termed the fuel-sensing enzyme of working muscle (Hawley & Spargo, 2007). AMPK plays a role in the adaptation of muscle in response to an exercise stimulus. Specifically AMPK is responsible for the regulation of mitochondrial biogenesis, glycogen storage, and glucose transporter (GLUT-4) translocation (Hawley, 2002). AMPK has been postulated to be the compound that signals an increase in GLUT-4 translocation to the muscle membrane in response to muscle contraction, independent of insulin (Langfort et al., 2003). AMPK upregulation may be the key to the benefits of exercise training in the reversal of the insulin resistance that occurs in type II diabetes patients.

All of these adaptations are the basis behind why endurance training or more specifically marathon training may enhance one's ability to use all available fuel sources in an efficient manner. Additional information related to trainability and performance

capability may be obtained if one were able to quantify how readily athletes' are able to use all fuel sources during endurance events.

2.8 Non-Linear RER Variability

RER has been shown to be highly variable even when looking at relatively homogeneous groups (Goedecke et al., 2000). Goedecke et al. (2000) found resting RER varied from 0.718-0.927 in 61 trained cyclists. This wide range of fuel utilization at rest indicates that RER may be highly variable even within trained athletes. The wide range of RER was maintained during exercise in these same subjects and the distribution of RERs can be seen in Figure 2 (Goedecke et al., 2000). Even an exercise intensity of 75% of the subjects' predetermined peak wattage yielded a range of RER values from approximately 0.88 to 1.06, indicating that each athlete may be using a fuel blend that is specific to their own metabolism (Goedecke et al., 2000). In fact, the distribution of the RER values follows a bell-shaped curve for all intensities of exercise as well as resting RER. This distribution suggests that further studies may be warranted to evaluate RER as a predictor of metabolic trainability in athletes. The researchers were able to account for 59% of the variance in RER using; muscle glycogen content, training volume, proportion of type I fibers, free fatty acid concentration, lactate concentration, and percent of dietary fat intake (Goedecke et al., 2000). This evidence suggests that the factors underlying average RER during a specified workload may be complex, and there is a need for a measure that can quantify the amount of variability in each subject's RER values.

RER variability measured by sample entropy.

Breath-to-breath metabolic gas data has previously shown to have both short and long term variability in a clinical setting (Cadena Méndez et al., 2008). Biltz et al. (2008) did a comparison of RER variability for obese and lean children and found that obese children tended to have lower SampEn scores (1.72 ± 0.13 vs. 1.49 ± 0.08, p =0.06) for RER variability than the lean children did during a 50W cycling test. The result was not statistically significant, but the trend indicates that the researcher's hypothesis that obese children should have lower variability scores than the healthy lean children may indeed be true. In the only known study analyzing RER variability scores in an athletic population found that trained female adolescent soccer players had higher SampEn scores (0.914 ± 0.433 vs. 0.564 ± 0.139, p = 0.026) than untrained controls during a 6-minute cycle protocol at 80% of their predetermined ventilatory threshold work rate (Biltz et al., 2011). This result also supported the researcher's hypothesis that healthy individuals should have a higher SampEn score for their RER during exercise. Training has an impact on substrate utilization and having the ability to utilize multiple fuels has a health and potential performance benefit. Figure 3 shows the RER signal plotted against time for a marathon runner in the trained (SampEn = 0.99) and untrained state (SampEn = 0.26), visually indicating the increased variability in the RER trace for the trained runner.

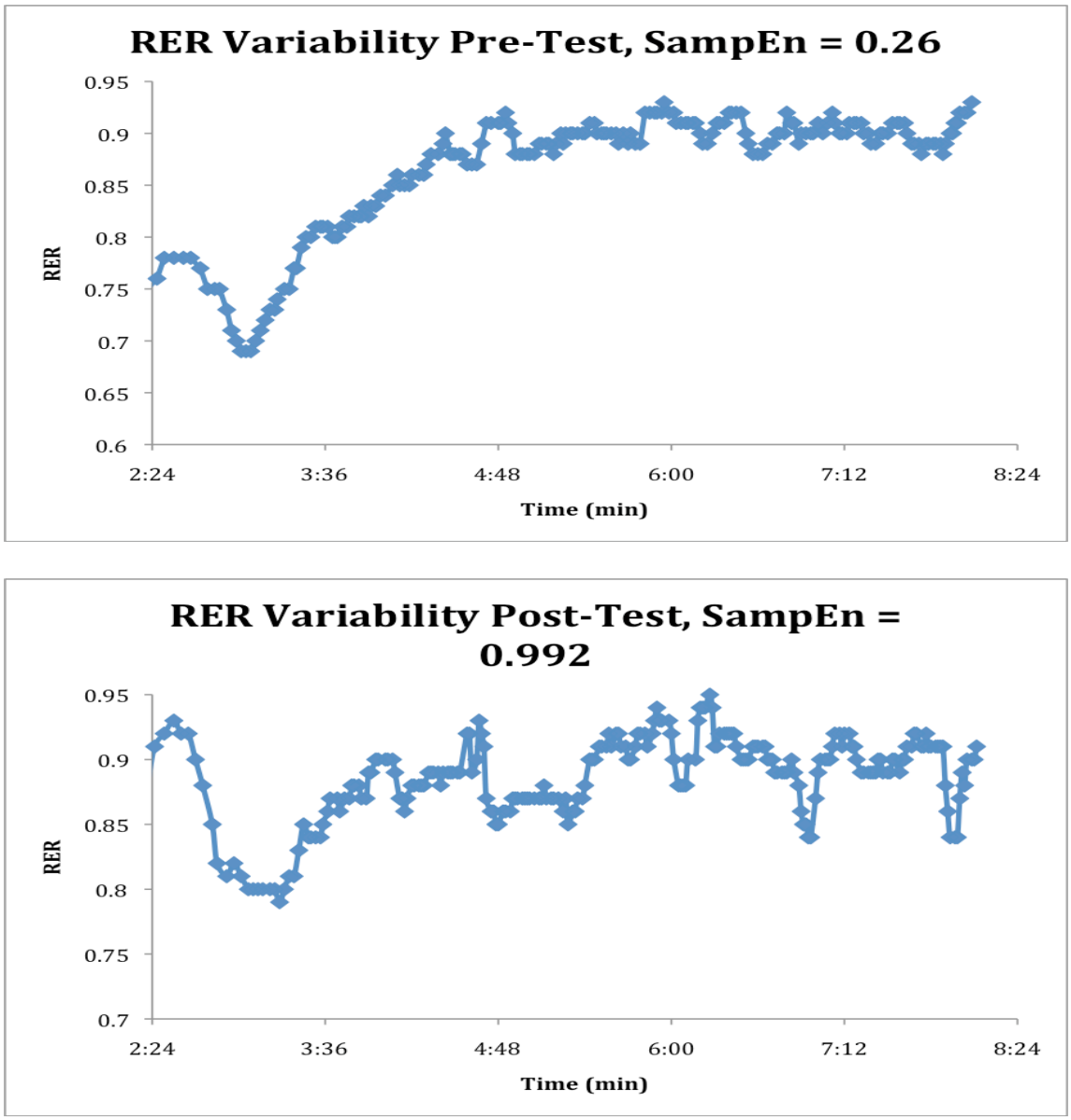

Figure 1: RER vs Time pattern for pre- and post-training for the marathon study. Lower variability can be seen in the RER tracing for pre-training (top) compared to the post-training (bottom), (Brown et al. 2013, unpublished data).

2.9 Summary

Variability analysis methods have found their way into mainstream health care and sports performance research. There are many non-linear variability methods that may be employed when analyzing physiological time series data. Careful consideration of the advantages and disadvantages of each method should be undertaken to ensure the method is actually analyzing the specific intent of the researcher. There has been minimal research investigating the use of non-linear methods on metabolism data, specifically respiratory exchange ratio. Substrate utilization is an important factor in both health and sports performance. Utilizing an analysis technique to assess metabolism non-invasively has the potential to benefit both clinical and sport outcomes of subjects. Analysis of btb variability in respiratory exchange ratio during exercise thus far has not been investigated or found in published research, so this novel analysis hopes to provide insight into the complexity of human metabolism.

CHAPTER 3: Methods

CHAPTER 3: Methods

The aim of this study was to determine the effect of marathon training on RER variability, as measured by SampEn, in healthy college-age novice marathon runners. A secondary aim is to determine if btb RER SampEn changes correlate with changes in average RER, body fat, 2-mile time, and VO_{2max} following 16 weeks of marathon training.

Subjects

Fifty-nine novice runners (39 females: age = 20.7 ± 1.1 yrs, height = 66.8 ± 3.8 in, weight = 65.0 ± 11.1 kg; 20 males: age = 18.9 ± 1.0 yrs, height = 67.8 ± 2.9 in, weight = 68.5 ± 10.5 kg), enrolled a marathon-training course in the Physical Activity Program at the University of Minnesota, volunteered to participate in the study. The subjects were healthy college-age students who had no known metabolic disorders and had not trained for a marathon in the last twelve months. All of the subjects completed the marathon training and ran the Eau Claire Marathon on May 6, 2012. The study was approved by the University of Minnesota Institutional Review Board, and all subjects gave written informed consent to participate.

Procedure for Data Collection

Subjects underwent testing for RER variability during a pre- and post-training VO_{2max} test. Subjects were required to arrive at the testing facility in the Human and Sports Performance Laboratory (HSPL) at the University of Minnesota on the day of their test in the fasted state (6 hours) and without consuming any caffeine prior to the test. Pre-testing started two-weeks prior to the initiation of the marathon-training protocol,

and post-testing took place two-weeks prior to the marathon. On testing days, height and weight were taken prior to testing. Body composition was assessed using hydrostatic underwater weighing, VO_{2max} testing was performed on a treadmill (Trackmaster, Newton, KS) and a btb gas samples were collected using a metabolic cart (Medgraphics, St. Paul, MN). The gas sensor was calibrated prior to data collection using two different gas mixtures, 21% O_2/79% N_2 and 5% CO_2/12% O_2/83% N_2, (Medgraphics, St. Paul, MN). Gas flow was calibrated using a three-liter syringe (Medgraphics, St. Paul, MN). Subjects were be fitted with a facemask and pneumatech (Medgraphics, St. Paul, MN). Heart rate was monitored during the testing using a heart rate monitor strap and s810 wrist computer (Polar, Kempele, Finland). The VO_{2max} protocol used in the study was developed and previously validated (Popp, 2009). The protocol included a one-minute warm-up session at 3.1 miles per hour, the subjects then ran at a treadmill speed corresponding to 65% of their pre-determined VO_{2max} pace (based on their initial 2-mile trial) for six minutes. The time frame of this stage ensured the subjects were exercising at a steady-state workload and generated enough time points for the non-linear analysis. After the 6-minute stage at 65% VO_{2max} pace, the speed and grade of the treadmill were increased until the subjects reached a maximum. Criteria for reaching maximum effort was achievement of a maximal heart rate (220-age), RER > 1.1, or reaching > 16 on a rate of perceived exertion scale. The same testing protocol was administered during post-testing, the treadmill speed was based on 65% of their post-training 2-mile time trial VO_{2max} pace.

Non-Linear Data Analysis

Collection and analysis of VO_{2max} data was completed using the Breezesuite software package (Medgraphics, St. Paul, MN). Raw data was checked for artifact prior to analysis. Sample entropy scores were calculated using Kubios Heart Rate Variability software Version 2 (University of Kupio, Kupio, Finland). Kubios was originally designed as a heart rate variability program, but can also be used for other biological time series data, such as RER (Tarvainen et al., 2009). Default values of m = 2 and r = 0.2*SD were used during the sample entropy analysis. SampEn analysis was chosen based on the lack of bias in the measure when compared to other entropy scores and due to the limited number of data points.

Statistical Analysis

All variables were checked for normality prior to statistical analysis. Pre- and post-RER variability (measured by SampEn), average RER, VO_{2max}, 2-mile time, and percent body fat were analyzed using a t-test for paired samples. Correlations between physiologic variables and performance variables were assessed using linear regression. There was no difference between males in females in regards to the variables measured, so they were considered together as one group. Results are presented as means +/- standard deviations. An α level of < 0.05 was considered to be statistically significant. Statistical analysis was completed using the statistical software R, version 2.9.2 (The R Foundation for Statistical Computing, USA).

CHAPTER 4: Pilot study: RER Variability Analysis by Sample Entropy: Comparing Trained and Untrained Adolescent Female Soccer Players

CHAPTER 4: Pilot study: RER Variability Analysis by Sample Entropy: Comparing Trained and Untrained Adolescent Female Soccer Players (for submission to the journal of Pediatric Exercise Science).

This research was conducted by Dr. George Biltz and myself (Scott Brown) by examining data obtained in collaboration with Dr. Vish Unnithan at the Centre for Sport, Health and Exercise Research at Staffordshire University, Stoke-on-Trent, UK.

RER Variability Analysis by Sample Entropy: Comparing Trained and Untrained Adolescent Female Soccer Players

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Introduction

Time series data for heart rate (HR), blood pressure, breathing frequency and a number of other physiologic variables exhibit moment-to-moment variability. As a general principle, this increased fine scale variability seems characteristic of healthy, adaptable physiology (West, 2006). The variability within the data contains potential information that is lost in the traditional calculation of a mean value. Variability analysis encompasses a variety of mathematical techniques that have been applied to physiologic time series data such as: time domain analysis, frequency domain analysis, fractal analysis and entropy analysis (Seely & Macklem 2004). Sample Entropy (SampEn), a nonlinear method for variability analysis of time series data, characterizes the inherent regularity of a data sequence (Richman & Moorman 2000). A higher entropy score

implies decreased predictability of sequential values – less self-similarity in the data. Sample entropy excels over other non-linear analysis techniques in that only a small amount of data points (50 points) are required for the analysis (Seely & Macklem, 2004). The requirement for such few data points make SampEn a useful metric when looking at physiologic time series data from a limited time frame. Sample entropy was first used to analyze heart rate variability (HRV) in neonates with sepsis (Lake, Richman, Griffin, & Moorman, 2002). The technique has been used in a wide variety of clinical research ranging from: fetal distress (Lake, Richman, Griffin, & Moorman, 2002), body posture (Lamoth, van Lummel, & Beek, 2008), atrial fibirilation (Alcaraz & Rieta, 2008), sleep apnea (Al-Angari & Sahakian, 2007) and respiratory exchange ratio (Biltz, Harmon, Dengel, Unnithan, & Witten, 2008).

Respiratory exchange ratio (RER) is the ratio of the volume of carbon dioxide expired divided by the volume of oxygen inhaled with each breath. RER is determined using indirect calorimetry during either a resting state or an exercising test. RER has been used to assess substrate utilization during metabolism. A low value (0.7) indicates fat as the primary fuel source where a high number (1.0) indicates a predominance of carbohydrate as a fuel source.

A previous study has shown that healthy humans rely primarily on fat as fuel at rest and under insulin stimulation or exercise stimulation the body switches from fat to carbohydrate (Kelley, Goodpaster, Wing, & Simoneau, 1999). Untrained and overweight individuals have been shown to lack the flexibility to switch from using fat as fuel at rest

and carbohydrate during insulin stimulated conditions (Goodpaster, Wolfe, & Kelley, 2002). These individuals simply cannot use fat as fuel in the same way that their lean counterparts do. This lack of flexibility has been coined metabolic inflexibility. The good news is that metabolic inflexibility may be able to be reversed with increased physical activity (Meex et al., 2010).

In a previous study, RER SampEn had a moderately negative correlation with percent body fat (Biltz et al., 2009). Obesity has been described as a state of metabolic inflexibility with a relatively similar ratio of carbohydrate and fat oxidation independent of the feeding or fasting state (Corpeleijn et al., 2009). Conversely, metabolic flexibility is observed in exercise, as RER changes from rest to progressive effort (Goedecke et al., 2000). Endurance training increases fat oxidation and lowers average RER during exercise (Jeukendrup & Wallis, 2005). The variability of breath-by-breath RER has not been investigated for potential effects of training, despite a wide range of resting average RER values reported for highly trained endurance cyclists. While the literature is sparse on using non-linear analysis techniques for analyzing RER variability, heart rate variability (HRV) has received much attention. It has been observed that HRV increased during sub-ventilatory threshold exercise in highly trained adolescent triathletes (Cottin et al., 2004). Aerobic training has also been reported to increase heart rate variability (HRV) in pre-pubertal children (Mandigout et al., 2002). Other studies have shown that obese children have shown favorable increases in HRV following the completion of an

endurance exercise program (Gutin, Owens, Slavens, Riggs, & Treiber, 1997; Gutin, Barbeau, Litaker, Ferguson, & Owens, 2000).

The aim of this study was to investigate soccer-training effects on RER variability during exercise. Specifically, we hypothesized that soccer training would increase RER variability, as measured by SampEn, during steady state, sub-maximal exercise. Training effects on RER variability would be analogous to previously reported training effects on HRV. This is a sub-study of a larger study on O_2 kinetics in youth female soccer players.

Methods

Experimental design

Eleven trained, female soccer players (14.6 ± 0.7 yrs) were recruited from two youth academies affiliated to two professional soccer teams in NW England. They regularly engaged in systematic training (10.3 ± 1.4 months, 5.2 ± 2.0 hrs/wk) with 5.9 ± 1.0 yrs playing competitive soccer. The control group contained nine untrained, but recreationally (no specific sport) active girls (15.1 ± 0.6 yrs) volunteered to participate in the study. Tanner staging by self-assessment of breast development ranged from 3-5 for both groups. All subjects performed an initial incremental cycle ergometer test (Lode Excaliber Sport, Groningen-The Netherlands) to volitional exhaustion. The protocol consisted of 3-min stages, with an initial workload of 35W and increments of 35 W/stage at a cadence of 60 rpm throughout the test. Cosmed K4b^2 (Rome, Italy) was used to obtain VE, VO_2, VCO_2, peak VO_2, and RER. Ventilatory threshold (VT) was determined for each subject by standard v-slope method conducted independently by 2

members of the research team. Subjects returned for evaluation of their VO_2 kinetics as part of a bigger study within the laboratory.

Determination of RER Sample Entropy

After 3 minutes of baseline pedaling at 10 W, subjects completed a 6-minute square wave transition maintaining 80% of their predetermined VT workload. Data intervals for RER SampEn analysis were matched to the data intervals selected for VO_2 kinetics evaluation by the UK research team. SampEn (m,r,N) is effectively a conditional probability: SampEn = -log A/B. Where B is the total number of matches of length m in a data series of length N. A is the total number of matches at length m+1 within the set of matches B. Data points within m and m +1 are said to match if they agree within a pre-selected tolerance r. A common value for r is 0.2*SD where SD is the standard deviation of N. (Richman & Moorman 2000). Since SampEn is fundamentally a measure of the regularity or self-similarity of a data sequence, it is a measure without units.

Statistical analysis

All SampEn scores were calculated using Kubios HRV version 2 software (University of Kupio, Kupio, Finland). The SampEn analysis feature in Kubios has preset values of m = 2 and r = 0.2*SD. Although developed for heart rate analysis, the non-linear analysis features in Kubios can be applied to other time series data as long as it has not been previously detrended (Tarvainen et al., 2008).

Independent sample t-tests were used to examine mean differences between the trained (n=11) and untrained (n =9) for SampEn, average RER, BMI and peak VO_2

using the statistical program R, an open access software. All results are presented as means [SD]. An alpha level of $p < 0.05$ was considered to be statistically significant.

Results

The trained soccer players had significantly higher RER SampEn scores for the 6-minute steady state pedaling interval compared to the untrained girls (0.914 ± 0.433 vs. 0.564 ± 0.139, $p = 0.026$). For the trained group, SampEn scores ranged from 0.031 to 1.558 demonstrating large inter-individual differences within the trained group. The untrained group RER SampEn scores were more homogeneous ranging from 0.402-0.841.

Trained players had significantly higher peak VO_2 compared to untrained (2.41 L/min ± 0.37 vs. 1.86 ± 0.25, $p < 0.05$).

The average RER during the 6-minute testing interval was calculated for each subject. For trained females the average RER was significantly lower than untrained females (0.935 ± 0.063 vs. 1.018 ± 0.041, $p = 0.002$). The average RER during exercise was also heterogeneous within the trained group ranging from 0.856-1.039. The untrained group had a more homogeneous average RER.

There was no significant difference in body mass index (BMI) between the trained and untrained group (21.80 ± 2.34 vs. 20.45 ± 2.15, $p = 0.139$). Differences in averaged RER and relative fat oxidation during exercise are not explained by differences in BMI.

	Trained	Untrained	p-value
RER SampEn	0.914 ± 0.433	0.564 ± 0.139	0.026
Average RER	0.935 ± 0.063	1.018 ± 0.041	0.002
VO_{2max} (L/min)	2.41 ± 0.37	1.86 ± 0.25	<0.05
BMI	21.80 ± 2.34	20.45 ± 2.15	0.139

Table 1: Physiological Variables Measured in Trained and Untrained Adolescent Female Soccer Players

Discussion

Soccer training significantly enhanced RER SampEn scores as predicted. As a group, trained females showed more variability, less self-similarity, in their sequential RER data during exercise. Trained subjects also exhibited higher peak VO_2 and lower average RER while pedaling indicating increased fat oxidation at 80% of VT compared to untrained girls. This is consistent with previously reported effects of training (Jeukendrup & Wallis, 2005).

Yet, there is large inter-individual variability in both RER SampEn score and average RER for trained subjects. The variability in RER SampEn could be interpreted as a sign of poor reliability of this measure for evaluating a training effect. However, a study of trained cyclists showed large inter-individual differences with resting RERs ranging from 0.718-0.927 and the RER diversity remained with increasing exercise intensity (Goedecke et al., 2000). Metabolic flexibility with exercise occurs on at least

two levels. Within an individual there is metabolic flexibility in the blending of carbohydrate and fats to match intensity of energy expenditure with available substrates. Between individuals, with seemingly similar levels of training and performance, there also appears to be metabolic flexibility. For example, lean body mass, estimated physical activity level, VO_{2max}, gender and fat mass together only accounted for 34% of the variance in peak fat oxidation (Jeukendrup & Wallis, 2005).

The variability found in RER SampEn scores for trained soccer players may reflect dynamic differences in metabolic state or metabolic flexibility. The fine scale variability pattern may be a marker for the underlying complexity occurring in exercise metabolism. RER SampEn may be useful for distinguishing relative metabolic inflexibility and subsets of subjects who may not respond well to training. Future studies will be needed to investigate applications of the potential information in RER SampEn.

This was the first study to show differences breath-to-breath variability in soccer-trained subjects' RER values during steady state exercise. This study is limited by the sample size along with the fact that the origina study was not specifically designed to look at RER variability. This study will serve as pilot data for future studies assessing RER variability on a breath-by-breath basis in humans.

CHAPTER 5: Respiratory Exchange Ratio Variability Increases With Marathon Training in Novice College-Age Runners

CHAPTER 5: Respiratory Exchange Ratio Variability Increases With Marathon Training in Novice College-Age Runners (for submission to the Journal of Sports Medicine and Exercise Science)

Respiratory Exchange Ratio Variability Increases With Marathon Training

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BROWN, S.R.; BILTZ, G.R.; RHODES, G.S.; LUNDSTROM, C.J., SCHAEFER, E.S.; and INGRAHAM, S.J. Breath-to-Breath Respiratory Exchange Ratio Variability Increases With Marathon Training in Novice College-Age Runners**.** *Med. Sci. Sports Exerc.* Vol. 45, No. 3, pp.444-460, 2013. **Purpose:** To assess the effects of 16 weeks of marathon training on breath-to-breath (btb) respiratory exchange ratio (RER) variability, measured by sample entropy (SampEn), in college-age, healthy novice marathon runners. **Methods:** Fifty-nine novice runners (39 female, 20 male, ages 18-24), from a marathon-training course offered at the University of Minnesota, volunteered to participate in the study. Subjects underwent 16 weeks of marathon training along with pre-and post-training lab testing, which included: 2-mile time trial, VO_{2max}, and body composition by hydrostatic underwater weighing. RER variability was determined pre- and post-training by a 6-minute steady state run at 65% of their predicted VO_{2max}, based on concurrent 2-mile time trial. Gas exchange data was collected and SampEn analysis of RER variability was calculated using Kubios software. Matched pair t-tests were used to compare average

RER and SampEn scores pre- and post-training. **Results:** SampEn measure of RER time series variability significantly increased from pre- to post-training (0.198 +/- 0.387, p = 0.0002). However, mean RER did not decrease with training (-0.012 +/- 0.079, p = 0.231). Subjects showed increased RER variability during sub-maximal steady state running after 16 weeks of marathon training. **Conclusion:** Average SampEn scores for participants increased with marathon training. SampEn analysis of RER time series may detect trainability, metabolic adaptations, or provide substrate utilization recommendations with endurance training.

Introduction

Indirect calorimetry has been used to gain insight into muscle metabolism, non-invasively, for many years (Edwards et al., 1934). During steady state exercise CO_2 output and O_2 input can be quantified btb, and RER, ratio of CO_2 expelled to O_2 inspired, can provide insight into substrate utilization patterns within working muscle (Jeukendrup & Wallis, 2005). Traditional methods used gas collection bags to monitor CO_2 output for exercising humans (Kasch & Wallace, 1976), but with increases in technology indirect calorimetry involves sophisticated btb gas detection methods for insight into metabolism during exercise (Goedecke et al., 2000; Naimark et al., 1964; Romijn et al., 1993). RER has been validated to assess changes in substrate utilization with endurance training (Bergman & Brooks, 1999; Romijn et al., 1993), as a predictor of mortality (Toubro, Sørensen, Hindsberger, Christensen, & Astrup, 1998; Zurlo et al., 1990), training status indicator (Ramos-Jiménez et al., 2008), and performance

prediction (Bellar & Judge, 2012). The results of RER studies have been inconclusive with respect to interpreting training response, and have not received the same attention as other physiologic variables such as VO_{2max} and lactate threshold.

Traditionally, RER has been analyzed either by calculating an average and standard deviation of RER or by detecting a maximum RER during a given steady-state exercise time frame (Friedlander et al., 1998; Klein et al., 1994; Messonnier et al., 2005). Goedecke et al. (2000) showed that both resting RER and RER during steady state exercise varied greatly from person to person in trained cyclists (Goedecke et al., 2000). These results support the idea that there is a great deal of individual variability in the metabolic response to exercise (McPhee et al., 2011; Vollaard et al., 2009).

RER values indicate a shift toward fat utilization with exercise training indicating a potential increase in storage, mobilization, transport, and utilization of fatty acids within working muscle (Egan & Zierath, 2013; Holloszy & Booth, 1976; Horowitz & Klein, 2000; Klein et al., 1994; Vollaard et al., 2009). This may be important to endurance athletes who will require fat and carbohydrate to power them through training and endurance events. There is not a consensus around fuel utilization (increase in fat utilization leading to glycogen sparing) changes with endurance training as RER has also been shown not to change following training (Bergman & Brooks, 1999). In fact, there has been a push to no longer suggest that there are any performance benefits to increased fat utilization through training and feeding (Burke & Kiens, 2006). Bergman et al. (1999) suggested that athletes do not utilize lipids in larger quantities than untrained

persons at intensities greater than 40% VO_{2max} (Bergman & Brooks, 1999). Essentially suggesting that fuel use patterns are fairly consistent within humans exercising at intensities greater than 40% VO_{2max}, which has been supported elsewhere (Brooks, 1998; Egan & Zierath, 2013; Weber, 2011). Traditional linear methods (average and standard deviation) of time series data analysis may not be able to capture metabolic changes, based on RER data, therefore exploration into non-linear analysis methods may help to provide additional insight into RER changes with exercise training.

Non-linear analysis methods of time series data have become common in clinical and exercise physiology (Bravi, Longtin, & Seely, 2011). Heart rate variability (HRV) studies have set precedent for establishing non-linear analysis techniques to quantify and correlate variability changes with the inherent physiology producing the signal. Variability studies seek to quantify the complex dynamics of a physiological process through mathematical reconstruction into a signal variable (Voss et al., 2009). HRV studies have shown that increases in variability following training are indicative of a healthy and adaptable heart (Tulppo et al., 2003). A lack of HRV has been tied to the beginning of disease states (Lake et al., 2002), training status (Manzi et al., 2009; Plews et al., 2012), and an indicator of overtraining in athletes (Mourot et al., 2004). There are multiple ways to assess the variability in time series data, but many of the non-linear analysis methods are limited in use for other physiologic variables due to the need for large time series data sets (Seely & Macklem, 2004). To overcome that limitation, a non-

linear analysis technique called sample entropy (SampEn) was developed to analyze data sets with as little as 50 data points (Voss et al., 2009).

Sample entropy seeks to quantify the inherent regularity of a system or the sequential pattern of data generation of a system (Richman & Moorman, 2000). This is accomplished through calculating a conditional probability that a set of points of length *m* will agree with the next set of points *m*+1 within a given tolerance of *r* (usually 0.2*SD). A higher entropy score implies decreased predictability of sequential values – less self-similarity of the data. HRV studies have shown that low sample entropy scores are an indicator of disease state, and that higher scores are reflected of healthy and adaptable physiology (Lake et al., 2002).

This is the first known published study that analyzes changes in SampEn btb RER changes with endurance training. Metabolic variability was previously confirmed in young patients in a clinical setting. Btb gas analysis suggested that the variability in the metabolic gas data was not noise, but indeed provided insight into the underlying metabolism generating the data (Cadena Méndez et al., 2008). Gas exchange variability was looked at in obese children who trended to have lower RER SampEn scores during steady-state exercise when compared to lean controls (Biltz et al., 2008). This supports the suggestion that obese individuals tend to become “metabolically inflexible” with a tendency to rely on carbohydrate as fuel at rest and during exercise with a decreased reliance on fat utilization at rest (Kelley & Mandarino, 2000). From data analyzed in the Human & Sport Performance Laboratory at the University of Minnesota, RER SampEn

scores during submaximal exercise were higher in trained adolescent female soccer players when compared with untrained controls (Biltz et al., 2011). The aim of this study was to determine the effect of marathon training on RER variability, as measured by SampEn, in healthy college-age novice marathon runners. A secondary aim is to determine if btb RER SampEn changes correlate with changes in average RER, body fat, 2-mile time, and VO_{2max} following marathon training.

Methods

Subjects

Fifty-nine novice runners (39 females: age = 20.7 ± 1.1 yrs, height = 66.8 ± 3.8 in, weight = 65.0 ± 11.1 kg; 20 males: age = 18.9 ± 1.0 yrs, height = 67.8 ± 2.9 in, weight = 68.5 ± 10.5 kg), enrolled a marathon-training course in the Physical Activity Program at the University of Minnesota, volunteered to participate in the study. The subjects were recruited during an initial informational meeting about the class. There were 84 students who initially volunteered, but only 59 met the exclusion criteria for the study. Subjects were medically cleared for marathon training and were excluded if they were older than 24 years of age, had a known metabolic disorder, or failed to complete both the pre- and post-testing. All of the subjects completed the marathon training and ran the Eau Claire Marathon on May 6, 2012. The study was approved by the University of Minnesota Institutional Review Board, and all subjects gave written informed consent to participate.

Procedure for Data Collection

Subjects underwent testing for RER variability during a pre- and post-training VO_{2max} test. Subjects were required to arrive at the testing facility in the Human and Sports Performance Laboratory (HSPL) at the University of Minnesota on the day of their test in the fasted state (6 hours) and without consuming any caffeine prior to the test. Pre-testing started two-weeks prior to the initiation of the marathon-training protocol, and post-testing took place two-weeks prior to the marathon. On testing days, height and weight were taken prior to testing. Body composition was assessed using hydrostatic underwater weighing, VO_{2max} testing was performed on a treadmill (Trackmaster, Newton, KS) and a btb gas samples were collected using a metabolic cart (Medgraphics, St. Paul, MN). The gas sensor was calibrated prior to data collection using two different gas mixtures, 21% O_2/79% N_2 and 5% CO_2/12% O_2/83% N_2, (Medgraphics, St. Paul, MN). Gas flow was calibrated using a three-liter syringe (Medgraphics, St. Paul, MN). Subjects were be fitted with a facemask and pneumatech (Medgraphics, St. Paul, MN). Heart rate was monitored during the testing using a heart rate monitor strap and s810 wrist computer (Polar, Kempele, Finland). The VO_{2max} protocol used in the study was developed and previously validated (Popp, 2009). The protocol included a one-minute warm-up session at 3.1 miles per hour, the subjects then ran at a treadmill speed corresponding to 65% of their pre-determined VO_{2max} pace (based on their initial 2-mile trial) for six minutes. The time frame of this stage ensured the subjects were exercising at a steady-state workload and generated enough time points for the non-

linear analysis. After the 6-minute stage at 65% VO_{2max} pace, the speed and grade of the treadmill were increased until the subjects reached a maximum. Criteria for reaching maximum effort was achievement of a maximal heart rate (220-age), RER > 1.1, or reaching > 16 on a rate of perceived exertion scale. The same testing protocol was administered during post-testing, the treadmill speed was based on 65% of their post-training 2-mile time trial VO_{2max} pace.

Non-Linear Data Analysis

Collection and analysis of VO_{2max} data was completed using the Breezesuite software package (Medgraphics, St. Paul, MN). Raw data was checked for artifact prior to analysis. Sample entropy scores were calculated using Kubios Heart Rate Variability software Version 2 (University of Kupio, Kupio, Finland). Kubios was originally designed as a heart rate variability program, but can also be used for other biological time series data, such as RER (Tarvainen et al., 2009). Default values of m = 2 and r = 0.2*SD were used during the sample entropy analysis. SampEn analysis was chosen based on the lack of bias in the measure when compared to other entropy scores and due to the limited number of data points.

Statistical Analysis

All variables were checked for normality prior to statistical analysis. Pre- and post-RER variability, measured by SampEn, average RER, VO_{2max}, 2-mile time, and percent body fat were analyzed using a t-test for paired samples. Correlations between physiologic variables and performance variables were assessed using linear regression.

There was no difference between males in females in regards to the variables measured, so they were considered together as one group. Results are presented as means +/- standard deviations. An α level of < 0.05 was considered to be statistically significant. Statistical analysis was completed using the statistical software R, version 2.9.2 (The R Foundation for Statistical Computing, USA).

Results

	Male (n= 20)	Range	Female (n= 39)	Range
Age (yrs)	18.9 ± 1.0	18 – 22	20.7 ± 1.1	18 – 23
Height (in)	67.8 ± 2.9	63.8 – 74.5	66.8 ± 3.8	61.3 – 76.5
Weight (kg)	68.5 ± 10.5	52.6 – 89.7	65.0 ± 11.1	45.7 – 89.5
% Body Fat	19.6 ± 6.4	7.4 – 36.4	19.4 ± 5.7	10.5 – 33.6
VO_{2max} (ml/kg*min^{-1})	49.4 ± 7.7	34.7 – 65.1	49.2 ± 7.1	31.3 – 61.8
2-mile time (min)	15:48 ± 2:18	12:20 – 20:25	16:12 ± 2:36	11:45 – 22:17

Table 2: Baseline characteristics of marathon training subjects.

RER SampEn scores during a six-minute steady-state exercise bout increased after 16 weeks of marathon training (0.52 ± 0.34 vs. 0.71 ± 0.41, p = 0.0002). Average RER trended to decrease, but the result was not statistically significant (0.89 ± 0.06 vs. 0.88 ± 0.04, p = 0.23).

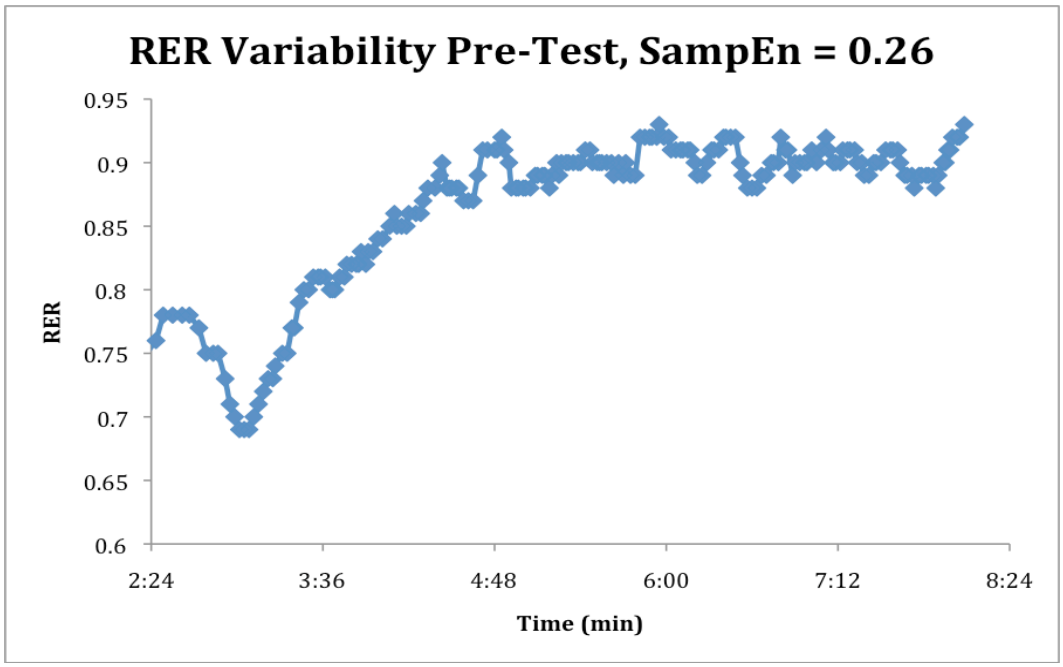
The subjects VO_2 max increased with training (49.2 ± 7.2 mL/kg*min vs. 53.8 ± 6.4 mL/kg*min, p = 3.0 x 10^{-7}) and their time to complete a two-mile time trial decreased (16:06 ± 2:28 min vs. 14:32 ± 2:01 min, p = 1.4 x 10^{-8}*). Percent body fat did not change during the training period (19.5 ± 5.9 vs. 19.2 ± 5.7, p = 0.30).

	Pre	**Range**	**Post**	**Range**	**p-value**
SampEn RER	0.52 ± 0.34	0.101 – 1.517	0.71 ± 0.41	0.202 – 1.836	< 0.01
Avg RER	0.89 ± 0.06	0.76 – 1.01	0.88 ± 0.04	0.79 – 0.96	0.23
% Body Fat	19.5 ± 5.9	7.4 – 36.4	19.2 ± 5.7	7.0 – 35.7	0.30
VO_{2max} (mL/kg*min)	49.2 ± 7.2	31.3 – 65.1	53.8 ± 6.4	43.6 – 69.9	< 0.01
2-mile time trial (min:sec)	16:06 ± 2:28	11:45 – 22:17	14:32 ± 2:01	11:16 – 19:02	< 0.01
Marathon time (hours:min)	4:31 ± 0:44	3:23 – 6:07			

Table 3: Pre- and post-training values for measured physiologic and performance variables in marathon training subjects.

There was no correlation between change in RER SampEn and change in avg RER ($p = 0.13$). There was no correlation between change in RER SampEn and change in 2-mile time, ($p = 0.94$). There was also no correlation between avg RER and change in 2-mile time ($p = 0.45$). Change in percent body fat positively correlated with change in 2-mile time ($p = 0.01$) and change in VO_2 max negatively correlated with change in 2-mile time ($p = 0.002$).

Example data of a low pre-test RER SampEn score and high post-test RER SampEn score (indicating increased RER variability post-training), a high pre-test RER SampEn score and high post-test RER SampEn score (without much overall change), and high pre-test RER SampEn score and low post-test RER SampEn score (indicating decreased RER variability post-training), can be seen in figures 4, 5, and 6.



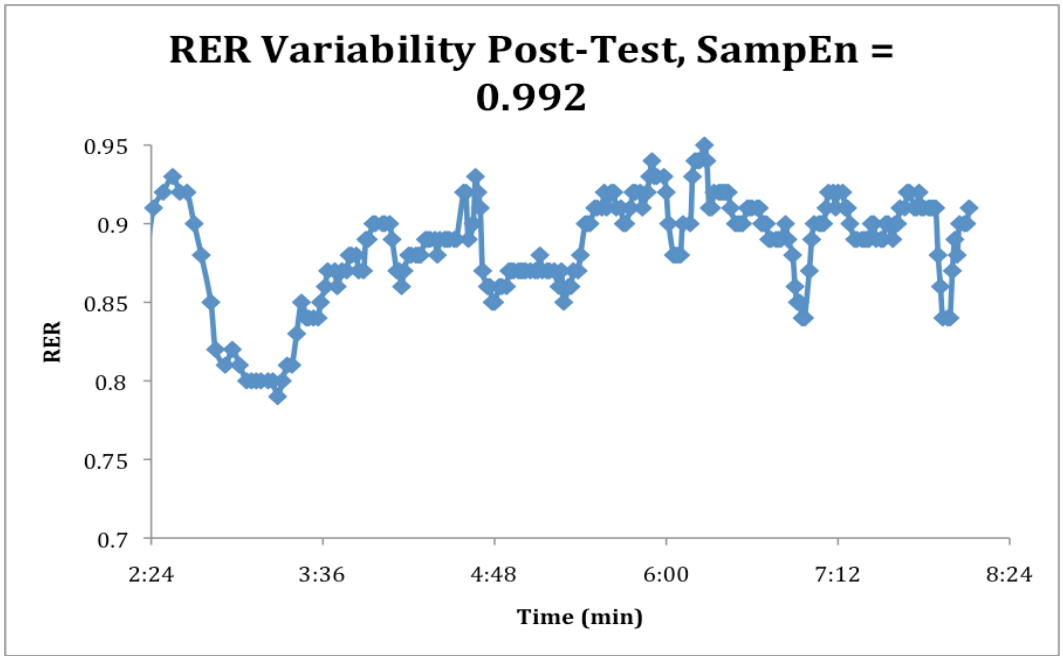
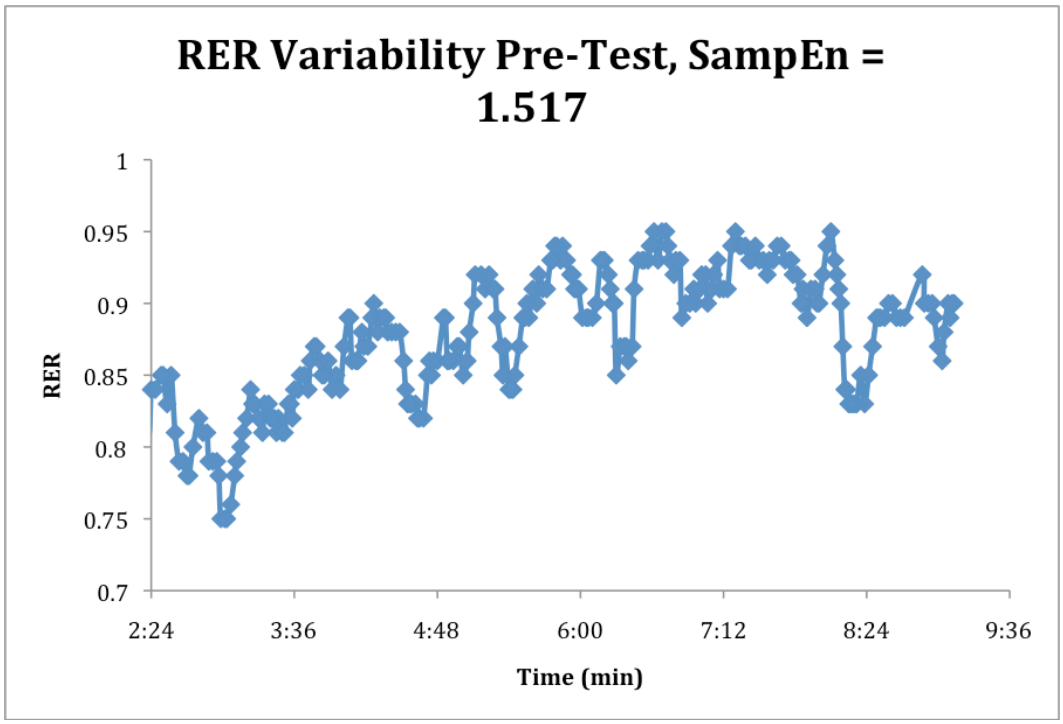


Figure 2: Example data from subject that started with low RER sampEn score (0.260) pre-training and transitioned to a higher RER sampEn score (0.992) post-training.



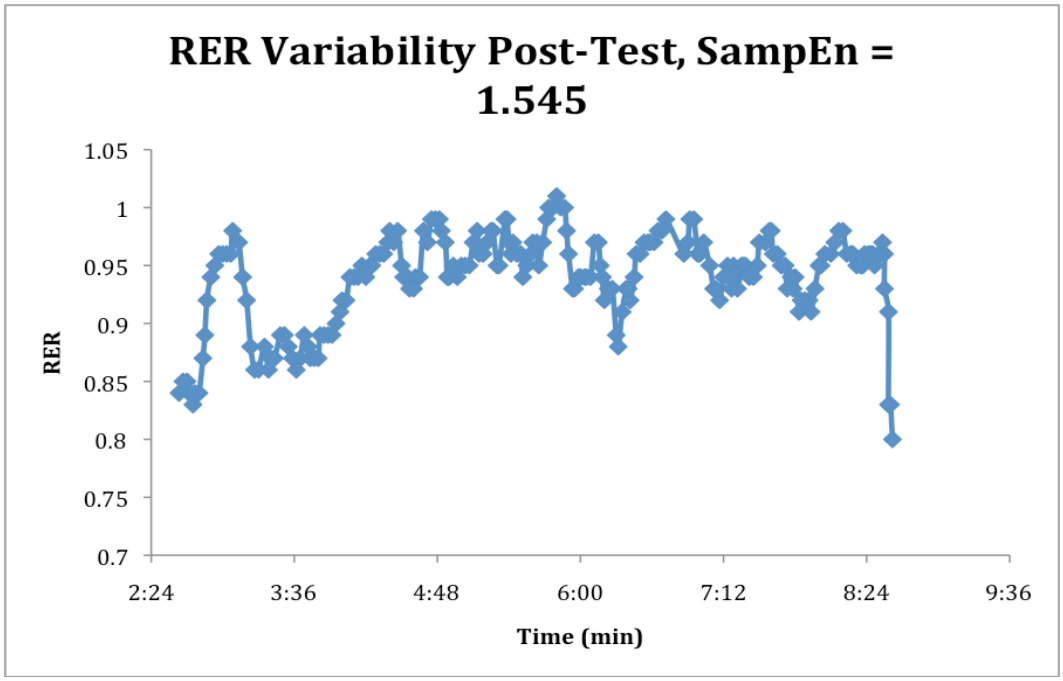
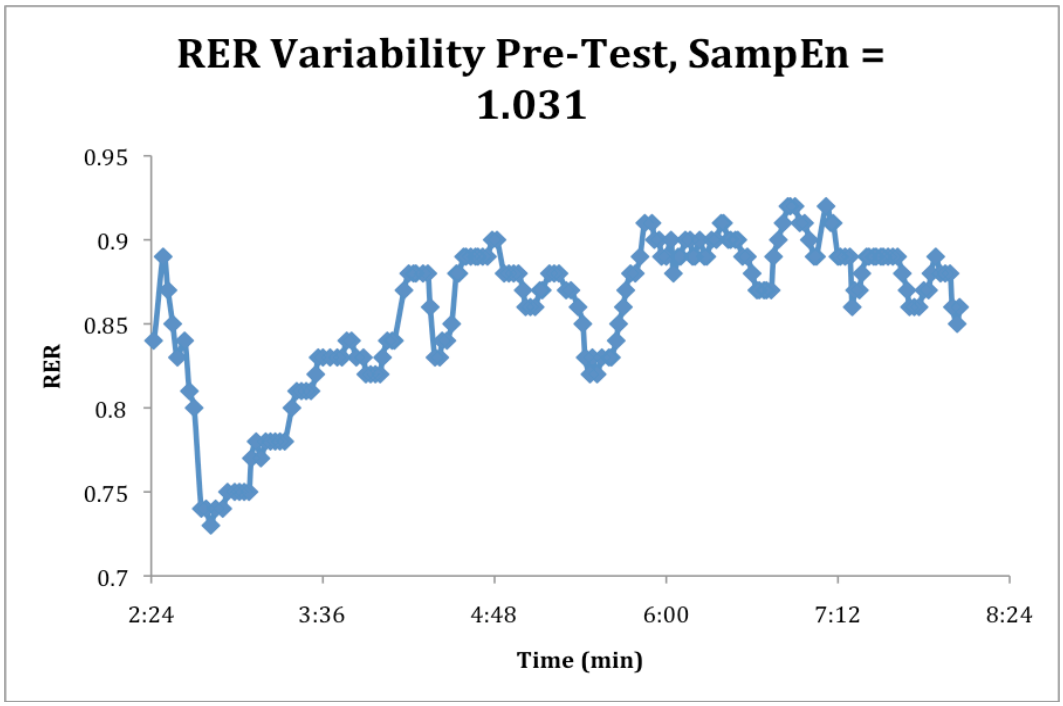


Figure 3: Example data from subject that started with high RER sampEn score (1.517) pre-training and transitioned to a higher RER sampEn score (1.545) post-training with low overall change.



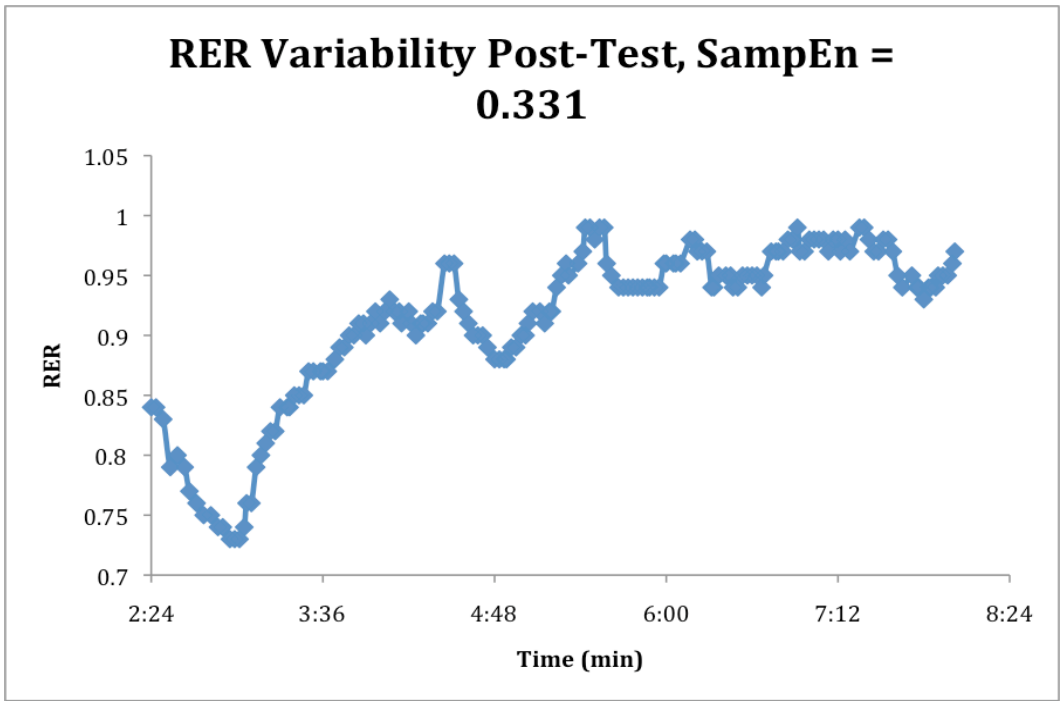


Figure 4: Example data from subject that started with high RER sampEn score (1.031) pre-training and transitioned to a lower RER sampEn score (0.331) post-training.

Discussion

This study provides an observation of the variability in metabolic changes that may occur with marathon training in a large cohort of healthy college-age students. Sample entropy analysis of RER time series data showed increased variability in the btb RER signal. These changes did not correlate with changes in VO_{2max} or 2-mile time. Non-linear analysis of physiological time series data may provide unique insight into the heterogeneity of metabolism changes in exercising humans.

Breath-to-breath RER variability, measured by SampEn, increased with marathon training, but there is a great deal of variability in the change in SampEn scores between individuals. A close examination of the data shows that there are three potential sub-groups within sample; those who had a large increase in RER SampEn, some with minimal change, and others whose RER SampEn decreased with training. This result may support previous research indicating large variability in absolute RER measured in a homogenous group of trained cyclists during rest and exercise (Goedecke et al., 2000). Data from the HERITAGE family study suggests that there may be responders and non-responders to any given endurance exercise stimulus (Bouchard et al., 1999). There is reason to assume that there will be a wide range of responses to any given exercise program, and consideration should be made when analyzing physiologic changes based on this.

There was a positive correlation between change in body fat and change in 2-mile time and a negative correlation between change in VO_{2max} and change in 2-mile

time, which supports the idea that reducing body fat and increasing one's ability to uptake and utilize oxygen should decrease the time it takes for an individual to finish a time trial run. The lack of correlation between RER SampEn and avg RER to 2-mile time suggests that these measures may be quantifying some different aspect of metabolism. RER SampEn scores may reflect substrate utilization patterns that do not have any effect on a short duration high intensity run like a 2-mile time trial. Substrate utilization is most likely not a limiting factor for exercise bouts less than 20 minutes with high intensity. If we were able to look at marathon time changes pre- and post-training we would most likely get a better idea if changes in RER SampEn predict long-term (during endurance events) fuel utilization patterns. The 2-mile time trial is most likely too short of a distance, too high of intensity, therefore subjects must utilize a consistent blend of fuel (high in carbohydrate) to allow for an adaptable and flexible metabolism to show any performance advantage.

The lack of correlation between avg RER and RER SampEn suggests that each of these may indeed be representing different physiological phenomena, and begs the question whether there is indeed more information to be gained by analyzing data sets using non-linear analysis techniques. It is speculated that the measured variability is not noise and may provide additional insight into metabolic changes following exercise training. Due to lack of correlations, the increase of RER SampEn, with training, appears to represent underlying physiologic changes that are not currently captured by changes in VO_{2max} and avg RER. This idea supports recent studies which have shown that there is

a disconnect between the changes in VO_{2max} and changes in mitochondrial enzymes and substrate utilization capabilities following endurance training (McPhee et al., 2011; Vollaard et al., 2009).

Increases in expression of mitochondrial enzymes, for both carbohydrate and fatty acid metabolism, were not shown to correlate with changes in VO_{2max} following six weeks of endurance cycling training (McPhee et al., 2011). Vollard et al. (2009) also demonstrated a large amount of metabolic variability in response to endurance training, and that metabolic marker changes did not correlate with changes in VO_{2max}. The researchers also concluded that much information is lost when looking at averages of time series physiological variables (Vollaard et al., 2009). Embracing the heterogeneity of muscle fibers (Pette, 1985), metabolism phenotypes (Assfalg et al., 2008), and variability in response to exercise (Goedecke et al., 2000) will become important as the field advances.

Muscle fiber type may also play a role in changes in RER SampEn. Adaptation to endurance training and substrate utilization will likely be different based on one's muscle fiber profile. If RER SampEn is indeed reflecting the btb variability in muscle fiber substrate utilization it seems plausible that there may be correlation between fiber type and RER SampEn score during steady-state exercise.

Variability in substrate utilization at rest and during exercise has been shown (Goedecke et al., 2000). Btb RER variability may have a close relationship to diet tendencies of the person being tested. Substrate utilization and diet have been linked in

exercise studies, and may provide additional information as to why there is so much variability between subjects btb RER SampEn scores.

Marathon performance is determined by the interaction of many factors (Hawley & Spargo, 2007; Rapoport, 2010), and substrate utilization is part of the performance equation. There is limited metabolic information that can be obtained from solely looking at VO_{2max} or substrate utilization alone, so looking at a multifactor model including RER and running economy may help correlate endurance performance specific to laboratory measures (Bellar & Judge, 2012; Goedecke et al., 2000).

Longer time series data sets may be needed in order to get a better understanding of the non-linear nature of btb RER. Other non-linear techniques (frequency domain) have been used to analyze btb RER time series data (Cadena Méndez et al., 2008), and there appears to be several different frequencies that appear over a longer data series, similar to what is seen in heart rate variability studies. Currently, only frequency and entropy non-linear measurements have been used to analyze btb metabolic gases. There are many other non-linear methods that exist though (Bravi, Longtin, & Seely, 2011), and the results of this study suggest that there may be plausibility to exploring the non-linear nature of metabolism on longer time series data sets.

In summary, this was the first study that demonstrated variability changes in breath-to-breath respiratory exchange ratio using a non-linear analysis method. The study is limited by a lack of a control group, but having pre- and post-training numbers

on the subjects provides data showing the metabolic changes that may occur with endurance training. Sample entropy analysis of RER time series data showed increased variability in the btb RER signal along with showing clear differences in the substrate utilization pattern after marathon training. These changes did not correlate with changes in VO_{2max} or 2-mile time. Non-linear analysis of physiological time series data may provide unique insight into the heterogeneity of metabolism changes in exercising humans.

CHAPTER 6: Training Effect on Heart Rate Variability During Exercise in Marathon Runners

CHAPTER 6: Training Effect on Heart Rate Variability During Exercise in Marathon Runners (for submission to the Journal of Strength and Conditioning Research)

Training Effect on Heart Rate Variability During Exercise in Marathon Runners

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Brown, S.R.; Biltz, G.R.; Rhodes, G.S.; Lundstrom, C.J., and Ingraham, S.J. Training Effect on Heart Rate Variability During Exercise in Marathon Runners. *Journal of Strength and Conditioning*. Vol., No., pp., 2013. **Purpose:** To assess the effects of 16 weeks of marathon training on time, frequency, and non-linear heart rate variability (HRV) measures during a steady-state treadmill run. **Methods:** Thirteen novice runners (9 female, 4 male, ages 19-24), from a marathon-training course offered at the University of Minnesota, volunteered to participate in the study. Subjects underwent 16 weeks of marathon training along with pre-and post-training lab testing, which included: 2-mile time trial, VO_{2max}, and body composition by hydrostatic underwater weighing. HRV variability was determined pre- and post-training by a 6-minute steady state treadmill run at 65% of their predicted VO_2 max, based on concurrent 2-mile time trial. Heart rate data was recorded using a Polar s810 heart rate monitoring system and HRV variability was calculated using Kubios software. Matched t-tests were used to compare HRV indices pre- and post-training. **Results:** There were no statistical significant changes in any mean HR the time domain measures along with most of the frequency measures. The ratio of SD1/SD2, from the Poincare plot, increased with training, (0.12 ± 0.05 to

0.24 ± 0.16, p=0.05. SD1 alone trended to increase (2.84 ± 0.95 to 5.98 ± 4.44, p=0.07) and a non-linear measure, SampEn, trended to increase following training (0.67 ± 0.40 to 0.91 ± 0.44, p=0.07). Subjects showed increased HRV during sub-maximal steady state running after 16 weeks of marathon training. **Conclusion:** The ratio of SD1/SD2 and average SampEn scores for participants trended to increase with marathon training. Assessing HRV at both rest and during exercise may provide insight to both parasympathetic and sympathetic adaptations to endurance training.

Introduction

Heart rate variability (HRV) analysis has become a common in both clinical and sports performance research (Seely & Macklem, 2004; Voss et al., 2009). Non-invasive measures of heart rate (HR) along with increases in heart rate monitoring technology allows for affordable monitoring of HR inside and outside the laboratory (Borresen & Lambert, 2008). Traditional linear analysis methods provide a quick and reliable indicator of training intensity based on heart rate response during exercise, but do not seek to elicit physiological information from the variability of the R-R time interval of a heart rate (Hautala, Kiviniemi, & Tulppo, 2009). Monitoring physiology during training, diagnosing overtraining, predicting race performance, assessing changes in physiology with training, and monitoring health outcomes are all possible with the help of HRV measures (Buchheit et al., 2010; Earnest et al., 2004; Hedelin, Wiklund, Bjerle, & Henriksson-Larsen, 2000; Manzi et al., 2009; Pagani & Lucini, 2009; Plews et al., 2012).

HRV analysis will continue to be important in monitoring both physiological changes in response to exercise and physiological readiness for exercise.

Traditional linear methods, calculating means and standard deviations, of analyzing physiological time series data are being supplemented with non-linear systems approach to analyzing physiology (Borresen & Lambert, 2008; West, 2006). The sum of the parts appears greater than any one variable, so an approach that uses both non-linear and linear analysis may be key to understanding the complexity of physiology. Physiology is multi-dimensional and interconnected, which presents challenges when studying physiology using only linear approaches to analyze a single variable (Bravi, Longtin, & Seely, 2011; Goldberger & West, 1987).

Variability analysis seeks to mathematically model the underlying multi-component physiology that takes place to generate one variable that may represent the underlying complexity (Voss et al., 2009). A review of the current methods of variability analysis recently published indicates that not only are non-linear measures becoming commonplace in physiology, but there are also new non-linear methods being developed (Bravi, Longtin, & Seely, 2011).

There are many ways to analyze R-R variability in heart rate, but most HRV studies focus on time, frequency, and non-linear domain measures (Bravi, Longtin, & Seely, 2011). Time domain analysis is the traditional way of looking at variability. In the case of HR, it is common to calculate a mean and standard deviation of the R-R signal (SDNN) during a given exercise period. Poincare plots give a visual representation of

time series data, and quantitative information can be obtained through the calculation of short-term (SD1) and long-term variability (SD2) along with the ratio of SD1/SD2 (Tulppo et al., 1996).

Frequency domain gives insight into the underlying frequency of the process generating the signal being measured (Manzi et al., 2009). Time series data can be converted to frequency data using a Fourier transformation, a breakdown of low and high frequency signals is obtained. The frequency data is completely dependent on the original time series data because it was transformed from the time series data.

Non-linear methods such approximate entropy (ApEn) and sample entropy (SampEn) assess the regularity of the time series data. Entropy scores were developed to measure the regularity of the underlying processes generating the signal, and provide the benefit of only needing 50 data points or more.

Sample entropy seeks to quantify the inherent regularity of a system or the sequential pattern of data generation of a system (Richman & Moorman, 2000). This is accomplished through calculating a conditional probability that a set of points of length m will agree with the next set of points m+1 within a given tolerance of r (usually 0.2*SD). A higher entropy score implies decreased predictability of sequential values – less self-similarity of the data. HRV studies have shown that low sample entropy scores are an indicator of disease state, and that higher scores are reflected of healthy and adaptable physiology (Lake et al., 2002).

HRV are now commonplace in exercise science with an increase in the number papers being published on the topic over the last five years (Hautala et al., 2009). Exercise studies typically analyze HRV in the morning while the subject is resting in a supine position or after exercise during a recovery phase (Earnest et al., 2004; Plews et al., 2012) while others have focused on HRV indices during exercise (Tulppo et al., 1996). Most exercise HRV studies have focused on using time and frequency domain measures with little attention paid to non-linear measures (Buchheit et al., 2010; Tulppo et al., 2003).

The wide range of potential application of HRV analysis in exercise science is shown in the literature. HRV parameters have been shown to signal overtraining in elite triathletes (Plews et al., 2012), elite cyclists (Earnest et al., 2004), marathoners (Kaikkonen et al., 2012), assess sympathetic changes in response to training in rowers (Iellamo et al., 2002), predict marathon performance (Manzi et al., 2009; Pagani & Lucini, 2009), monitor fitness levels in basketball players (Esco & Williford, 2011), predicting and monitoring trainability in healthy sedentary subjects partaking in endurance training (Buchheit et al., 2010; Tulppo et al., 2003), and examining age effects on vagal response to exercise training (Tulppo, Mäkikallio, Seppänen, Laukkanen, & Huikuri, 1998).

The literature is lacking studies analyzing changes in heart rate variability due to marathon training. The aim of the current study is to measure the effect of 16 weeks of

marathon training on time, frequency, and non-linear HRV measures during a steady-state treadmill run.

Methods

Subjects

Thirteen novice runners (9 females: age = 20.3 ± 1.5 yrs; 4 males: age = 20.5 ± 1.3 yrs), enrolled a marathon-training course in the Physical Activity Program at the University of Minnesota, volunteered to participate in the study. The subjects were recruited from a population of 59 students enrolled in a metabolic study conducted in our lab. Subjects were medically cleared for marathon training and were excluded if they were older than 24 years of age or failed to complete both the pre- and post-testing. Due to a data acquisition error only 13 subjects completed both the pre- and post-testing for this study. All of the subjects completed the marathon training and ran the Eau Claire Marathon on May 6, 2012. The study was approved by the University of Minnesota Institutional Review Board, and all subjects gave written informed consent to participate.

Procedure for Data Collection

Subjects underwent testing for HRV variability during a pre- and post-training VO_2 max test. Subjects were required to arrive at the testing facility in the Human and Sports Performance Laboratory (HSPL) at the University of Minnesota on the day of their test in the fasted state (6 hours) without consuming any caffeine prior to the test. Pre-testing started two-weeks prior to the initiation of the marathon-training protocol, and

post-testing took place two-weeks prior to the marathon. On testing days, height and weight were taken prior to testing. The subjects' body composition was assessed using a Floataweigh hydrostatic underwater weighing (Exertech, Dresbach, MN) and calculated using the Brozek equation (Brožek et al., 1963), VO_{2max} testing was performed on a treadmill (Trackmaster, Newton, KS) and a breath-by-breath gas samples were collected using a metabolic gas analyzer (Medgraphics, St. Paul, MN). The gas sensor was calibrated prior to data collection using two different gas mixtures, 21% O_2/79% N_2 and 5% CO_2/12% O_2/83% N_2, (Medgraphics, St. Paul, MN). Gas flow was calibrated using a three-liter syringe (Medgraphics, St. Paul, MN). Subjects were fitted with a facemask and pneumatech (Medgraphics, St. Paul, MN).

HRV was monitored during the 6-minute steady state treadmill run using a Polar s810 heart rate monitoring system (Polar, Kempele, Finland). The VO_{2max} protocol used in the study was developed and validated previously (Popp, 2009). The protocol included a one-minute warm-up session at 3.1 miles per hour, the subjects then ran at a treadmill speed corresponding to 65% of their estimated VO_{2max} pace (based on their initial indoor 2-mile trial (Mello et al., 1988)) for six minutes. The time frame of this stage ensured the subjects were exercising at a steady-state workload and generated enough time points for the non-linear analysis. After the 6-minute stage at 65% VO_2 max pace, the speed and grade of the treadmill were increased until the subjects reached a maximum. Criteria for reaching maximum was achievement of a maximal heart rate (220-age), RER > 1.1, or reaching > 16 on a rate of perceived exertion scale. The same

testing protocol was administered during post-testing, once again, the treadmill speed was based on 65% of their post-training 2-mile time trial VO_{2max} pace.

Heart Rate Variability Analysis

Raw data was checked for outlying data points prior to analysis using Polar Protrainer 5 software (Polar, Kempele, Finland). Subjects' HRV was assessed during a 6-minute steady-state treadmill run at 65% of their predetermined VO_{2max} pace. Time domain, frequency domain, and non-linear results were calculated using Kubios Heart Rate Variability software Version 2 (University of Kupio, Kupio, Finland) (Tarvainen et al., 2009). A value of m = 2 and r = 0.2*SD were used during the sample entropy analysis.

Statistical Analysis

All variables were checked for normality prior to statistical analysis. All HRV methods were analyzed comparing pre-test and post-test values using a t-test for paired samples. There was no difference between males in females in regards to the variables measured, so they were considered together as one group. Results are presented as means +/- standard deviations. An α level of < 0.05 was considered to be statistically significant. Statistical analysis was completed using the statistical program R, version 2.9.2 (The R Foundation for Statistical Computing, USA).

Results

There were no statistical significant changes in any of the time domain measures along with most of the frequency measures. The ratio of SD1/SD2, from the Poincare

plot, increased with training, p=0.05. SD1 trended to increase, p=0.07 and a non-linear measure, SampEn, trended to increase following training, p=0.07.

	Male (n=4)	Female (n=9)
Age (yrs)	20.3 ± 1.0	19.8 ± 1.6
Height (in)	74.1 ± 1.8	65.4 ± 2.4
Weight (kg)	82.0 ± 7.1	63.9 ± 7.0
% Body Fat	16.5 ± 3.0	21.5 ± 4.2

Table 4: Baseline anthropometric data for heart rate variability marathon subjects.

	Pre	Post	p-value
Mean HR (beats/min)	163 ± 11	161 ± 12	0.34
Mean RR (ms)	370.6 ± 25.7	378.6 ± 32.5	0.20
SDNN (ms)	17.1 ± 5.7	21.7 ± 11.3	0.24

Table 5: Time domain HRV results.

	Pre	Post	p-value
LF (Hz)	0.059 ± 0.019	0.074 ± 0.036	0.24
HF (Hz)	0.246 ± 0.088	0.227 ± 0.073	0.48
SD1 (ms)	2.84 ± 0.95	5.98 ± 4.44	0.07
SD2 (ms)	24.0 ± 8.1	28.6 ± 13.7	0.33
SD1/SD2	0.12 ± 0.05	0.24 ± 0.16	0.05*

Table 6: Frequency domain HRV results.

	Pre	Post	p-value
SampEn	0.67 ± 0.40	0.91 ± 0.44	0.07

Table 7: Non-linear HRV results.

Discussion

There was no change specific to the traditional linear measures of mean HR and SDNN during the 6-minute exercise bout. Short-term heart rate variability may increase with marathon training indicated by the increasing trend in SD1 of the Poincare plot. The ratio of SD1/SD2 increased with training, which has also reflects an increases in short-term variability. SampEn changes in R-R variability, previously, have not been quantified during exercise in response to endurance training. In the current study, SampEn trended to increase with training, which gives support that HR becomes more adaptive with the various stresses imposed on the body while training outside. These results match previous research findings; there is increased sympathetic response with exercise training, especially during exercise (Tulppo et al., 1996). There is a great deal of variability in the absolute SampEn scores of the subjects, which supports that individual response to exercise is highly variable (Vesterinen et al., 2011). Studies also support the high degree of variability when it comes to cardiovascular and metabolic adaptations associated with aerobic training (Goedecke et al., 2000; Tulppo et al., 2003; Vollaard et al., 2009). This variability must be recognized and taken into account when interpreting a training response.

Tulppo et al. (1996) used linear and non-linear analysis methods to assess HRV changes in healthy males during exercise. Time domain parameters were found to only give a glimpse of the underlying physiology, whereas non-linear measures such as approximate entropy (ApEn) captured information lost by only taking a linear approach

to time series data analysis. Using atropine administration to block parasympathetic tone they found that changes in ApEn, SD2, and SD1/SD2 did not correlate with parasympathetic control, but SD1 of the Poincare plot did correlate with vagal modulation (Tulppo et al., 1996). There was a slight correlation between ApEn and sympathetic tone towards the end of exercise. This may suggest that entropy scores are best used to represent the complexity of the underlying physiological mechanisms generating a HR signal.

Interpretation of HRV data is dependent on the physiological state the subject, so future attention may be given to look at a combination of HRV measurements under differing physiological conditions (Buchheit, Simon, Piquard, Ehrhart, & Brandenberger, 2004; Vesterinen et al., 2011). It appears plausible that both parasympathetic and sympathetic pathways both improve their ability to act on the heart after endurance training. HRV measures showed increased sympathetic dominance in world-class junior rowers following high intensity training (Iellamo et al., 2002). For exercise, changes in sympathetic response during exercise may have positive health and performance outcomes due to the increased ability for the heart to deliver blood to working muscle (Buchheit et al., 2010). Parasympathetic response may become more important during rest or recovery and monitoring of each of these parameters may provide insight to the underlying physiology (Buchheit et al., 2010). Measuring HRV during exercise only pose problems due to the lack of stationarity during most exercise conditions, and there is no consensus around the specific HRV adaptation to training (Buchheit et al., 2004; Earnest

et al., 2004; Tulppo et al., 1996). Performing HRV measurements both at rest and during exercise may give a more complete picture of the physiological adaptation to exercise training.

The results of the study provide novel insight into HRV changes during exercise associated with marathon training in health college-age subjects. Limitations to the study include having a small sample size along with not having a simultaneous control group. With that said, the subjects' trended towards increased sympathetic heart rate response during exercise following endurance training. These findings suggest that there is increased heart rate variability following marathon training, and suggest an increase in sympathetic response during exercise. HRV parameter changes following marathon training contain a great deal of individual variability, which must be accounted for when designing and interpreting training programs and results. Examining heart rate variability not only at rest, but also during exercise may provide a more complete picture of sympathetic and parasympathetic changes in response to endurance exercise of an individual.

CHAPTER 7 - References

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Chapter 8: Appendices

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8.1 Statistical Analysis

Description of data

There is data from N=59 marathon runners. Each runner was assessed for physiologic variables and performance measures prior to 16 weeks of marathon training and after 16 weeks of training.

The physiologic variables measured at each time point were:

- Respiratory exchange ratio sample entropy (SampEn)
- Average respiratory exchange ratio (avg RER)
- % body fat
- VO_2 max

The performance measures used in this analysis are:

- 2-mile time (minutes)
- Marathon time (post-only)

Our goal is to examine whether there are changes in these physiologic variables and determine if these changes are associated with changes in 2-mile time.

Marathon time was excluded since it was measured only after 16 weeks of training.

Characteristics of sample

Table 8 shows age and sex distributions for the sample.

Age	Number
18	4 (7%)
19	13 (22%)
20	15 (25%)
21	15 (25%)
22	10 (17%)
23	1 (2%)
24	1 (2%)
Mean (SD)	20.4 (1.3)
Median (Range)	20 (18-24)
Sex	
Female	39 (66%)
Male	20 (34%)

Table 8: Age and sex distribution for sample.

Changes in physiologic and performance variables

Table 9 shows the mean differences (pre-post) for each physiologic variable. A paired t-test was used to test for significant differences.

Variable	N	Mean (SE)	p-value
Samp En	59	0.20 (0.05)	0.0002
Avg RER	59	-0.01 (0.01)	0.23
Body fat %	50	-0.29 (0.28)	0.30
VO_2 max	58	4.44 (0.78)	<0.0001

Table 9: Mean differences (pre-post) for each physiologic variable.

The results indicate that SampEn and VO_2 max significantly increased, on average, from pre to post-training.

Figure 7A-7D show the changes for each runner in graphical form.

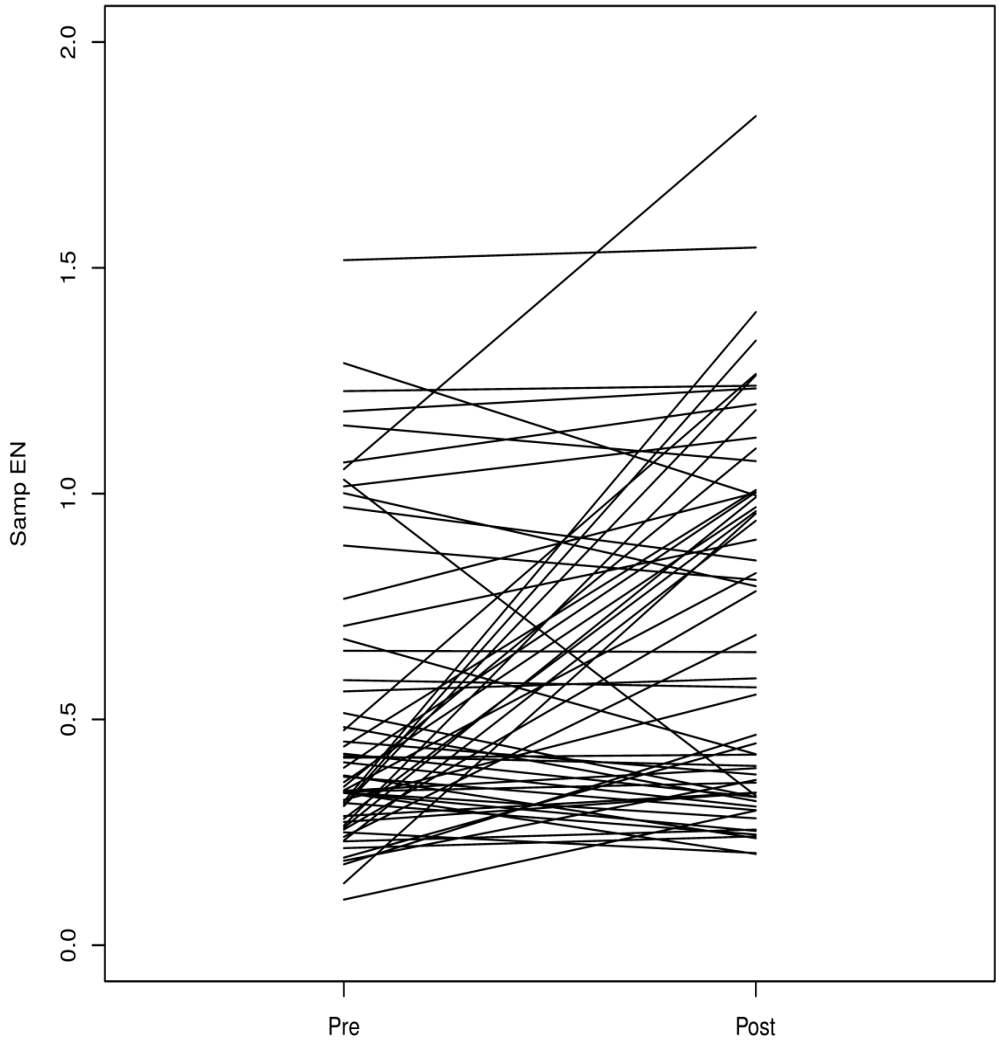

Figure 5A: Pre and post-training measurements for SampEn for each runner.

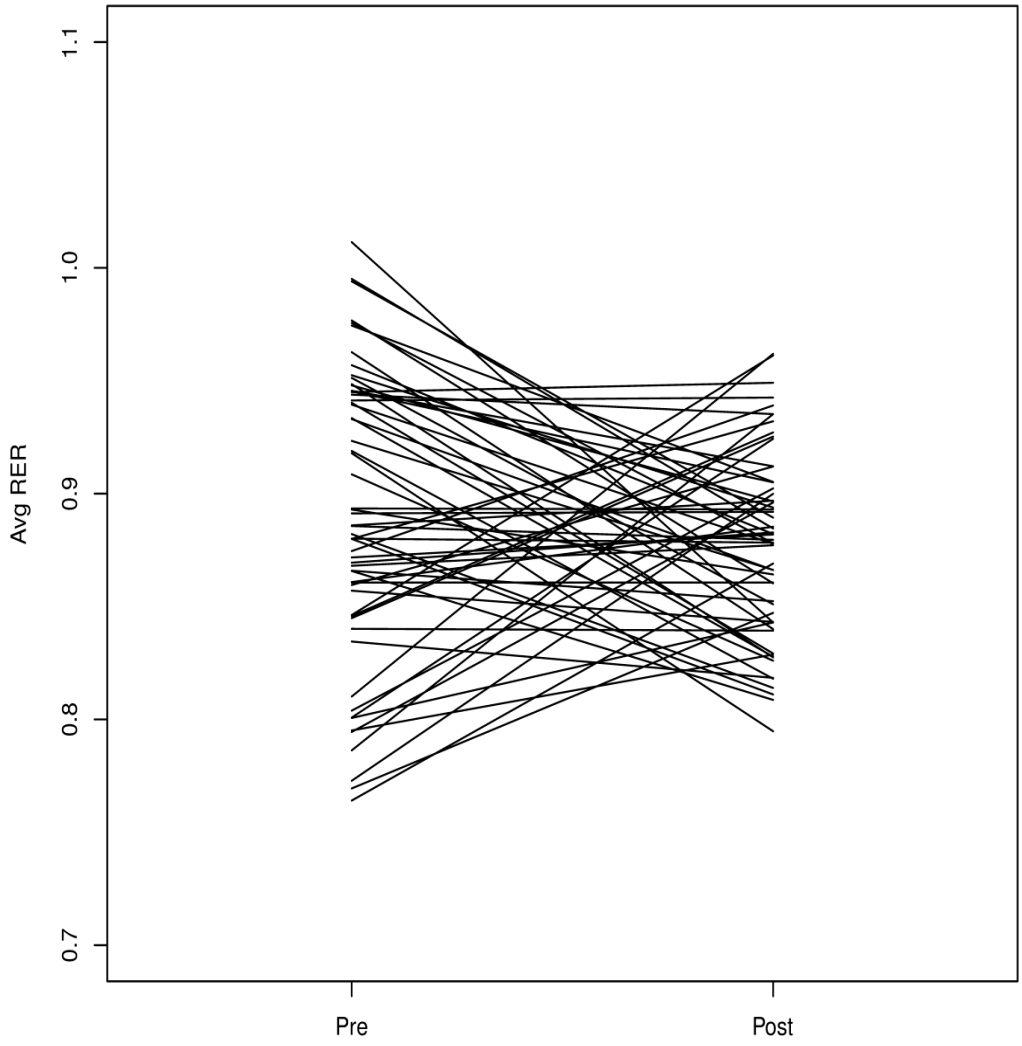

Figure 5B: Pre and post-training measurements for avg RER for each runner.

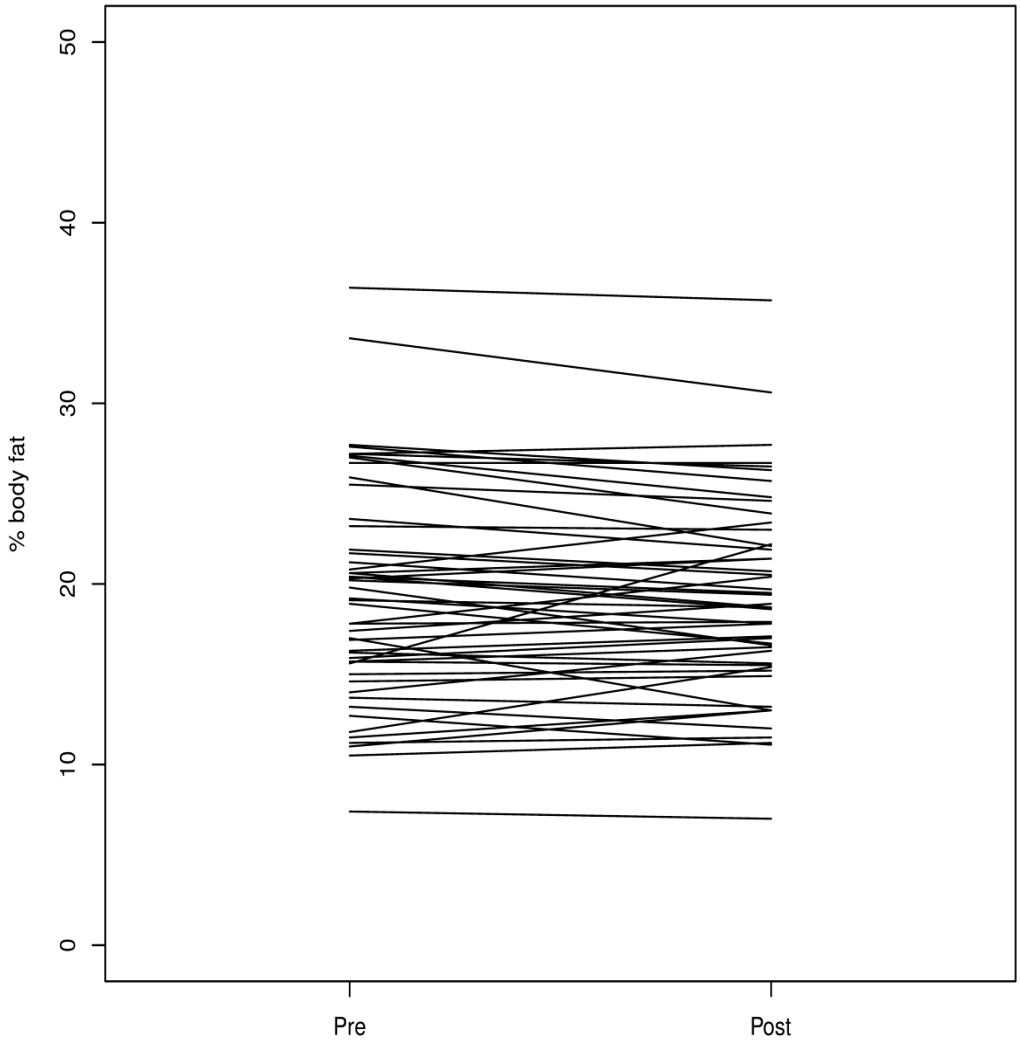

Figure 5C: Pre and post-training measurements for %body fat for each runner.

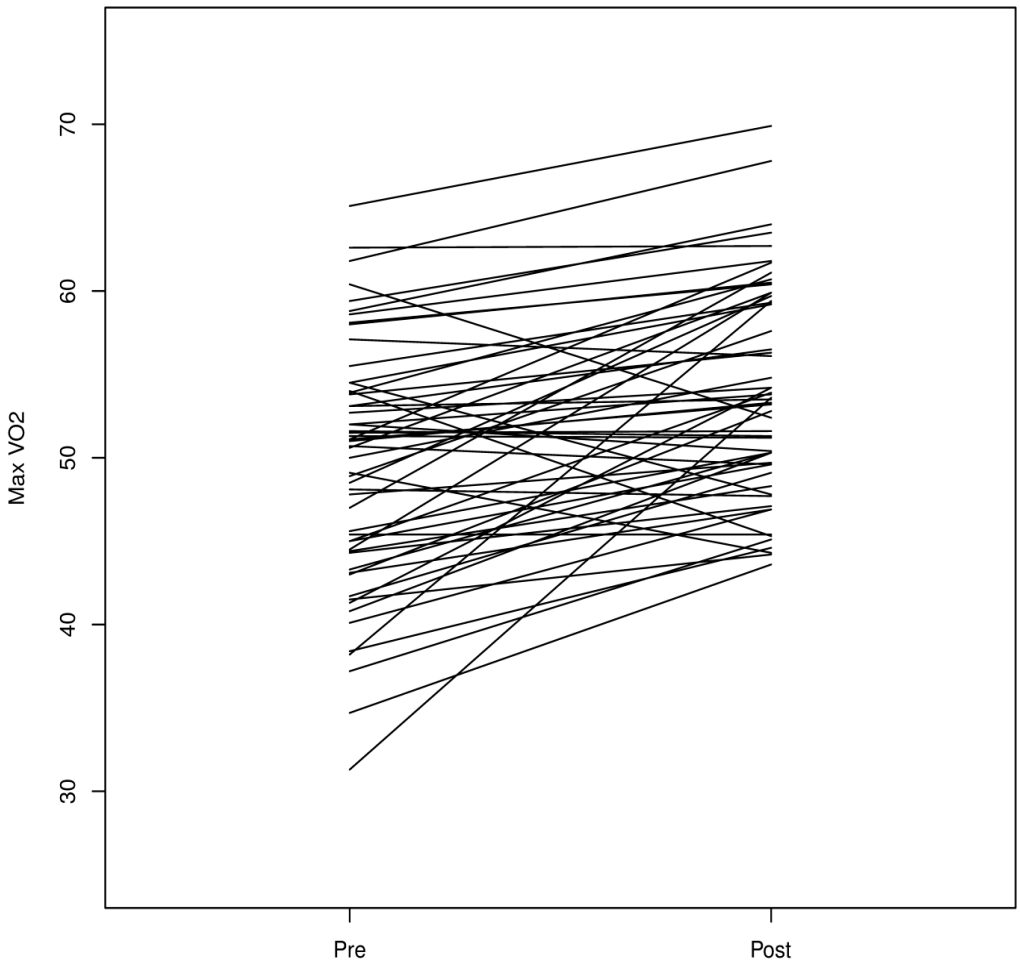

Figure 5D: Pre and post-training measurements for VO_2 max for each runner.

Table 10 shows the mean difference (pre-post) for our only performance measure, 2-mile time. A paired t-test was used to test for a significant difference.

Variable	N	Mean (SE)	p-value
2-mile time (minutes)	59	-1.5 (0.15)	<0.0001

Table 10: Mean differences (pre-post) for 2-mile time.

The results indicate that, on average, runners decreased their 2-mile time by 1.5 minutes.

Figure 6 shows the 2-mile time changes for each runner in graphical form.

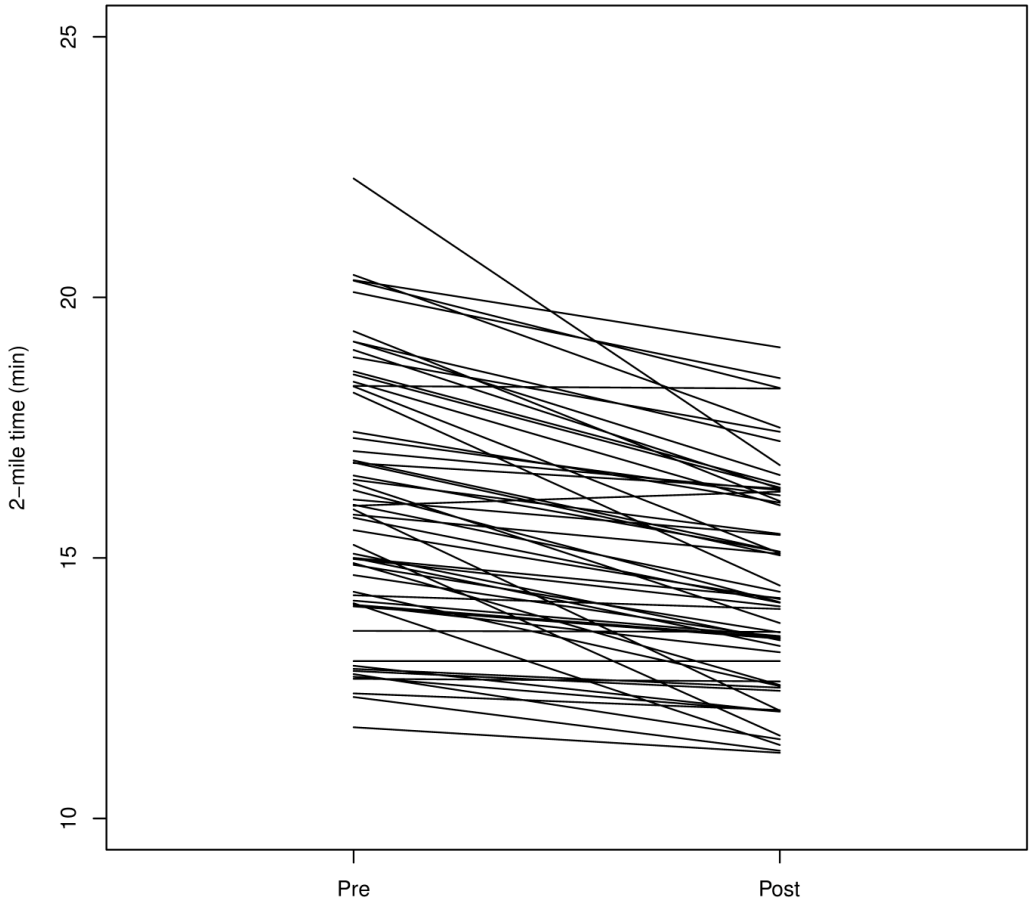

Figure 6: Pre and post-training measurements for 2-mile time for each runner.

Differences in RER physiologic variables by sex

Figure 7A and 7B show the changes in RER variables (pre-post) by sex. These figures indicate that the changes are similar between sexes.

T-tests comparing these RER variables by sex had p-values of 0.77 for change in sampEn and 0.99 for change in avg RER, indicating no evidence that these variables differed by sex.

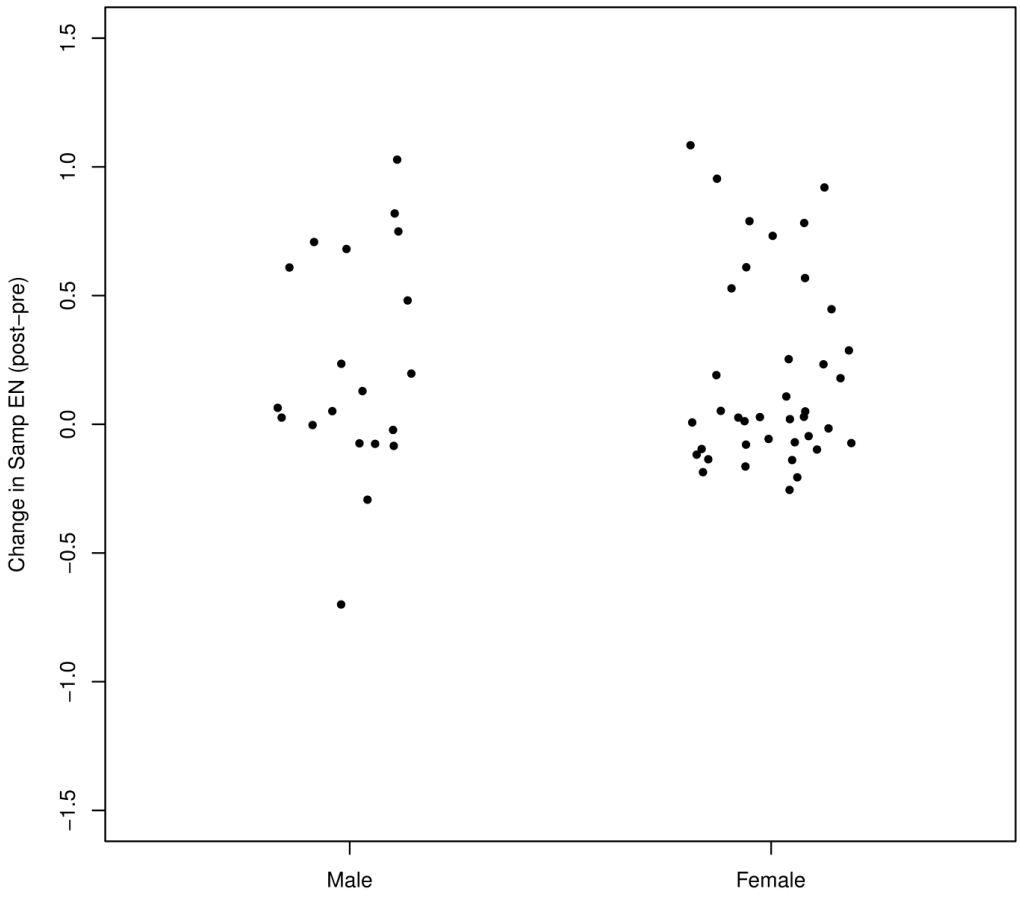

Figure 7A: Change in SampEn by sex. Points are jittered horizontally for easier identification.

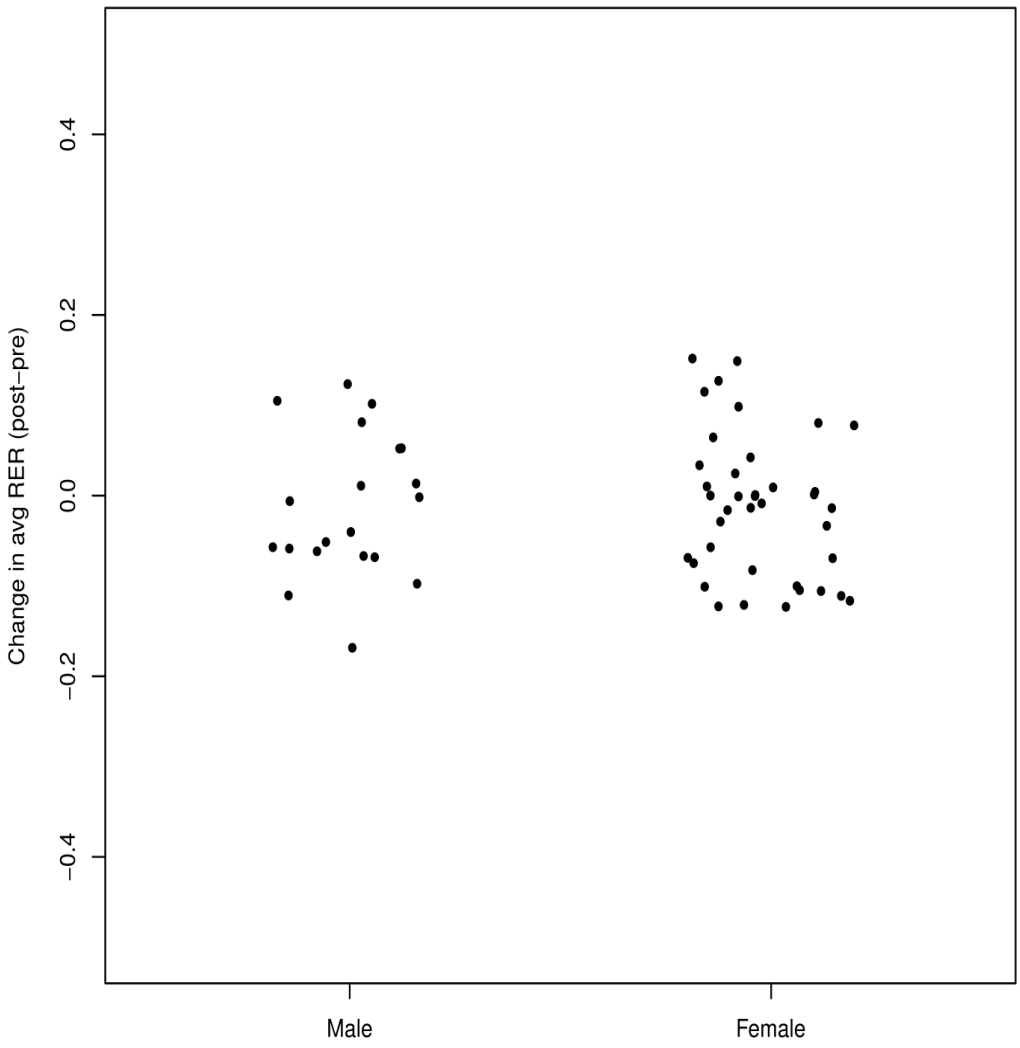

Figure 7B: Change in avg RER by sex. Points are jittered horizontally for easier identification.

Relationship between change in SampEn and change in avg RER

Figure 8 shows a scatter plot of the change in SampEn by the change in avg RER with simple linear regression curve and 95% confidence interval for the mean overlaid.

The plot indicates a slightly positive relationship between the variables. However, the slope of the line is non-significant (p=0.13) in the linear regression model.

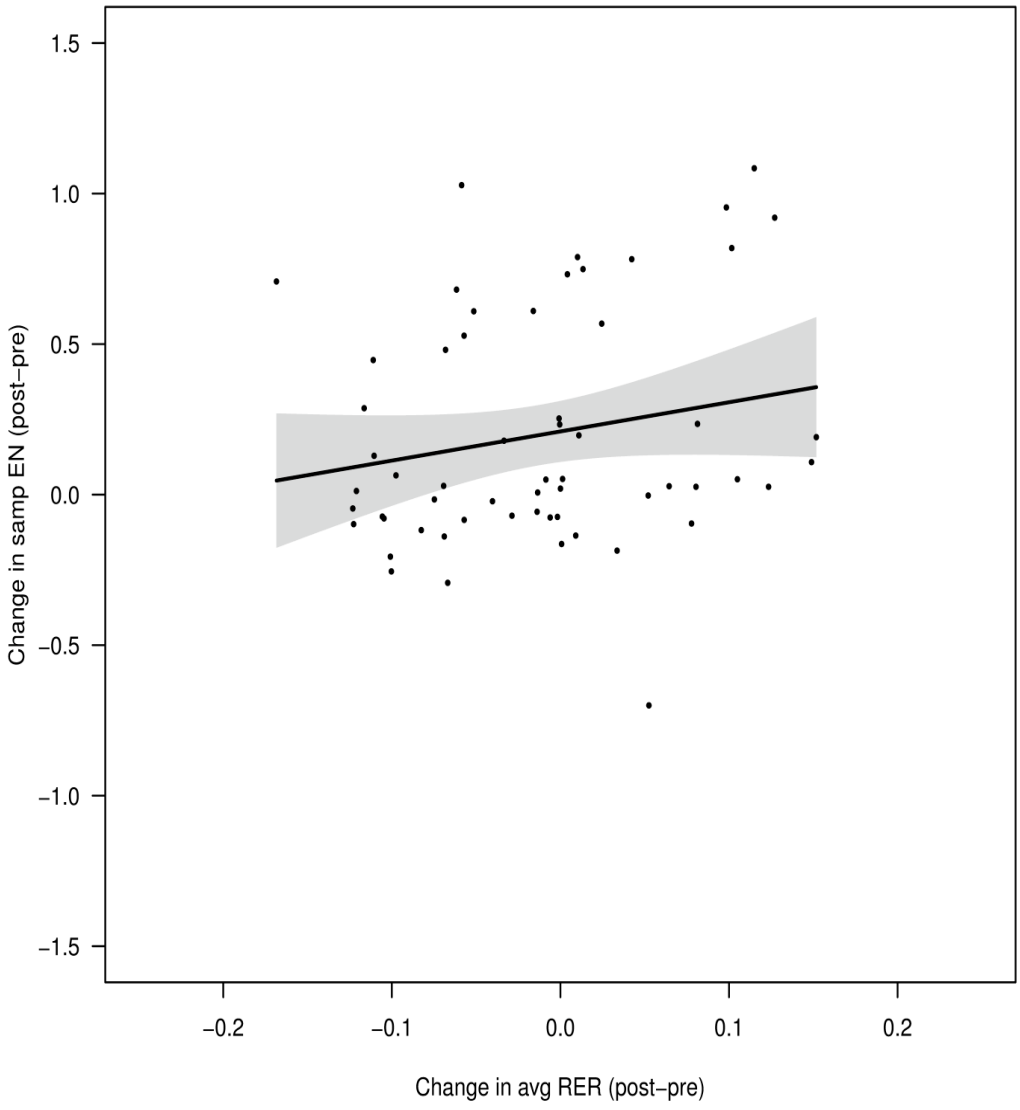

Figure 8: Scatter plot of change in avg RER by change in SampEn with simple linear regression fit (and 95% confidence interval for the mean) overlaid.

Relationship between changes in physiologic variables and changes in 2-mile time

In the following section, we show scatter plots of the change in each physiologic variable by the change in 2-mile time. We also fit a simple linear regression model that related 2-mile time (outcome) to each physiologic variable. The fitted line is shown on the scatter plot, and the estimates from the model are shown in the accompanying table.

The results of the fitted linear model are reported for a change in physiologic variable at a convenient value near the SD (instead of the standard 1-unit change, which is sometimes misleading). The reported effect for each variable is shown below:

- SampEn diff: 0.50
- Avg RER diff: 0.10
- % body fat: 2
- VO_2 max: 5

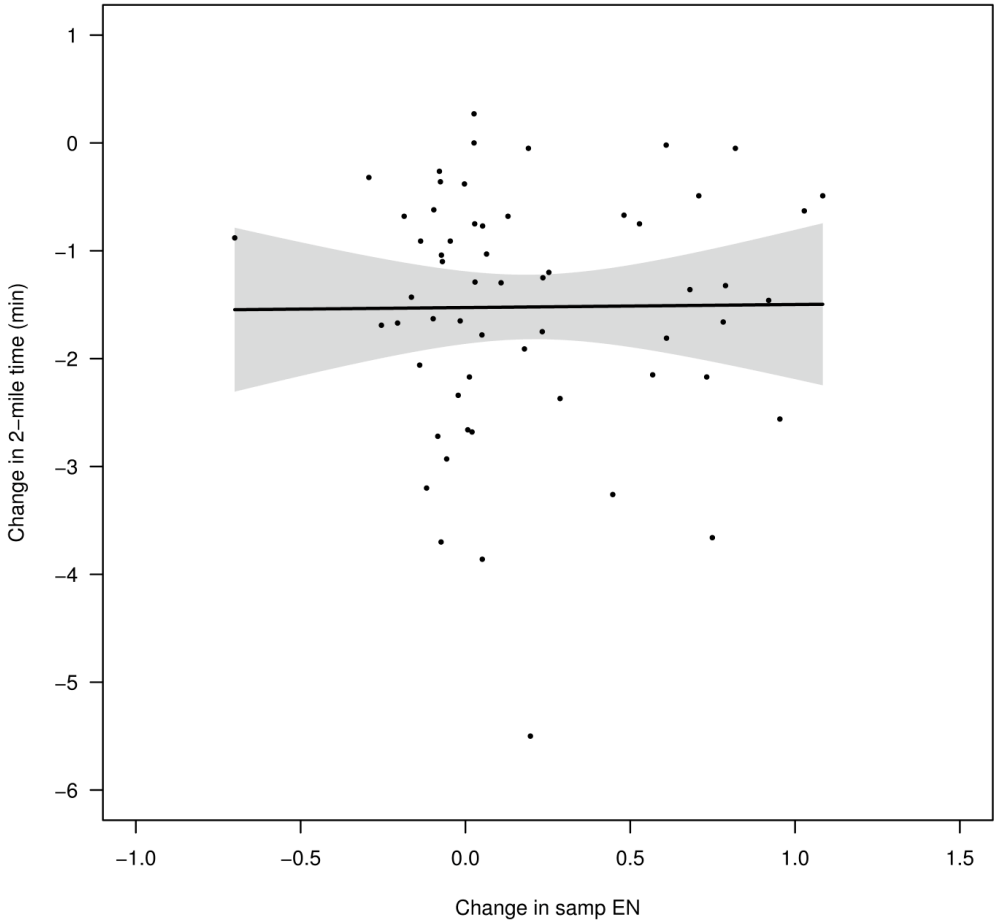

Figure 9A: Change in 2-mile time by change in SampEn, with best fitting linear regression line and 95% confidence interval overlaid.

Variable	Estimate (standard error)	p-value
Intercept	-1.53 (0.17)	<0.001
Change in Samp EN, 0.5-unit increase	0.01 (0.19)	0.94

Table 11A: Parameter estimates from simple linear regression model.

The results from above indicate that change in SampEn was not significantly associated with change in 2-mile time. In particular, a 0.5-unit increase in SampEn was associated with 0.01 minute larger 2-mile time (essentially no change as indicated in the Figure), on average.

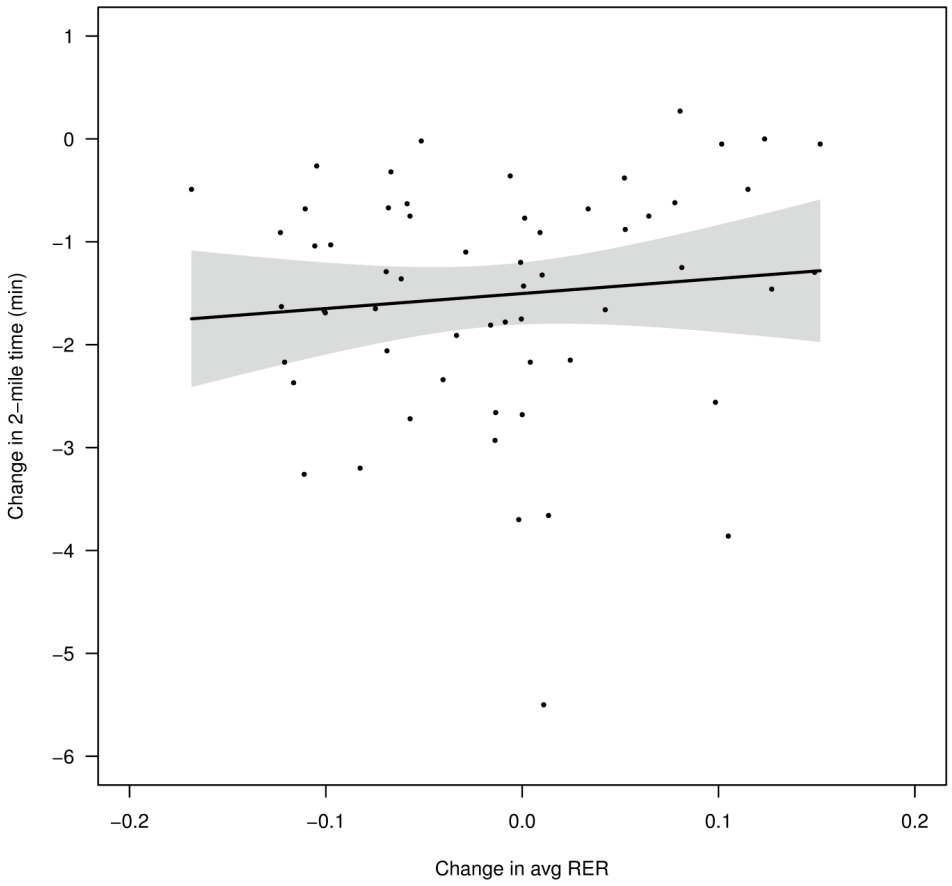

Figure 9B: Change in 2-mile time by change in avg RER, with best fitting linear regression line and 95% confidence interval overlaid.

Variable	Estimate (standard error)	p-value
Intercept	-1.50 (0.15)	<0.001
Change in avg RER, 0.1-unit increase	0.15 (0.19)	0.45

Table 11B: Parameter estimates from simple linear regression model.

The results from above indicate that change in avg RER was not significantly associated with change in 2-mile time. In particular, a 0.1-unit increase in avg RER change was associated with 0.15 minute larger 2-mile time, on average.

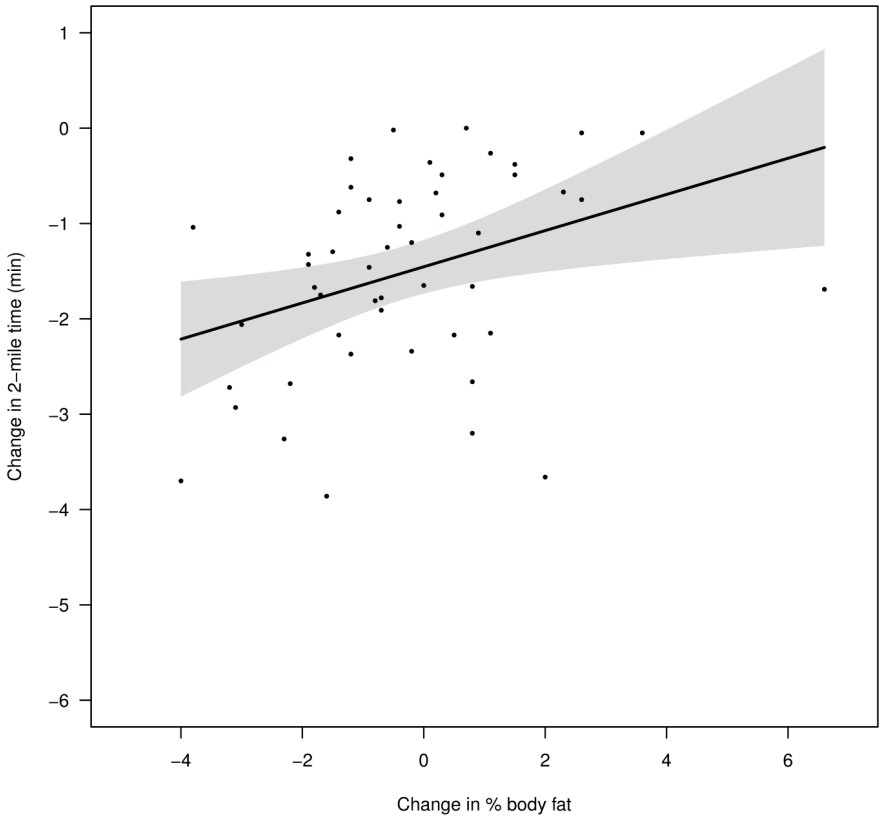

Figure 9C: Change in 2-mile time by change in % body fat, with best fitting linear regression line and 95% confidence interval overlaid.

Variable	Estimate (standard error)	p-value
Intercept	-1.45 (0.14)	<0.001
Change in %body fat, 2-unit (%) increase	0.38 (0.14)	0.011

Table 11C: Parameter estimates from simple linear regression model.

The results from above indicate that a 2 percentage point change in body fat was significantly associated (p=0.011) with an increase in 2-mile time. In particular, a 2 percentage point increase in body fat was associated with 0.38 minute larger 2-mile time, on average. (Or a 2 percentage point decrease in body fat was associated with a 0.38 decrease in 2-mile time.)

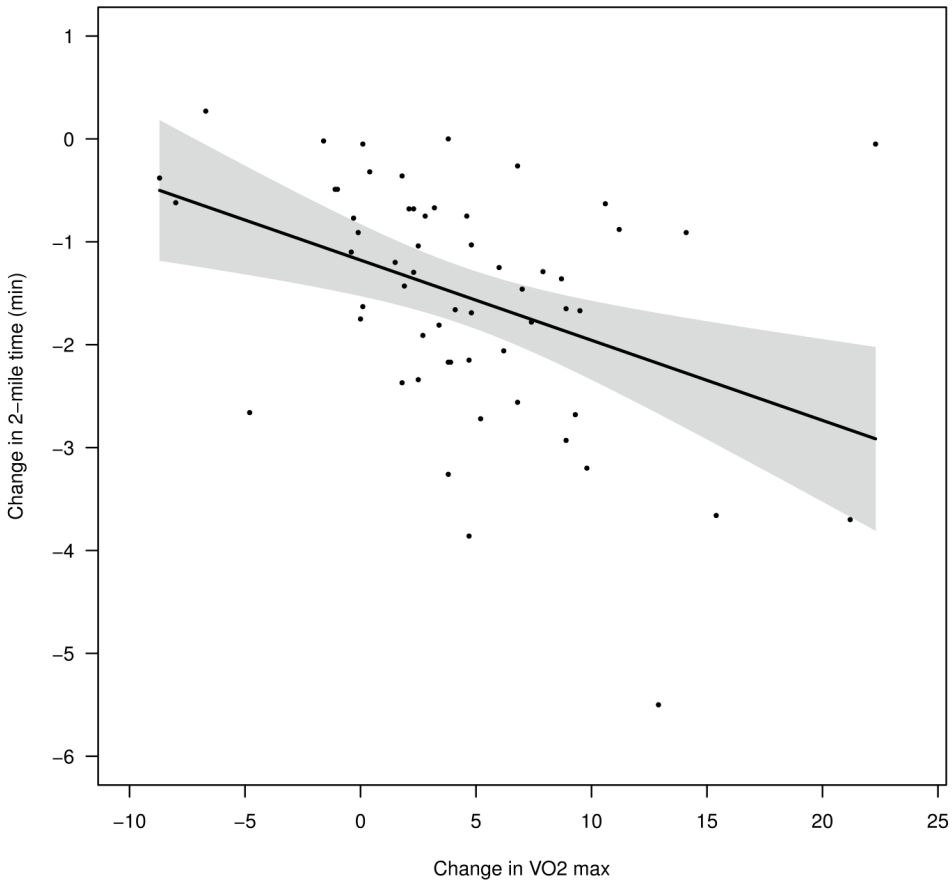

Figure 9D: Change in 2-mile time by change VO_2 max, with best fitting linear regression line and 95% confidence interval overlaid.

Variable	Estimate (standard error)	p-value
Intercept	-1.18 (0.17)	<0.001
Change in VO2 max, 5-unit increase	-0.39 (0.12)	0.002

Table 11D: Parameter estimates from simple linear regression model.

The results from above indicate that change in VO_2 max was significantly associated (p=0.002) with change in 2-mile time. In particular, a 5-unit increase in VO_2 max was associated with 0.39 lower 2-mile time, on average.

Multivariable models

In the models above, we have considered only one physiologic variable for each model. In this section, we use all physiologic variables to construct a multivariable model. Our outcome remains the change in 2-mile time.

Since SampEn and avg RER were both derived from same lung data and both attempt to measure (in different ways) the same underlying process, we fit 2 separate models, one for each measure. Each model adjusts for age, sex, change in % body fat, and change in VO_2 max.

A total of 10 runners had missing values for either % body fat or VO_2 max. Therefore, the multivariable models use only N=49 runners with complete information on all covariates.

The parameter estimates and statistical tests for each model are shown in Tables 12A and 12B below.

Variable	Estimate (standard error)	p-value
Intercept	-1.06 (0.27)	<0.001
Change in SampEn, 0.5-unit increase	-0.11 (0.20)	0.59
Change in body fat, 2-point increase	0.37 (0.15)	0.014
Change in VO2 max, 5-point increase	-0.27 (0.12)	0.031
Sex (F vs. M)	-0.15 (0.30)	0.61
Age, 1-year increase	-0.09 (0.11)	0.41

Table 12A: Parameter estimates from simple linear regression model that included change in SampEn.

The fitted model indicates that change in body fat and change in VO_2 max were the only variables significantly associated with change in 2-mile time.

Variable	Estimate (standard error)	p-value
Intercept	-1.10 (0.26)	<0.001
Change in avg RER, 0.1-unit increase	0.15 (0.17)	0.38
Change in body fat, 2-point increase	0.33 (0.14)	0.024
Change in VO2 max, 5-point increase	-0.26 (0.12)	0.034
Sex (F vs. M)	-0.13 (0.29)	0.65
Age, 1-year increase	-0.10 (0.10)	0.36

Table 12B: Parameter estimates from simple linear regression model that included change in avg RER.

The fitted model indicates that change in body fat and change in VO_2 max were the only variables significantly associated with change in 2-mile time.

8.2: Example Kubios Files

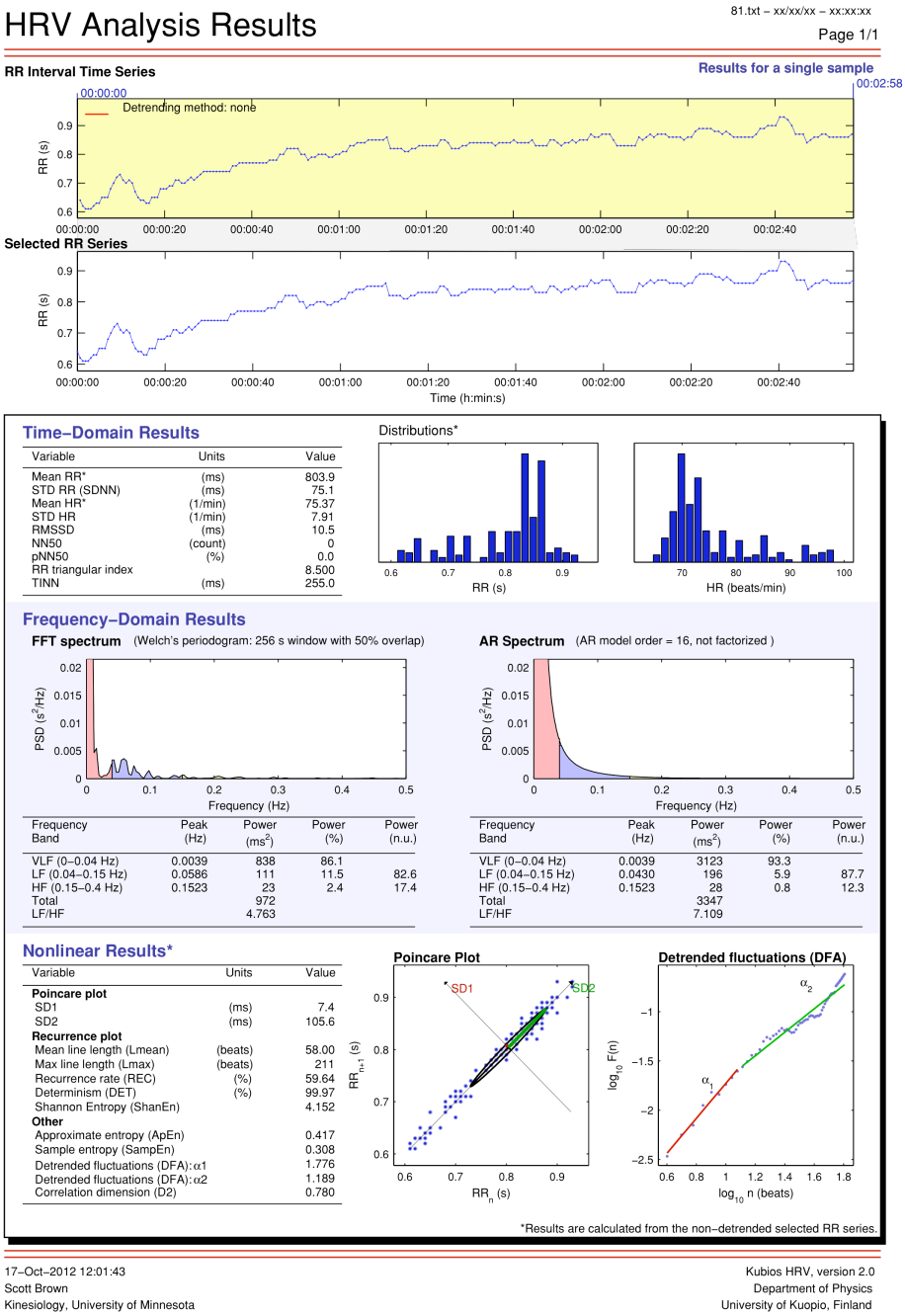

Figure 10: Example pre-training Kubios data analysis file report sheet.

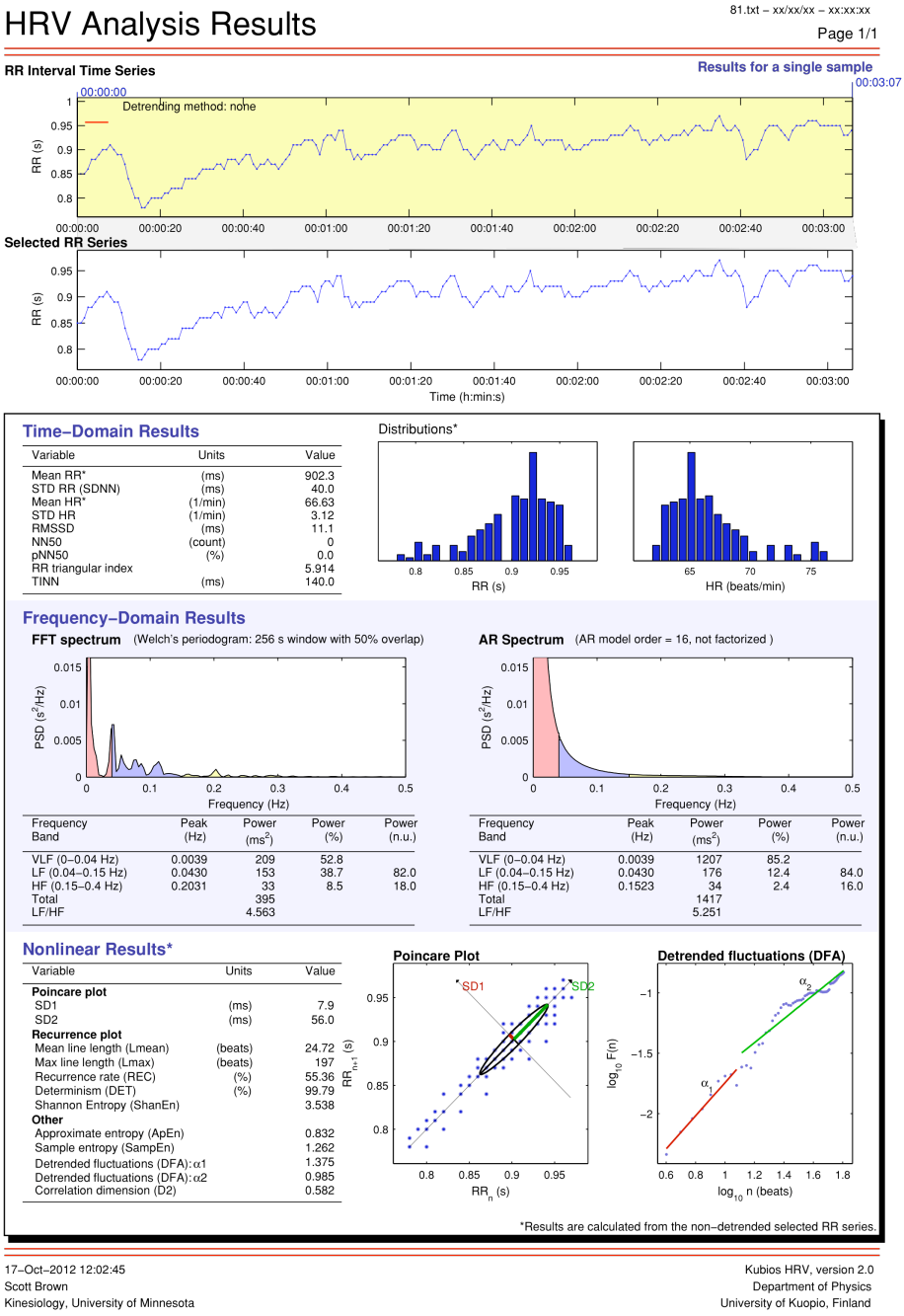

Figure 11: Example post-training Kubios data analysis file report sheet.