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MATHEMATICAL MODELING OF HEART RATE DEFLECTION POINT IN RELATION TO RESPIRATORY COMPENSATION AND TREADMILL RUNNING PERFORMANCE

by

KAYLA M. BAKER B.S. University of Central Florida, 2016

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Educational and Human Sciences in the College of Education and Human Performance at the University of Central Florida Orlando, Florida

Spring Term 2016

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ABSTRACT

Heart rate deflection point (HRDP), identified as the second breakpoint in the "intensity / heart rate" relationship, is indicative of the anaerobic threshold (AT). This point can be determined via bi-segmental linear regression (2SEG) or through use of the maximal distance model (D-max); however, the relationship between these methods has yet to be investigated 2. **Purpose:** To compare the use of 2SEG and D-max methods to determine HRDP and to examine the relationship between these values with a metabolic threshold, respiratory compensation point (RCP), as well as running performance [5,000 meter treadmill time trial (5K_{time})]. Methods: Nineteen recreationally active men (n=9, 25.56±3.17 y, 1.77±0.05 m, 83.52±6.77 kg, 48.98±7.37 ml·kg·min⁻¹) and women (n=10, 22.78±2.11 y, 1.64±0.07 m, 62.28±6.20 kg, 42.32±4.13 $ml \cdot kg \cdot min^{-1}$) were recruited for this study. Participants completed two experimental trials, consisting of a graded exercise test to exhaustion (GXT) and 5,000m time trial on the treadmill. Estimates of HRDP and RCP were calculated from data collected during the GXT. One-way repeated measures analysis of variance was used to compare HRDP found through 2SEG (HRDP_{2SEG}), HRDP found through D-max (HRDP_{D-max}), and RCP. Pearson product moment correlations were used to examine the relationship between variables (HRDP_{2SEG}, HRDP_{D-max}, RCP, and 5K_{time}). **Results:** No differences were found between HRDP_{2SEG} (176.70±9.40 bpm), $HRDP_{D-max}$ (178.18±6.85 bpm), and RCP (176.92±6.63 bpm) (p = 0.533). Strong correlations were found between HRDP_{2SEG} and HRDP_{D-max} (r = 0.831, p < 0.0001), RCP and HRDP_{2SEG} (r =0.650, p = 0.003), and RCP and HRDP_{D-max} (r = 0.619, p = 0.005). No relationship was found between 5K_{time} and HRDP_{2SEG} (r = 0.419, p = 0.074), HRDP_{D-max} (r = 0.241, p = 0.321), or RCP (r = 0.193, p = 0.429). Similar limits of agreement were found for all comparisons (HRDP_{2SEG} and RCP, p = 0.070; HRDP_{D-max} and RCP, p = 0.868; HRDP_{2SEG} and HRDP_{D-max}, p = 0.029),

however, systematic bias was found between $HRDP_{2SEG}$ and $HRDP_{D-max}$. **Conclusion:** Based on these results, $HRDP_{2SEG}$ and $HRDP_{D-max}$ could potentially be used interchangeably as methods to determine HRDP. However, the potential for systematic bias must be taken into consideration. Additionally, the results indicate that RCP and HRDP may provide similar estimates of the anaerobic threshold, but none of these thresholds appear to be related to $5K_{time}$ on a treadmill.

ACKNOWLEDGMENTS

First, I would like to thank my family and friends for being so supportive of me throughout this process. I would especially like to thank my mom, Rita Baker, for being my best friend and my rock. Her support and words of wisdom have helped me through many tough days. I would also like to thank my brother, Adam Baker, for being the most loving, caring, fun, and sweet brother and best friend I could have ever dreamed of having. Also, thank you to my dad; although he is no longer physically by my side, he still lives in my heart, and I am reminded by his words on a daily basis to always stand tall, no matter what the circumstance.

I would also like to thank my colleagues at the University of Central Florida for all of the help they have given throughout these last couple of years. Thank you to David Church, Michael La Monica, Josh Riffe, Mattan Hoffman, and Alyssa Varanoske for being such huge parts of this study. Without you, this project would not have been possible. Thank you to the rest of my colleagues, past and present: Adam Jajtner, Mike Redd, Tyler Muddle, Jeremy Townsend, Carleigh Boone, Ran Wang, Amie Miramonti, Dr. AJ Wells, Dr. Jerry Mangine, and Dr. Adam Gonzalez. Every single one of you inspires me in different ways on a daily basis and I am so lucky to work and have worked with such amazing people. I would also like to send a special thanks to Dr. Bill McCormack for not only teaching me so much during some of the busiest times of his Doctorate, but also for inspiring me four years ago to pursue this journey I am on now.

Lastly, thank you to my committee advisors: Dr. Jeffrey Stout and Dr. Jay Hoffman. I am so appreciative for all of your guidance and support. I would especially like to thank my committee chair, Dr. David Fukuda, for making this entire experience an enjoyable and

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unforgettable one. I have learned and grown so much from this experience. Thank you, Dr. Fukuda, for inspiring me and believing in me.

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OPERATIONAL DEFINITIONS

Heart rate deflection point – indicative of the anaerobic threshold; the second breakpoint in		
linearity when examining a "speed or time / heart rate" relationship curve		
Peak oxygen consumption – the highest amount of oxygen able to be consumed per minute,		
typically determined during a graded exercise test		
Minute ventilation - volume of oxygen inhaled (or carbon dioxide exhaled) per minute		
Respiratory exchange ratio – the ratio between the volume of oxygen inhaled and volume of		
carbon dioxide exhaled in one breath		
Conconi test – a non-invasive test to determine anaerobic threshold (or heart rate deflection		
point) which is determined by the relationship between running velocity and heart rate		
Graded exercise test – an exercise test which utilizes increasing intensities until complete		
exhaustion		
Exhaustion – the point at which the participant can no longer continue running as the designated		
treadmill speed and incline		
Running performance – the time in which it takes each runner to complete a 5,000-meter time		
trial on the treadmill		
Bi-segmental linear regression method – a method of determining heart rate deflection point (or		
the second break point in linearity) by utilizing an algorithm applied to heart rate and time,		
which allows for analysis of the moment that a change in values (or break point in linearity)		
which allows for analysis of the moment that a change in values (or break point in linearity) occurs and divides the "time / heart rate" relationship into two linear regression segments,		
occurs and divides the "time / heart rate" relationship into two linear regression segments,		

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of the "time / heart rate" relationship curve, where the point on the polynomial regression curve that produces the maximal perpendicular distance to the straight line is considered the heart rate deflection point

LIST OF ACRONYMS/ABBREVIATIONS

2SEG	bi-segmental linear regression method
bpm	beats per minute
D-max	maximum distance method
GXT	graded exercise test
HPL	Human Performance Laboratory
HR	heart rate
HRDP	heart rate deflection point
HRDP _{D-max}	heart rate deflection point determined by D-max method
HRDP _{2SEG}	heart rate deflection point determined by 2SEG method
mph	miles per hour
PAR-Q	Physical Activity Readiness Questionnaire
RCP	respiratory compensation point
RER	respiratory exchange ratio
RPE	Ratings of Perceived Exertion
TT	5000-meter treadmill time trial
TT _{time}	5000-meter treadmill time trial time
VCO ₂	volume of expelled carbon dioxide
VE	minute ventilation
VO ₂	volume of consumed oxygen
VO ₂ peak	peak oxygen consumption
VT	ventilatory threshold

CHAPTER I: INTRODUCTION

The anaerobic threshold is highly correlated to endurance performance and is often used to determine an athletes training intensity (Buchheit, Solano, & Millet, 2007; Carey, 2002, Kara et al., 1996). Optimal training intensities vary between individuals and training goals. Researchers have investigated and defined four main exercise intensity domains, including moderate, heavy, severe, and extreme intensities (Hill et al, 2002; Wilkerson et al., 2004; Jones & Poole, 2005). Moderate intensity is defined as exercise performed below the lactate threshold, with oxygen consumption and blood lactate remaining at low steady state levels. During heavy exercise intensity, lactate threshold is surpassed and an increase in oxygen consumption and blood lactate steady state occurs, which indicates a continuous increase in oxygen consumption and blood lactate accumulation until maximum oxygen consumption is reached (Poole et al., 1988; Beneke & von Duvillard, 1996). As extreme exercise intensity is reached, voluntary exhaustion and fatigue occur (Hill et al, 2002; Wilkerson et al., 2004).

During a graded exercise test, heart rate and exercise intensity theoretically increase at a linear rate. Breakpoints in this linearity, however, have been observed and are likely relevant when determining training intensities (Brooke & Hamley, 1972). These breakpoints occur when heart rate departs from linearity in the "speed or time / heart rate" relationship curve. The first breakpoint on this graph has been shown to be indicative of the aerobic threshold, or point where the body begins to utilize the anaerobic energy system in addition to the aerobic system (Aunola & Rusko, 1992). This transition from moderate to heavy exercise intensity is also considered the "first lactate turn point," where blood lactate accumulation begins to increase while the amount

of oxygen inhaled increases at a disproportionate rate to the amount of carbon dioxide exhaled (Davis et al., 1983). This first breakpoint in linearity is also deemed the ventilatory threshold, where respiratory gases begin to increase at a disproportionate level during incremental exercise (Mourot et al., 2011; Marques-Neto, Maior, Neto, & Santos, 2012). The second breakpoint in linearity has been shown to be indicative of the anaerobic threshold, or point where the body begins to utilize a higher percentage of the anaerobic energy system. This transition from severe to extreme exercise intensity is also referred to as several other fatigue thresholds, such as second lactate turn point, respiratory compensation point, and heart rate deflection point (Svedahl & Macintosh, 2003; Binder et al., 2008; Davis et al., 1983, Billat, 1996; Kara et al., 1996). The "second lactate turn point" is considered to be the point at which blood lactate is produced in the body at a faster rate than what can be processed, as well as the point at which the amount of oxygen being inhaled and the amount of carbon dioxide being exhaled are further increased (Aunola & Rusko, 1992; Davis et al., 1983). The second breakpoint in linearity is also considered the respiratory compensation point, where respiratory gases continue to increase disproportionately to the excretion of carbon dioxide (Mourot et al., 2011; Marques-Neto, Maior, Neto, & Santos, 2012), as well as the heart rate deflection point.

Although the explanation behind the occurrence of the heart rate deflection point is not fully understood, researchers have investigated the relationship between heart rate deflection point and blood lactate levels, potassium levels, parasympathetic drive, epinephrine levels, and norepinephrine levels, though no relationship was found in most cases (Pessenhofer et al., 1991; Pokan et al., 1995; Pokan et al., 1998; Tulppo et al., 1998; Hofmann et al., 1999). Hofmann and colleagues (1999) examined blood lactate and potassium levels as potential causes of heart rate deflection point during incremental exercise. No relationship was found between blood lactate

and heart rate deflection point, or between blood lactate and cycling performance, but a potential relationship was found between potassium levels and heart rate deflection point (r = -0.328, p =0.051), indicating that potassium may influence heart rate during incremental exercise (Hofmann et al., 1999). In a study conducted by Pokan and colleagues (1993), function of the heart in relation to heart rate deflection point was examined, and it was found that heart rate deflection point corresponded with left ventricular ejection fraction, or the volume of blood pumped per contraction (r = -0.672, p < 0.01). Pokan et al. (1993) found that the highest level of left ventricular ejection fraction occurred at the same breakpoint as heart rate deflection point, and once this breakpoint was reached, heart rate and left ventricular ejection fraction simultaneously began to stabilize (Pokan et al., 1993). Other studies have examined parasympathetic drive as a potential cause of heart rate deflection point with mixed results, potentially due to the major influence of the sympathetic nervous system on heart rate above 60% of maximal oxygen consumption (Hofmann et al., 1994; Tulppo, Makikallio, Seppanen, Laukkanen, & Huikuri, 1998; Pokan et al., 1998). Subsequent research has demonstrated changes in the heart rate response to increasing exercise intensities when parasympathetic drive was blocked, resulting in a shift from heart rate deflection to linearity, and linearity to heart rate inflection (Pokan et al., 1998). However, researchers were unable to find a relationship between epinephrine and norepinephrine and heart rate deflection point during incremental exercise testing on a cycle ergometer (Pokan et al., 1998). While the exact mechanisms of the heart rate deflection point remain unclear, its relationship with performance and other fatigue thresholds demonstrate its potential utility for laboratory and field-based evaluation investigations.

Because the second breakpoint in linearity is also identified as the heart rate deflection point, determination of this point can simultaneously measure anaerobic threshold. Coaches,

athletes, and practitioners may benefit from the knowledge of understanding heart rate deflection points and how they can be measured non-invasively in order to better determine proper training intensities through use of percentages of the anaerobic threshold, as well as monitor endurance training adaptations through changes in heart rate deflection point (Plews, Laursen, Stanley, Kilding, & Buchheit, 2013).

The "Conconi test", created by Conconi, Ferrari, Ziglio, Droghetti, & Codeca (1982), was developed to non-invasively investigate the "speed / heart rate" relationship and to determine the heart rate deflection point as a proxy measure of the anaerobic threshold. Conconi and colleagues (1982) conducted graded exercise tests, or tests utilizing increments of increasing intensity, on a 400-meter outdoor track utilizing slight increases in velocity (approximately 0.5 kilometers per hour) every 200 meters, with no more than an 8 beats per minute increase in heart rate, until exhaustion. The Conconi test has since been used and tested by many researchers, some of which have shown mixed results (Jones & Doust, 1995; Jones & Doust, 1997). However, the majority of researchers have concluded that the use of the Conconi test is an accurate non-invasive way to determine anaerobic threshold (Droghetti et al., 1985; Petit, Nelson, & Rhodes, 1997; Vachon, Bassett, & Clarke, 1999; Sentija, Vucetic, & Markovic, 2007; Bodner, Thodes, Martin, & Coutts, 2002; Cabo, Martinez-Camblor, & Valle, 2011).

Because there is no standardized method of identifying the breakpoint in heart rate linearity, researchers have utilized different approaches to identify the heart rate deflection point, with some of the most common methods being bi-segmental linear regression and a maximum distance model. The bi-segmental linear regression method has been shown to provide strong correlations between heart rate deflection points and performance measures (Higa et al., 2007; Grazzi et al., 2008). For example, Grazzi et al. (2008) found that heart rate deflection point

strongly correlated with anaerobic threshold when both were determined via bi-segmental linear regression (r = 0.980, p < 0.0001). Similarly, the maximum distance method has been shown to provide accurate estimates of heart rate deflection points (Kara et al., 1996; Siahkouhian, Azizan, & Roohi, 2012; Ferreira et al., 2015) and strong relationships with running performance (Da Silva, Peserico, & Machado, 2014; Pereira et al., 2015). The current study examined the relationship between the heart rate deflection point determined from bi-segmental linear regression and the maximum distance method. To the best of our knowledge, this comparison has yet to be examined in previous research.

Along with heart rate deflection point, respiratory compensation point may be a potential indicator of anaerobic threshold. The respiratory compensation point defines the point at which ventilation, or breathing, increases at a disproportionate rate with respect to the excretion of carbon dioxide during a graded exercise test and is determined with gas exchange analysis (Meyer, Faude, Scharhag, Urhausen, & Kindermann, 2004). This point may be indicative of the body's inability to continue buffering exercise bi-products at a consistent rate, leading to a more anaerobic state. Respiratory compensation points can be found by identifying the inflection point in the "ventilatory equivalent / volume of expelled carbon dioxide" relationship (Binder et al., 2008). This break in linearity has also been shown to take place at approximately 80% of a participant's maximal oxygen consumption (Bergstrom et al., 2013). In previous research, respiratory compensation point have been found to be consistently higher than the anaerobic threshold, signifying that the two should not be used interchangeably (Beaver, Wasserman, & Whipp, 1986). However, more recent studies have shown respiratory compensation point to be indicative of the anaerobic threshold (Svedahl & Macintosh, 2003).

The purpose of the current study investigated the potential differences between heart rate deflection point estimation using the maximum distance and the bi-segmental linear regression methods. In order to fully evaluate these methods, the relationship with a potentially similar fatigue threshold, respiratory compensation point, and 5,000-meter treadmill running performance were also examined.

Purpose of the Study

The following are the purposes of this study:

- To examine two different methods of identifying heart rate deflection points, including a D-max method (using exponential-plus-constant regression) and a bi-segmental linear regression method.
- 2. To compare the heart rate deflection point estimation methods to respiratory compensation point.
- To determine the relationship between heart rate deflection point estimation methods and 5,000-meter treadmill running performance.

Research Question

The following are the research questions for this study:

- 1. Will the use of the D-max method and bi-segmental linear regression method provide similar estimates of heart rate deflection points?
- 2. Will heart rate deflection points strongly correlate with respiratory compensation points?
- 3. Is there a strong relationship between heart rate deflection point and 5,000-meter treadmill running performance?

Hypothesis

The following are hypotheses for this study:

- 1. The use of the D-max method and bi-segmental linear regression method will provide similar estimates of heart rate deflection points.
- The heart rate deflection points will correlate strongly with respiratory compensation points.
- 3. A strong relationship will be found between heart rate deflection points and 5,000-meter treadmill running performance.

Delimitations

The following are the delimitations of this study:

- 1. Nine males and ten females were recruited to participate in this study.
- 2. Participants' ages ranged from 18-35 years old.
- 3. All participants were required to complete a medical history, exercise questionnaire, and statement of informed consent prior to testing.
- Participants had no history of cardiovascular, metabolic, renal, hepatic, or musculoskeletal disorders.
- 5. Participants were not currently on any medications.
- 6. Participants had a minimum peak oxygen consumption of 35 mL·kg⁻¹·min⁻¹.
- 7. Participants consumed at least 200 milligrams of caffeine per day.
- All testing was performed in the Human Performance Laboratory at the University of Central Florida (Orlando, Florida).

Limitations

- Maximal effort was expected of the participants during graded exercise tests and 5,000meter treadmill time trials.
- 2. Menstrual cycle of female participants was not accounted for.
- 3. Participants completed self-reported food logs.
- 4. There was no criteria for body composition of participants.

Assumptions

- 1. Participants provided accurate information with regard to activity level, supplement use, and medical history.
- 2. All participants were capable of running 5,000 meters without walking or stopping
- 3. Proper calibration and placement of equipment was executed by the researchers.

CHAPTER II: LITERATURE REVIEW

Evaluation of the Heart Rate Deflection Point

Conconi, Ferrari, Ziglio, Droghetti, and Codeca, 1982

Determination of the Anaerobic Threshold by a Noninvasive Field Test in Runners

One of the initial research studies investigating the "speed / heart rate" relationship in incrementally increasing exercise was conducted by Conconi et al. in 1982, who created a noninvasive test to determine anaerobic threshold, termed the Conconi test. The heart rate deflection point (or point at which heart rate departs from linearity in a graded exercise test) was theorized to be a good indicator of the anaerobic threshold. In order to test this theory, a sample size of 210 well-conditioned, middle and long distance male runners (15-65 years, ranging from amateur to elite) were examined during a graded exercise test. Participants completed each trial (or Conconi test) twice. Trials took place on a 400-meter outdoor track and began with subjects running at a velocity of 12-14 kilometers per hour (a low to moderate running intensity, at approximately 70% of the participants' heart rate max). Participants increased velocity in slight increments as they reached submaximal running intensities, which were determined based on each individual runner's abilities (an increase of approximately 0.5 kilometers per hour every 200 meters with no more than an 8 beats per minute increase in heart rate). Each trial was completed when participants reached exhaustion (an average time of 15-20 minutes, or 8-12 laps of 400 meters). Heart rate and running speed were recorded in order to observe a possible relationship. An electrocardiogram was used to determine heart rates of each runner. In examining the relationship between running speed and heart rate, Conconi and researchers (1982) detected a deflection from linearity at submaximal running speed in each participant, where heart rates ceased to increase proportional to running speed.

Conconi et al. (1928) discovered that the heart rate deflection point in the "running speed / heart rate" relationship coincided with the velocity at the anaerobic threshold, which was established by blood lactate measurements, in ten runners, via regression analysis. Correlations were also found between velocity at the heart rate deflection point and average running speed for a 5,000-meter run (r = 0.93), marathon (r = 0.95), and one-hour race (r = 0.99). Researchers concluded that in noninvasively determining the heart rate deflection point in the "running speed / heart rate" relationship, anaerobic threshold could be measured.

Droghetti, Borsetto, Casoni, Cellini, Ferrari, Paolini, Ziglio, and Conconi, 1985

Noninvasive Determination of the Anaerobic Threshold in Canoeing, Cross-Country Skiing, Cycling, Roller and Ice Skating, Rowing, and Walking

Droghetti and colleagues (1985) aimed to create and test adaptations to the Conconi test in order to make it applicable among a broader spectrum of sports other than running, and become a more universal method. In this study, participants included 172 male and 18 female athletes of whom were all well-trained and considered to be at an intermediate athletic level, at minimum. Because researchers aimed to look at a range of different sports, these participants included canoeists (n = 4, 16-22 years), rowers (n = 32, 18-31 years), cross-country skiers of the Italian National Team (n = 42, 17-31 years), cyclists (n = 72, 14-47 years), ice skaters of the Italian National Team (n = 9, 17-26 years), roller skaters (n = 10, 15-23 years), and walkers, of which 14 were on the Italian National Team (n = 20, 15-37 years).

Although each graded exercise test was created differently for each sport in order to maintain specificity, a 15-30 minute warmup was required of all subjects prior to any testing. During all tests, speed, velocity, and heart rate were monitored, and blood draws were taken periodically. Speed was increased incrementally during graded exercise tests. For the group

containing canoeists and rowers, subjects performed a graded exercise test over a distance of 2,400 meters at a velocity of 36 meters per hour, with speed increases every 200 meters. Crosscountry skiers performed an exercise test on an uphill asphalt road when snow was not available, wearing roller-skis. This test was completed over a distance of 1,680 meters with speed increases every 140 meters. When snow was present, skiers completed the test on a frozen lake, 200 meters in length, with speed increases after every lap, or 200 meters (lasting an average of 10-14 laps). Cyclists performed the graded exercise test on a velodrome, 335 meters in distance, with speed increases after every lap, or 335 meters (lasting an average of 12-16 laps). Ice skaters were placed in an artificially frozen track, 400 meters in length, with speed increases after every lap, or 400 meters (lasting an average of 10-12 laps). Roller skaters completed the test on an asphalt skating track, 325 meters in length, with speed increases after every lap, or 325 meters (lasting an average of 10-12 laps). Finally, the walkers performed the graded exercise test on a 400-meter track with speed increases every 200 meters (lasting an average of 10 laps).

In evaluating the "speed / heart rate" relationship in these athletes, researchers were able to find a linear relationship between speed and heart rate in all cases, as well as a deflection point once higher intensities began. Because blood was drawn in this study, Droghetti et al. (1985) were able to identify the onset of blood lactate accumulation, indicating the athletes' anaerobic threshold. This value was shown to be highly correlated with the heart rate deflection point found in the "speed / heart rate" relationship, therefore signifying that the adaptations to the Conconi test in an effort to make this method more applicable to other sports are as accurate and useful as the original Conconi test for runners.

Jones and Doust, 1995

Lack of Reliability in Conconi's Heart Rate Deflection Point

In order to assess the test-retest reliability of the Conconi test, researchers simulated the original outdoor test procedure in a laboratory setting. Jones and Doust (1995) believed that if the heart rate deflection point was a physiological occurrence, it could be replicated in the laboratory. Fifteen well-trained male distance runners (22.5±3.3 years) were recruited and familiarized with treadmill running and testing procedures. An initial graded exercise test to determine peak oxygen consumption was performed by each participant, utilizing a constant speed of 4.44 meters per second and a 1% increase in grade per minute until volitional exhaustion. Researchers were able to distinguish a plateau in oxygen consumption in twelve out of fifteen participants.

Participants completed two Conconi tests on a motorized treadmill. Participants were required to complete each test at four hours post-prandial, wearing the same shoes and clothing for both tests, and at the same time of day. To account for the change in resistance from an outdoor track to an indoor treadmill, Jones and Doust (1995) set the treadmill to a constant 1% incline in grade. Participants began the Conconi test by gradually (within 1-2 minutes) reaching a running velocity of 3.33 meters per second and incrementally increasing velocity by 0.14 meters per second every 200 meters until exhaustion. Heart rate monitors were used to record heart rates throughout the test.

Heart rate deflection points were analyzed through use of bi-segmental linear regression and visual inspection performed by two experienced physiologists. Jones and Doust (1995) were only able to determine a heart rate deflection point in both Conconi tests for six of the fifteen participants, where there was a strong correlation between running velocity and heart rate

deflection point (r = 0.89). Five of the fifteen participants only revealed a heart rate deflection point in one of the Conconi tests performed. In four of the fifteen participants, heart rate deflection point was not determined in either of the Conconi tests. Researchers concluded that the Conconi test may not be reliable in reproducing heart rate deflection points from test to test in a laboratory setting.

Conconi, Grazzi, Casoni, Guglielmini, Borsetto, Ballarin, Mazzoni, Patracchini, & Manfredini, 1996

The Conconi Test: Methodology after 12 Years of Application

Due to previously discovered lack of reliability in the Conconi test, Conconi et al. (1996) created a modification to the test in order to more accurately predict anaerobic threshold from graded exercise tests. Instead of increasing running velocity every 200 meters on an outdoor track, the new test called for increased velocity in increments of time instead of distance. It has been shown that at lower velocities, the "speed / heart rate" relationship is more linear, whereas greater velocities produce a more curvilinear relationship. Along with a new testing protocol, participants were asked to perform a proper warm-up prior to completing any of the experimental trials, which ultimately allowed subjects to reach a higher maximal heart rate than if they had not warmed up. Subjects were also required to have not done any strenuous activity, taken any medication, or drank any coffee within 48 hours prior to testing.

Conconi et al. (1996) used data that had been collected from over 5,000 tests within the past 12 years of research that coincided with the modified Conconi test. Researchers chose 300 of the 5,000 tests at random to use for analysis. Each trial began with a speed that the participant believed was moderate for their specific running abilities, with extremely slight increases in speed for each time interval. Heart rates were monitored via heart rate monitors and were not

meant to exceed an increase of 8 beats per minute as each minute of the test went by. Gradual increases in speed were required for the modified test in order to decrease the initial lactic acid oxygen deficit that may have been seen in previous studies (Tokmakidis, Leger, Fotis, & Roy, 1987). Conconi et al. (1996) considered an unsuccessful Conconi test as one where (1) heart rate deflection point is unidentifiable, (2) r < 0.98 for the straight-line equation of the "speed / heart rate" relationship, (3) heart rate increases at a rate higher than 8 beats per minute every minute, or (4) the acceleration of the final stage is not at a high enough speed.

Researchers developed a computer program to graph the "speed / heart rate" relationship and obtain the correlation coefficient r, intercept on the y-axis, and slope of the straight-line equations attained with the addition of each successive data point. Where the linear phase transitions into a curvilinear phase is where the heart rate deflection point is identified. In observing the heart rate deflection point of these runners, Conconi et al. (1996) noticed that the speed at which this point occurs is much lower than the acceleration speed of the final phase, which demonstrates that the break from linearity occurs much earlier than in the final acceleration phase, which the test protocol calls for. Because of this, researchers concluded that the athlete's acceleration is based on time and not distance, which allows for heart rate adaptation at each stage and a better identification of a deflection in linearity which coincides with exercise intensity above blood lactate increases.

Sentija, Vucetic, and Markovic, 2007

Validity of the Modified Conconi Running Test

Sentija, Vucetic, & Markovic (2007) aimed to validate the modified Conconi test and examine the validity of a shorter, faster ramp treadmill protocol, as an alternative to the more gradual protocol proposed by Conconi et al. (1996). While the original Conconi test aimed to see

increases in heart rate at no more than a rate of 8 beats per minute every minute, Sentija et al. (2007) aimed to see heart rates increase at a much faster and higher rate. Researchers also chose treadmill running over an outdoor track because of the accuracy and ease of controlling participants' running speed. Fifty-one trained, male runners (22.3 ± 5.5 years) were recruited to perform two incremental exercise tests on the treadmill. Participants included sprinters, middle distance runners, and long distance runners of varying abilities. The experimental trials consisted of a standard incremental treadmill test (similar to the one used by Conconi et al., 1996) with speed increases of 1 kilometer per hour every 30 seconds, until exhaustion. Both treadmill protocols utilized a constant treadmill incline of 1.5%. Heart rate during the standard incremental treadmill test, which lasted approximately 18 minutes, reached increases of 6.3 ± 1.2 beats per minute every minute. Heart rate during the fast incremental treadmill test, which lasted approximately 10 minutes, reached increases of 10.2 ± 1.8 beats per minute every 30 seconds.

Sentija and researchers (2007) found that heart rate deflection points from the slow treadmill protocol were reached at an average of 90.4% of participants' maximum heart rate, while heart rate deflection points from the fast treadmill protocol were reached at an average of 91.8% of participants' maximum heart rate. Therefore, heart rate deflection point found during the fast treadmill protocol were significantly higher than those found during the slow treadmill protocol (p < 0.001). Researchers also found a very strong correlation between heart rate deflection point recorded during the slow and fast treadmill protocols (r = 0.92, p < 0.001), as well as no significant difference between the two protocols (p = 0.79). In addition, Bland Altman plots were created in order to determine the limits of agreement for each protocol. A proportional bias indicating low levels of agreement was found in the slow treadmill protocol, but was not

evident in the fast treadmill protocol. Because of these findings, Sentija et al. concluded that incremental exercise treadmill tests utilizing faster increases in speed and shorter total test duration may be an acceptable protocol for determining heart rate deflection point in trained runners.

Identifying the Breakpoints in Linearity

Marques-Neto, Maior, Neto, and Santos, 2012

Analysis of Heart Rate Deflection Points to Predict the Anaerobic Threshold by a Computerized Method

In order to identify the thresholds that occur during the "time / heart rate" relationship, Marques-Neto, Maior, Neto, & Santos (2012) evaluated a computerized method of determining heart rate deflection points. Researchers aimed to compare participants' heart rate deflection points with other sought after thresholds, such as ventilatory threshold and lactate threshold. Ventilatory threshold has been defined as the point at which respiratory gases begin to increase at a disproportionate level during a graded exercise test. Ventilatory threshold has also been shown to be strongly correlated with long-distance sports performance and associated fatigue, as well as anaerobic threshold.

Twenty-four male professional soccer players (22±5 years) performed an incremental treadmill test while heart rate and gas exchange were monitored. Testing was done at the same time of day for each trial, and included a warm-up for three minutes. Once the incremental treadmill test began, subjects experienced increases in speed and incline every three minutes until exhaustion. Gas exchange analysis and blood lactate concentrations were also documented during the experimental trials.

A computer algorithm was used to create a line of best fit for time and heart rate. Two break points were found, which researchers deemed the "first heart rate deflection point" and the "second heart rate deflection point". The second heart rate deflection point was found in all participants. In order to determine these break points in linearity, the computerized program was used to fit three adjoining linear segments along the "time / heart rate" relationship. The two intersections of these linear segments were identified as the first and second heart rate deflection points. Unfortunately, neither deflection point was found to be correlated with the first lactate threshold (r = 0.26, p < 0.0001) or second lactate threshold (r = 0.49, p < 0.0001). However, the second heart rate deflection point was found to be highly correlated with the respiratory compensation point (r = 0.98, p > 0.05), which may indicate a relationship with cardiorespiratory fatigue and endurance performance.

Ribeiro, Fielding, Hughes, Black, Bochese, and Knuttgen, 1985

Heart Rate Break Point May Coincide with the Anaerobic and Not the Aerobic Threshold

Ribeiro et al. (1985) conducted a study evaluating the relationship between heart rate deflection point and both aerobic and anaerobic thresholds. Participants were broken up into two separate groups (Group 1 and Group 2). Group 1 included 11 male participants (23.9±2.8 years), ranging from sedentary adults to highly trained athletes. Participants performed a graded exercise test on a cycle ergometer, starting at a load of 30 Watts for four minutes. Subjects pedaled at a constant rate of 70 revolutions per minute while increments of 30 Watts were added to each 60-second stage, until subjects could no longer maintain 70 revolutions per minute. Gas exchange was measured while blood samples were collected throughout the test. Each minute, heart rate was monitored via electrocardiogram, and blood lactate was tested.

Group 2 consisted of 16 physically active male participants (22.4±2.0 years) who performed two separate graded exercise tests in order to evaluate test-retest reproducibility. Tests were performed with a period of 1-2 weeks separating them. Subjects began each test with a load of 25 Watts for three minutes and a constant pedaling rate of 70 revolutions per minute. Increments of 25 Watts were added to each 60-second stage, until the subjects could no longer maintain 70 revolutions per minute. Gas exchange was measured every 30 seconds while blood samples were collected throughout the test. Heart rate was monitored via electrocardiogram and recorded every 30 seconds.

In Group 1, the "blood lactate / power output" relationship was fitted with three straight lines and two break points. Researchers examined these break points via visual inspection. Aerobic threshold was defined as the point at which blood lactate levels began to increase above resting values. Aerobic threshold was also deemed the first break point. Anaerobic threshold was defined as the point just prior to the quick rise in blood lactate. Anaerobic threshold was also deemed the second break point. In Group 2, the anaerobic threshold was defined as the point where the fraction of expired carbon dioxide began to decrease consistently in the "fraction of expired carbon dioxide began to decrease consistently in the "fraction of expired carbon dioxide began to deviate from linearity in the "power / heart rate" relationship.

In Group 1, anaerobic threshold and heart rate deflection point had the strongest correlation (r = 0.97, p = 0.01) when compared to aerobic threshold and heart rate deflection point (r = 0.89, p = 0.01) and aerobic threshold and anaerobic threshold (r = 0.92, p = 0.01). There was no significant differences between anaerobic threshold and heart rate deflection point (p = 0.34), and researchers found that the aerobic threshold was significantly lower than the heart

rate deflection point (p = 0.001), which suggests that the heart rate deflection point may be a good predictor of anaerobic threshold. In Group 2, however, Ribeiro et al. (1985) were only able to find a heart rate deflection point in eight out of the sixteen participants in both of the graded exercise tests.

Hofmann, Pokan, Von Duvillard, Seibert, Zweiker, and Shmid, 1997

Heart Rate Performance Curve during Incremental Cycle Ergometer Exercise in Healthy Young Male Subjects

One of the main focuses of Hofmann et al. (1997) in this research study was to examine the relationship between the heart rate deflection point and lactate turn point. Two hundred twenty-seven males (23±4.0 years), who specialized in various athletic sports, performed a graded exercise test on a cycle ergometer. Each test began with an initial intensity of 40 Watts, with increasing increments of 20 Watts per 60-seconds until exhaustion. Heart rate was measured every five seconds. Blood samples were also taken periodically throughout the test. Hofmann and colleagues (1997) assessed heart rate deflection point through use of a computerized program, utilizing linear regression analysis. Deflection points were determined by calculating the point of intersection of two regression lines (between first lactate turn point and maximal power). First lactate turn point was defined as the first increase of blood lactate concentration above resting level and was deemed the first break point. The second lactate turn point was defined as the second increase of blood lactate concentration (~4 millimole per liter) and was deemed the second break point.

Participants were split into three separate groups including those who showed a regular deflection point (85.9% of participants), those who did not show a deflection point (6.2% of participants), and those who showed an inverted deflection point (7.9% of participants). In those

participants whose heart rate deflection points were identifiable (93.8% of participants), researchers found no significant difference between heart rate deflection point and second lactate turn point (or second break point), regardless of a regular or inverted deflection point, and heart rate deflection point was significantly correlated with second lactate turn point (r = 0.889, p < 0.001). This indicates that heart rate deflection point may be a good estimate of the second lactate turn point, or anaerobic threshold. However, because a heart rate deflection point was not found in every participant, an individualized method of heart rate deflection point determination may be a more beneficial technique for researchers.

Methods of Determining Heart Rate Deflection Point

Visual Inspection Method

Vachon, Bassett, and Clarke, 1999

Validity of the Heart Rate Deflection Point as a Predictor of Lactate Threshold during Running

The method of visual inspection has been deemed the most simple and practical method of heart rate deflection point determination (Ribero et al., 1985), while mathematical models have been shown to be less practical, but more reliable (Kara et al., 1996, Tokmakidis & Leger, 1992). The purpose of this study by Vachon, Bassett, & Clarke (1999) was to investigate whether the same heart rate deflection point could be identified using different test protocols (slower speeds versus higher speeds) through visual inspection. Another purpose of this study was to examine the relationship between heart rate deflection point and lactate threshold. Vachon and colleagues (1999) recruited eight trained runners who performed four separate tests, including (1) a graded exercise test on the treadmill to determine maximal oxygen consumption, (2) a Conconi test, (3) a continuous treadmill run, and (4) a continuous lactate threshold treadmill test.

For the graded exercise test, incline was set to increase by 1% every minute. Ventilation was measured and heart rate was monitored via heart rate monitor watch. The Conconi test took place on a 400-meter outdoor track and utilized speed increases at a rate of 0.5 kilometers per hour every 200 meters, starting at 10-14 kilometers per hour and increasing to final speeds of 18-22 kilometers per hour. The continuous treadmill run included 16-20 stages of increasing speeds, which increased at a rate of 0.5 kilometers per hour every minute. Speeds began around 11-12 kilometers per hour and increased up to speeds around 18-21 kilometers per hour. Finally, the lactate threshold test included 8-10 stages of increasing speeds, at a rate of 0.8 kilometers per hour every three minutes. Blood samples were taken throughout each test.

Vachon et al. (1999) used a third-order polynomial equation to create a "speed / heart rate" relationship curve for each test performed. A third-order polynomial equation was chosen because of the significantly better curve it provided when compared to a linear or second-order polynomial equation. Lactate threshold was defined as the running speed at which blood lactate concentrations increased from baseline and was determined by visual inspection. Researchers discovered that a heart rate deflection point was identified in all participants who performed the Conconi test via the visual inspection method. However, heart rate deflection points of only 50% of the participants were able to be identified from the treadmill tests. Maximal running speed was also found to be higher during the Conconi test than any of the treadmill tests, but maximum heart rate was similar between tests. Researchers believe that because the stages of speed increases during the Conconi test were shortened, runners were able to increase running speed beyond the point of maximal heart rate deflection point and lactate threshold were found to be significantly different, with speed at heart rate deflection point and lactate threshold were found to be

Because of this, it was concluded that heart rate deflection point may be inaccurate in estimating lactate threshold. However, Vachon et al. (1999) further concluded that the Conconi test was more likely to produce heart rate deflection points than treadmill tests, and the visual inspection method may be a good way to identify these points.

Candido, Okuno, da Silva, Machado, and Nakamura, 2015

Reliability of the Heart Rate Variability Threshold using Visual Inspection and D-max Methods

Candido et al. (2015) conducted this study in order to compare the heart rate deflection point from six graded exercise tests, and to examine the reproducibility of the tests. Heart rate deflection point was represented as "heart rate variability threshold" in this study. Researchers hypothesized that the D-max method would provide better results when compared to the visual inspection method to create reproducibility. Twelve males (26.8 ± 5.0 years) performed six graded exercise test until exhaustion on a cycle ergometer. Tests were separated by a period of 2-4 days. Beginning the trial, intensity was set to 25 Watts, with increasing intensity in increments of 25 Watts every three minutes. Participants were required to maintain a rate of 60 revolutions per minute. Heart rate was monitored via heart rate monitor. Two methods were used for determination of the heart rate variability threshold: visual inspection method and D-max method. Candido et al. (2015) used a third-order polynomial curve in order to determine the threshold point by the D-max method: $a + bW + cW^2 + dW^3$ (W = Watts; a,b,c = curveparameters determined using SPSS).

Researchers found no difference between the six graded exercise tests (p > 0.05, ICC = 0.98), and the visual inspection method presented the highest level of reproducibility of heart rate variability threshold (ICC = 0.83) when compared to the D-max method (ICC = 0.63). Therefore,

the visual inspection method may be an important method to consider using when determining heart rate deflection point.

D-max Method

Kara, Gokbel, Bediz, Ergene, Ucok, and Uysal, 1996

Determination of the Heart Rate Deflection Point by the D-max Method

The purpose of this study was to compare use of the D-max method to the conventional linear regression method used in the Conconi test for calculating heart rate deflection point. Researchers observed thirty two untrained males (18-22 years) during a maximal exercise test on a cycle ergometer. Participants began each test with a three minute warm up at an intensity of 40 Watts, and then proceeded to perform the maximal exercise test with increasing intensities every 60 seconds at a constant rate of 60 revolutions per minute. Gas exchange variables were measured and recorded every 15 seconds. Heart rate was monitored throughout each test via heart rate monitor.

Heart rate deflection points were calculated using a linear regression method and a thirdorder curvilinear regression method (D-max). Kara et al. (1996) used minimum heart rate values of 140 beats per minute and the maximum heart rate values available for the linear regression method. Two regression curves were drawn from the original values between these minimum and maximum heart rate values. The heart rate deflection point was defined as the merge of the points of these curves. The same cutoff point of 140 beats per minute was used for the D-max method, and the two end points of the "time / heart rate" relationship curve were connected by a straight line. The heart rate deflection point was defined as the most distant point from the curve to the straight line.

In nine of the thirty two participants, a heart rate deflection point was not identified in the linear regression method. However, when using the D-max method, heart rate deflection points were found in all participants. All recorded maximum heart rate values were close to estimated maximum heart rate (approximately 90% of maximum heart rate). Strong relationships were found between heart rate deflection point expressed as work rate (r = 0.97, p < 0.001), oxygen uptake (r = 0.93, p < 0.001), and heart rate (r = 0.93, p < 0.001) between the linear regression and D-max methods. However, Kara et al. (1996) concluded that the D-max method may be more useful than the more commonly used linear regression method due to heart rate deflection points seen in more participants through use of D-max.

Siahkouhian, Azizan, and Roohi, 2012

A New Approach for the Determination of Anaerobic Threshold: Methodological Survey on the Modified D-max Method

Siahkouhian, Azizan, & Roohi (2012) investigated the relationship between heart rate using a modified D-max method and heart rates measured at anaerobic threshold by continuous respiratory gas measurements. The modified D-max method included using a parallel straight line slope mathematical model and the use of the Narita target heart rate equation (Siahkouhian, Azizan, and Roohi, 2012). The parallel straight line slope mathematical model was used to calculate the upward and downward inflections of the heart rate performance curve, as well as define the maximum distance between line and curve without use of a formula. The Narita target heart rate equation also took into consideration resting heart rate and gender in order to determine target heart rate for exercise.

Participants in this study included eight females (19.3±1.70 years) who performed an incremental treadmill test until exhaustion. Gas exchange and heart rate values were measured

throughout the test. Anaerobic threshold of each participant was calculated from the continuous respiratory gas measurements. Lactate threshold was calculated by use of the D-max method which was determined by identifying the point on the polynomial regression curve that produced the maximal perpendicular distance to the straight line which connected the first and second lactate turn points.

Anaerobic threshold was also calculated via a mathematical model with only one point in the curve with the same slope as that of the straight line between the two end points of the "time / heart rate" relationship curve, which should give an equal value to that of the maximum distance found from the D-max method. This distance was found by use of a formula which was written by the authors of the computerized mathematical model: [74.8 + (0.76)(resting heart rate) - (0.27)(age) + (7.3)(gender), where male = 0 and female = 1.

Bland Altman plots revealed high levels of agreement between heart rate at anaerobic threshold measured through continuous respiratory gas exchange and heart rate found through use of the modified D-max methods. No significant difference was found between heart rates measured at anaerobic threshold via respiratory gas measurements and heart rates determined by the modified D-max methods (167.00 ± 9.22 vs. 165.25 ± 6.32 beats per minute), therefore indicating that the D-max method may be a reliable and useful method to determine heart rate deflection points in young females.

Bi-segmental Linear Regression Method

Higa, Silva, Neves, Catai, Gallo, and Silva de Sa, 2007

Comparison of Anaerobic Threshold Determined by Visual and Mathematical Methods in Healthy Women

Higa and colleagues (2007) examined two different methods of determining anaerobic threshold, which has been found to be indicated by the heart rate deflection point. Higa et al. (2007) compared the anaerobic threshold determined by visual inspection and anaerobic threshold determined by bi-segmental linear regression, using heart rate and carbon dioxide values. Thirteen young women (24±2.63 years) and sixteen older women (57±4.79 years) volunteered as participants for this study and underwent a graded exercise test on a cycle ergometer. This test consisted of a warm-up lasting four minutes at an intensity of 4 Watts, with intensity increases of 10-20 Watts per minute. Heart rate via electrocardiogram and gas exchange were recorded throughout each test.

The visual inspection method was performed by three experienced researchers, which has been considered to be the gold standard for determining anaerobic threshold. Anaerobic threshold was determined as the point at which a disproportionate amount of carbon dioxide was increased in relation to the linear increase in oxygen consumption. The bi-segmental linear regression model was executed using an algorithm applied to heart rate and carbon dioxide values, which allowed for analysis of the "carbon dioxide / heart rate" relationship, identification of the moment that a change in values occurs, and detection of the intersection point of two adjustment phases of data sets. In comparing the two methods of identifying anaerobic threshold, researchers found a significant correlation between the visual inspection method and the bi-segmental linear regression method when heart rate and carbon dioxide data were used (r = 0.78,

p < 0.05). In conclusion, Higa et al. suggested that the bi-segmental linear regression method is a useful tool for detecting anaerobic threshold.

Cabo, Martinez-Camblor, and Valle, 2011

Validity of the Modified Conconi Test for Determining Ventilatory Threshold during On-Water Rowing

The purpose of this study by Cabo, Martinez-Camblor, and Valle (2011) was to use the Conconi test in order to design an original field test to determine ventilatory threshold of rowers. Researchers also aimed to test reliability and validity of the new test which was geared towards rowers. Sixteen male lightweight rowers (22.1±2.3 years) performed a modified Conconi test, specific to on-water rowing. Tests were done on large bodies of water (4-5 kilometers) in which athletes could row in a straight line for a continuous time of 12-15 minutes. The athletes' beginning stroke rate started at 18 strokes per minute and continued to increase in strokes by 2 strokes per minute. This equated to a speed increase of approximately 12 kilometers per hour every minute. In order to follow the guidelines of the modified Conconi test, athletes could not increase their heart rate by more than 8 beats per minute. Tests were completed when rowers could not maintain stroke rhythm or reached exhaustion. Within 72 hours of the first trial, athletes performed a second test in order to establish reproducibility. Gas exchange was measured throughout the tests via a portable metabolic system which could be carried in a backpack, and heart rate was monitored via heart rate monitor and portable metabolic system. Second ventilatory threshold was determined as the second break in linearity and an increase in respiratory equivalents for oxygen and carbon dioxide. Heart rate deflection point was determined by linear regression (as is done in the Conconi test) between heart rate, stroke rate, and average boat speed.

Researchers found that the correlation between heart rate and stroke rate were highly correlated (r = 0.96) as well as between heart rate and speed (r = 0.97). In examining the test-retest data, heart rate deflection points were only found in 88.8% of participants, but the differences between tests were very slight with low total errors. Researchers tested validity of the modified Conconi test with the new field test for rowers by comparing heart rate deflection point with ventilatory threshold. There were no significant differences found for different parameters (heart rate, oxygen consumption, stroke rate, and speed) of heart rate deflection point and ventilatory threshold. Cabo et al. (2011) concluded that the modified Conconi test geared towards rowing performance is valid and reliable when determining second ventilatory threshold.

Identifying Accuracy of Cutoff Points to Determine Heart Rate Deflection Point

When examining heart rates on a graph from an graded exercise test, linearity may be lost in the beginning stages due to fluctuations and quick rises in heart rate (for example, during a warm-up stage). Because of this, researchers have used heart rate cutoff points in order to account for these inconsistencies during the initial stages of an exercise test. Previous studies have used cutoff points at arbitrary numbers, such as 110 and 140 beats per minute. Other studies have used cutoff points at ventilatory threshold, which has also been considered to be the first lactate turn point or the first break in linearity before the heart rate deflection point. In using a cutoff point at ventilatory threshold (or the first breakpoint), researchers are able to disregard the heart rates that occur before this first breakpoint, leaving the next breakpoint in linearity to be indicative of the heart rate deflection point.

Cutoff Point: 110 beats per minute

Vucetic and Sentija, 2008

The Relationship between Heart Rate Deflection Point and the Ventilatory Anaerobic Threshold in Runners with Different Aerobic Capacity

Vucetic and Sentija (2008) aimed to examine the possibility of the heart rate deflection point accurately estimating the anaerobic threshold of runners. This study utilized a sample size of forty eight nationally ranked male runners (21.7±5.1 years) with a variety of running disciplines and abilities. Participants included ten sprinters, fifteen 400-meter runners, ten middle distance runners, and thirteen long distance runners. Subjects were asked to complete a graded exercise test on a treadmill until exhaustion. Each test began at a speed of 7 kilometers per hour with a 1 kilometer per hour increase in speed per minute. The treadmill's incline was set at a constant grade of 1.5% incline. Researchers measured gas exchange and heart rate (via heart rate monitor) every 30 seconds. Anaerobic threshold was assessed by use of the V-slope method, which includes observing a second disproportionate increase of expired carbon dioxide in relation to oxygen consumption. The "speed / heart rate" relationship contained heart rates between 110 beats per minute and maximum heart rate achieved. Heart rate deflection point was determined by use of visual inspection of a break point in linearity and was identified in all forty eight participants.

Vucetic and Sentija (2008) found that the running speeds at heart rate deflection point were strongly related and almost identical to the running speeds at anaerobic threshold (r = 0.85, p < 0.01), and that there was no difference between heart rates at heart rate deflection point and heart rates at anaerobic threshold (p = 0.61). Researchers concluded that finding the intensity at

heart rate deflection point is an accurate, non-invasive method to detect intensity at anaerobic threshold.

Cutoff Point: 140 beats per minute

Siahkouhian and Meamarbashi, 2013

Advanced Methodological Approach in Determination of the Heart Rate Deflection Point: S.D.-max versus L.D.-max Methods

One of the main purposes of Siahkouhian and Meamarbashi (2013) was to compare two different cutoff points in the determination of heart rate deflection point. In previous studies, researchers have used cutoff points at different thresholds, or at arbitrary values such as 140 beats per minute or 110 beats per minute. Other studies have shown the use of no cutoff points at all. Siahkouhian and Meamarbashi wanted to examine the use of a cutoff point at 140 beats per minute and the use of no cutoff points, with plasma lactate measurements as the criterion, when determining a heart rate deflection point that would accurately correspond to anaerobic threshold. Fifteen males (20-24 years) performed a graded exercise test on a cycle ergometer. Subjects completed a warm up at an intensity of 30 Watts for 10-12 minutes. They then completed a maximal test at a constant pedaling rate of 70 revolutions per minute. Load increases began at an initial intensity of 140 Watts and increased by increments of 20 Watts every 30 seconds. Gas exchange and heart rate values via heart rate monitor were recorded during the test. Blood samples were also taken.

Researchers were able to find a heart rate deflection point in all participants. No difference was found between heart rate deflection point with a cutoff at 140 beats per minute and lactate measurements (p = 0.86), while a significant difference was found between heart rate deflection point with no cutoffs and lactate measurements (p = .001). This difference could

potentially be due to the large fluctuations in heart rate that are seen in the beginning of a graded exercise test, therefore resulting in an inaccurate heart rate deflection point. Researchers also found a high level of agreement between the 140 cutoff point method and lactate measurements, while there was no agreement between the no cutoff point method and lactate measurements, through use of Bland Altman plots. A strong correlation was seen between the 140 cutoff point method and lactate measurements (r = 0.944, p < 0.001), while there was no correlation between the non-cutoff point method and lactate measurements (r = 0.944, p < 0.001), while there was no correlation between the non-cutoff point method and lactate measurements (r = 0.158, p = 0.575). From this information, Siahkouhian and Meamarbashi (2013) concluded that a cutoff point at 140 beats per minute may be a more accurate and reliable method to determine heart rate deflection point compared to using no cutoff points.

Cutoff Point: Ventilatory Threshold

In previous studies, researchers have equated the ventilatory threshold to the heart rate deflection point, while others have considered there to be two separate ventilatory thresholds: one that happens well before the heart rate deflection point, and one that happens at the same time as the heart rate deflection point (Mourot et al., 2011).

Condello, Reynolds, Foster, de Koning, Casolino, Knutson, and Porcari, 2014

A Simplified Approach for Estimating the Ventilatory and Respiratory Compensation Thresholds

In a study by Condello et al. (2014), ventilatory threshold was considered to be the first breakpoint in linearity that occurred before the heart rate deflection point. One of the primary purposes of this study was to determine if ventilatory threshold could be determined based off of a percentage of a person's maximal running speed. Researchers also looked at the determination of respiratory compensation point via percentage of maximal running speed, as well. This study was completed in two phases, in which different participants were used. Thirty one recreational and well-trained male (n = 16; 22.2 \pm 2.2 years) and female (n = 15; 21.8 \pm 2.5 years) athletes participated in Phase 1 of this study, including competitive recreational runners and college level runners, soccer players, and basketball players. Initially, participants completed a graded exercise test to exhaustion, which included a constant incline of 1% and a 0.22 meters per second increase in speed every minute, starting at 1.56 meters per second. Researchers measured respiratory gases every 30 seconds, and ventilatory thresholds and respiratory compensation points were determined by visual inspection. Ventilatory threshold was found to be reached at $67\pm9\%$ of maximal running speed, where respiratory compensation point was reached at $84\pm6\%$ of maximal running speed.

For Phase 2 of this study, twenty recreational and well-trained male (n = 10; 21.8 \pm 2.6 years) and female (n = 10; 21.9 \pm 1.8 years) athletes were recruited. Subjects completed the same graded exercise test as those in Phase 1, followed by two 30-minute steady state sub-maximal treadmill running trials at 64% and 86% of their maximal running speed (used to predict ventilatory threshold and respiratory compensation point). Blood lactate and heart rate measurements were taken throughout the run.

There were no differences between males and females when determining ventilatory threshold and respiratory compensation point as a percentage of maximal running speed, so all participants' data were combined regardless of gender. Researchers found that running at 64% and 86% of maximal running speed accurately predicted conditions consistent with running at or less than ventilatory threshold and at or greater than respiratory compensation point, respectively. It was suggested that running at 64% of maximal running speed may be too high,

and a speed slightly slower than 64% may be optimal, further greatening the distance between ventilatory threshold and heart rate deflection point.

Although treadmill speeds are not used to determine heart rate deflection points in the current study, ventilatory thresholds are used as cutoff points, indicating the first breakpoint in linearity. This study by Condello et al. (2014) supports the idea of using ventilatory threshold as a cutoff point to indicate the first breakpoint because of the lower percentage of maximal running speed used, compared to the higher percentage of maximal running speed used to determine respiratory compensation point.

Heart Rate Deflection Point in Relation to Respiratory Compensation Point

Redkva, Zagatto, Batista, Kalva-Filho, Loures, Kaminagakura, Da Silva, and Papoti, 2003 Correlation between Heart Rate Deflection Point and Respiratory Compensation Point in Brazilian Army Runners

This study aimed to examine the possible correlation between heart rate deflection point and respiratory compensation point during running. Researchers considered the respiratory compensation point to be equal to the second ventilatory threshold. Subjects included 11 welltrained men with national level military training and experience (19.8±1.7 years). Researchers required participants to remain consistent with their food intake on the days of the testing trials, which were each performed at the same time of day. Before each maximal graded exercise test, participants completed five minutes of warm-up on the treadmill at a moderate intensity, followed by five minutes of light intensity warm-up. Each test began at a speed of 12 kilometers per hour, with speed increases of 1 kilometer per hour every three minutes, with a constant treadmill incline at 1%. Breath-by-breath measures were taken and reduced to 15-second averages. Second ventilatory threshold and heart rate deflection point were found by determining the last point where inflection occurred in the "ventilatory equivalent / volume of expelled carbon dioxide" curve and by determining the point where the loss of deflection occurred with the increase in intensity, respectively. These points were determined by visual inspection. Intensities at second ventilatory threshold and heart rate deflection point were moderately correlated (r = 0.71, p = 0.01), which allowed researchers to conclude that heart rate deflection points may not be an accurate tool to measure respiratory compensation point. However, more research needs to be done on this topic.

Heart Rate Deflection Point in Relation to Athletic Performance

Zacharogiannis and Farrally, 1993

Ventilatory Threshold, Heart Rate Deflection Point, and Middle Distance Running Performance

Zacharogiannis and Farrally (1993) conducted this study in order to better examine the relationship between laboratory measurements (specifically ventilatory threshold and heart rate deflection point) with middle distance running time. Participants included ten trained male runners and two trained female runners (25.9 ± 6.24 years). Before subjects performed a 3000-meter time trial, they completed an incremental treadmill test in the laboratory. Participants began the test with a five minute warm up at a speed of 9.25 ± 1.71 kilometers per hour. The increments used for this test consisted of a 1 kilometer per hour increase in speed every three minutes, with no change in incline. Researchers measured gas exchange and heart rate throughout each test. Each experimental trial was completed twice per subject and test-retest reproducibility was highly correlated (r = 0.93). After two graded exercise tests on the treadmill, participants performed a 3000-meter time trial on an outdoor track. Five subjects were retested

for the 3000-meter time trial on a different day under different weather conditions, and test-retest results had a very strong correlation (r = 0.98), which indicated that their performance times were almost identical under separate weather conditions.

In order to determine heart rate deflection points, Zacharogiannis and Farrally (1993) created a "speed / heart rate" relationship curve and visually identified the point where heart rate broke away from linearity. Running velocity was assessed via mathematical interpolation. Test-retest correlations for heart rate deflection point for the five most experienced runners was highly correlated (r = 0.97), as well as the test-retest correlations for ventilatory threshold for the five most experienced runners (r = 0.94).

Ventilatory threshold and heart rate deflection points were highly correlated when expressed as speed (r = 0.96), but were significantly different when expressed as speed, volume of oxygen consumed, percent of maximum oxygen consumption, heart rate, and percent maximum heart rate (p < 0.01). Velocity was found to correspond to ventilatory threshold. Ventilatory threshold and heart rate deflection point were negatively correlated with 3000-meter running performance (r = -0.984, r = -0.94, respectively, p < 0.01). Speed corresponded with heart rate deflection point, and speed and maximal oxygen consumption were highly correlated with 3000-meter running performance (r = 0.94), as well as heart rate deflection point and maximal oxygen consumption (r = 0.82). Researchers concluded that these physiological measures of heart rate deflection point and ventilatory threshold may be good assessments of middle distance running performance.

Petit, Nelson and Rhodes, 1997

Comparison of a Mathematical Model to Predict 10-km Performance from the Conconi Test and Ventilatory Threshold Measurements

Petit et al. (1997) aimed to validate a mathematical model that predicts 10-kilometer race time and evaluate heart rate deflection point. Researchers also examined the relationship between ventilatory threshold and heart rate deflection point, when determined through laboratory tests. Twelve males and five females were recruited to participate in this research study (19-33 years). Participants performed a modified Conconi test, which included an increase in speed by 2 seconds every 200 meters until exhaustion. Runners began the test at a pace of 10.3 kilometers per hour for the initial 200 meters. Heart rate was monitored and recorded every 200 meters throughout the tests.

Researchers utilized a computerized mathematical model (based on a logistics function curve) to determine heart rate deflection points via the "speed / heart rate" relationship curve. The data was fit by use of the following formula:

$$y = 1/(abx + c)$$

which was then transferred into linear form by using the following formula:

$$(1 / H_s) - (1 / m) = abs$$

where "H_s" is defined as the following:

$$H_{\rm s} = 1 / [abs + (1 / m)]$$

where "m" is the subject's maximum heart rate. The log of each side of the second equation were taken in order to create the linear format that would be used in a regression of the "speed / heart rate" relationship data in order to find the intercept (a) and slope (b):

$$H_s = a + bs$$

where $H_s = ln[1 / H_s) - (1 / m)]$, a = lna, and b = lnb. Heart rate deflection point was calculated using the following formula:

$$[(H_x - H_x - 1) / (H_o - H_f)] / [(S_x - S_x - 1) / (S_o - S_f)]$$

where "S" is speed, "H" is heart rate, "x" is the point on the curve of current derivation, "o" is the first point of derivation (11 kilometers per hour), and "f" is the final point of the derivation (the final speed of the test). The prediction of 10-kilometer race pace was determined by multiplying 1.03 by the speed at deflection.

Researchers were able to find a strong correlation between the mathematically predicted 10-kilometer running time and the actual time (r = 0.98, p < 0.01). There was no significant difference seen between treadmill data and the mathematically calculated data for heart rate, running speed, or predicted time (p > 0.05). A strong relationship was also found between speed at ventilatory threshold and heart rate deflection point (r = 0.95, p < 0.01). Petit et al. (1997) concluded that the computerized, mathematical model used to predict 10-kilometer running performance is a valid assessment and is closely related to the laboratory measures. *Bodner, Rhodes, Martin, and Coutts, 2002*

The Relationship of the Heart Rate Deflection Point to the Ventilatory Threshold in Trained Cyclists

Along with running performance, heart rate deflection point has been studied in relation to cycling performance, as well. The researchers involved in this study investigated the relationship between heart rate deflection point and ventilatory threshold in trained cyclists, which included twenty one extremely well-trained male subjects (26.2±5.6 years). A graded exercise test to exhaustion was performed by all participants on a cycle ergometer. A ramp protocol was used, which included an initial warm-up, a beginning intensity of 50 Watts, and an

increasing intensity of 30 Watts per minute. Prior to any testing, researchers demonstrated that the ramp protocol chosen for this study followed the guidelines set forth by the Conconi test. This meant that increases in intensity were gradual enough to allow an 8 beats per minute increase in heart rate per minute or stage. Participants were also told to keep a constant pedaling cadence of approximately 80-90 revolutions per minute. Before testing, subjects were asked to abstain from heavy exercise for 24 hours, as well as refrain from eating or drinking for two hours prior to testing.

Data was measured every breath and included minute ventilation, volume of oxygen uptake, volume of expelled carbon dioxide, heart rate, and respiratory exchange ratio. Ventilatory threshold was determined via examination of the excess carbon dioxide elimination curve, in which two trained exercise physiologists analyzed this break point independently through use of visual inspection. Heart rate deflection point was calculated via the same mathematical model used in the previously mentioned study (Petit, Nelson, & Rhodes, 1997). This method utilized the natural log of the heart rate data points in order to create a curvilinear fit, and, when applied to incremental exercise on a cycle ergometer, has shown high reproducibility and reliability (Bodner, Rhodes, & Coutts, 1998). Researchers used a cut-off point at a power output at 60% of maximal heart rate.

Bodner et al. (2002) found no significant difference between heart rate at heart rate deflection point (171.7±9.6 beats per minute) and heart rate at ventilatory threshold (169.8±9.9 beats per minute) (p > 0.01), as well as no significant difference between volume of oxygen uptake at heart rate deflection point (53.6±4.2 milliliters per kilogram per minute) and volume of oxygen uptake at ventilatory threshold (52.2±4.8 milliliters per kilogram per minute) (p > 0.01). A significant difference was found, though, between power at heart rate deflection point

(318.7±30.7 Watts) and power at ventilatory threshold (334.8±36.7 Watts) (p < 0.01). Significant relationships were found between heart rate deflection point and ventilatory threshold, using heart rate (r = 0.92, p < 0.001), volume of oxygen uptake (r = 0.72, p < 0.001), and power (r = 0.77, p < 0.001). In conclusion, heart rate deflection point may be a good indicator of ventilatory threshold in well-trained cyclists, which is important to understand when prescribing training programs and monitoring progress.

Grazzi, Mazzoni, Casoni, Uliari, Collini, Van Der Heide, and Conconi, 2008

Identification of a VO₂ Deflection Point Coinciding with the Heart Rate Deflection Point and Ventilatory Threshold in Cycling

Grazzi et al. (2008) sought to explore the possibility of a deflection point when examining volume of oxygen consumption during a graded exercise test, corresponding to the heart rate deflection point and ventilatory threshold in trained cyclists. Twenty four world-class professional cyclists participated in this study (26±3.2 years). Researchers asked participants to refrain from any cycling races for 48 hours, as well as food or drink for 2-3 hours, prior to testing. Participants were also asked to avoid caffeine, in order to eliminate anything that could potentially influence heart rate.

Testing sessions included a graded exercise test on a wind-load simulator, where the subjects' bicycle was mounted onto. Breath-by-breath gases were measured, as well as heart rate. The initial warm-up consisted of 15 minutes of light cycling at an intensity of 100-150 Watts and a cadence from 60 revolutions per minute to 95 revolutions per minute, followed by three bouts of near-maximal intensity for 10 seconds, with a one minute recovery. After this prolonged warm-up, participants rested for a few minutes of easy cycling, then proceeded to complete the exercise test. The testing protocol consisted of a cadence of 60 revolutions per minute and an

intensity of 100-120 Watts. Then, cadence was increase by 1 revolution per minute every 30 seconds until maximal exercise intensity was reached. Heart rate increased at a rate under 8 beats per minute each minute, which was consistent with the Conconi test.

Bi-segmental regression was used to analyze the "intensity / heart rate" relationship, while ventilatory threshold was found using the V-slope method. Heart rate deflection point was found via visual inspection. Researchers found that the "oxygen consumption / heart rate" relationship was identical to the "intensity / heart rate" relationship, and the oxygen consumption deflection point was found to coincide with the heart rate deflection point and ventilatory threshold (r = 0.99, p < 0.0001).

Mikulic, Vucetic, and Sentija, 2011

Strong Relationship between Heart Rate Deflection Point and Ventilatory Threshold in Trained Rowers

Mikulic, Vicetic, & Sentija (2011) investigated the relationship between heart rate deflection point and performance variables (heart rate, power output, and oxygen uptake), as well as the relationship between ventilatory threshold and those same performance variables in trained rowers. They believed that the performance variables corresponding to heart rate deflection point were not significantly different from the corresponding variables at ventilatory threshold, which could demonstrate heart rate deflection point as being a good indicator of anaerobic threshold (which Mikulic, Vucetic, & Sentija, (2011) assessed as ventilatory threshold). Eighty nine male trained rowers (21.2±4.1 years) performed a graded exercise test on a rowing ergometer until exhaustion. Participants began by completing a 15-minute warm up, which included ergometer rowing and stretching. Testing intensity began at a power output of 150 Watts and incrementally increased by either 20 Watts (for subjects < 19 years) or 25 Watts (for subjects > 19 years) every

minute. Stroke rates were determined by each rower, but were encouraged to reach maximal performance.

Once a "power / heart rate" relationship was established, Mikulic et al. (2011) determined heart rate deflection points by use of two methods: visual inspection method and a computerized regression analysis method. For the computerized method, a linear regression breakpoint analysis was used to aid in the visual analysis. For the visual inspection method, three experienced researchers were used to identify the break points from linearity. They defined the heart rate deflection point as the point where the slope values of the linear part of the "power / heart rate" relationship began to decline while the values of the intercept on the y-axis began to increase. For the determination of ventilatory threshold, researchers used a combination of three methods (V-slope, excess carbon dioxide, and ventilatory equivalent of oxygen and carbon dioxide) in order to improve the accuracy of ventilatory threshold identification.

A heart rate deflection point was successfully found in all participants involved. There was found to be a strong relationship between performance variables (heart rate, power output, and oxygen uptake) and heart rate deflection point, as well as between said performance variables and ventilatory threshold (r = 0.79-0.96, p < 0.001). Heart rate at the heart rate deflection point was found to be significantly higher (but only slightly) than the heart rate at ventilatory threshold (174.5 beats per minute versus 172.8 beats per minute, p = 0.003). Heart rate deflection point and ventilatory threshold did not significantly differ with respect to the observed performance variables in trained rowers (p > 0.011). Researchers concluded that the use of heart rate deflection points may serve as a simple and noninvasive method of anaerobic threshold assessment in trained rowers.

Da Silva, Peserico, and Machado, 2014

Relationship between Heart Rate Deflection Point Determined by D-max Method and 10km Running Performance in Endurance Recreationally-Trained Female Runners

Da Silva et al. (2014) conducted this study in order to examine the effect of different models and heart rate point cutoffs used to determine heart rate deflection point. Researchers also investigated the relationship between the speed at heart rate deflection point with 10kilometer running performance and the speed at lactate threshold with 10-kilometer running performance, using the D-max method. A graded exercise test was used on thirteen endurancetrained female runners (41.3±7.2 years). Beginning the test, speed was set at 7 kilometers per hour with increases in speed at increments of 1 kilometer per hour every three minutes. Increments were kept small in order to coincide with the recommendations of the Conconi test. Between each stage, a 30-second rest was given in order to collect blood samples. Heart rate was monitored via heart rate monitor. One month after completing the graded exercise test, participants performed a 10-kilometer running time trial.

Heart rate deflection point and lactate threshold were determined by using these two methods: exponential-plus-constant regression model and third-order polynomial regression model. The exponential-plus-constant model was used to determine heart rate deflection point from heart rate and speed. The following equation was used to fit the data points:

$$HR(s) = a + (b * exp(c * s))$$

where "s" is speed, and "a", "b", and "c" were parameters determined by nonlinear regression in SPSS. The slope was found as the first derivative of the above equation. The calculation of heart rate deflection point was based on the following equation:

$$HRDP = (ln((exp(c * max s) - exp(c * initial s)))/((c * max s) - (c * initial s))))/c$$

Where "s" is speed, and "c" is the parameter of the exponential-plus-constant equation.

The third-order polynomial regression model was also used to determine heart rate deflection points from heart rate and speed. Data was fitted by using this formula:

$$HR(s) = d_0 + d_1 \cdot s + d_2 \cdot s^2 + d_3 \cdot s^3$$

where "s" is speed, and " d_{0} ", " d_{1} ," " d_{2} ," and " d_{3} " are parameters determined by nonlinear regression in SPSS. Using this model, the heart rate deflection point occurs where the slope of the regression curve equals the slope of the straight line that connects the first and last points of the curve. The slope was found as the first derivative of the above equation. Heart rate deflection point was calculated based on the following equation:

$$HRDP = \{-d_2 + d_2^2 - 3 \cdot d_3 (d_1 - \Delta) \frac{1}{2}\} / (3 \cdot d_3)$$

Where d_1 , d_2 , and d_3 are parameters of the third-order polynomial equation, and Δ is the slope of the straight line connecting the first and last points of the curve, which is defined by the following formula:

$$\Delta = (HR_{\text{final}} - HR_{\text{initial}}) / (s_{\text{f}} - s_{\text{i}})$$

Heart rate deflection points were also calculated based on two different cutoff points for minimum heart rate values: heart rates above 140 beats per minute and the use of all heart rate values without a minimum cutoff point.

Researchers found that the calculation of heart rate deflection point using the exponential-plus-constant regression model strongly correlated with the runners' 10-kilometer time when using all heart rate values (r = 0.96) as well as using only heart rate values above 140 beats per minute (r = 0.79). Heart rate deflection points calculated via the exponential-plus-constant regression model showed higher correlations than when heart rate deflection points were calculated using the third-order polynomial regression model for either cutoff method, as

well as the lactate threshold calculations via D-max method. Heart rate deflection point calculated from the exponential-plus-constant regression model with no heart rate cutoff showed higher correlations with lactate threshold calculations by the same model than did the calculation of heart rate deflection points by the exponential-plus-constant regression model utilizing heart rates only above 140 beats per minute. However, the heart rate deflection points calculated by the exponential-plus-constant regression model using heart rate cutoffs above 140 beats per minute. However, the heart rate cutoffs above 140 beats per minute were better correlated with lactate thresholds calculated by the third-order polynomial regression model than the heart rate deflection points calculated by the exponential-plus-constant regression model with no cutoff points. Lastly, heart rate deflection points calculated via third-order polynomial regression using heart rates above 140 beats per minute showed higher correlations with lactate threshold calculations by exponential-plus-constant regression and lactate threshold calculations by exponential-plus-constant regression and lactate threshold calculations by third-order polynomial regression than heart rate deflection points by third-order polynomial regression.

From these findings, Da Silva and researchers (2014) concluded that heart rate deflection point calculated via exponential-plus-constant regression model using either the minimum cutoff of 140 beats per minute or no cutoff at all are both good predictors of 10-kilometer running performance in endurance-trained females, and that the when using the D-max method, the calculation of heart rate deflection point and lactate threshold is effected by the type of regression model used.

Baiget, Fernandez, Iglesias, and Rodriguez, 2015

Heart Rate Deflection Point Relates to Second Ventilatory Threshold in a Tennis Test

Baiget and colleagues (2015) investigated the relationship between heart rate deflection point and second ventilatory threshold in tennis players. In previous research, second ventilatory

threshold has been considered to be the second break point in linearity that corresponds to heart rate deflection point. The game of tennis involves high-intensity sprints with low-intensity recovery in between. Because ventilatory threshold has been positively correlated with muscle fatigue and endurance performance, tennis players could benefit from the knowledge of heart rate deflection point determination, if it does indeed coincide with the second ventilatory threshold (Matsumoto, Ito, & Moritani, 1991, Esteve-Lanao, San Juan, Earnest, Foster, & Lucia, 2005).

Thirty-five competitive male tennis players (18.2±1.3 years) participated in this study. Unlike the tests that have been done in the studies all previously mentioned, Baiget et al. (2015) created a tennis test to cater to the specificity of the sport. The testing protocol consisted of hitting tennis balls from a ball machine with alternating forehand and backhand strokes, as well as alternating cross court or down the line. Balls were initially shot at a rate of 9 balls per minute, followed by an increase of 2 balls per minute every two minutes. Once the subject was not able to complete the strokes with the correct technique and precision, the test was terminated. During each trial, respiratory gases and heart rate were measured continuously, via portable measurement unit and heart rate monitor. Heart rate deflection points were determined by using 1 minute averages of heart rate points and was assessed by two experienced researchers via visual inspection. Second ventilatory threshold was determined through analysis of breaks in linearity of ventilatory parameters.

Heart rate deflection points were found in almost all subjects (91.4%). Researchers found no difference between heart rate at heart rate deflection point (178.9 \pm 8.5 beats per minute) and heart rate at ventilatory threshold (177.9 \pm 8.7 beats per minute), but found significant differences between heart rate deflection point and ventilatory threshold when using volume of consumed

oxygen (p < 0.001), time, frequency of balls ejected, and testing stages (p \leq 0.05-0.001). Strong correlations were found, though, for all variables corresponding to heart rate deflection point and ventilatory threshold (r = 0.79-0.96, p < 0.001). Therefore, researchers suggested that the heart rate deflection point found via frequency of balls ejected can be a practical performance variable to prescribe sport-specific training near ventilatory threshold. Researchers also concluded that heart rate deflection point found from a tennis test can be used to estimate ventilatory threshold.

Heart Rate Deflection Point in Relation to the Myocardium

Pokan, Hofmann, Preidler, Leitner, Dusleag, Eber, Schwaberger, Fuger, and Klein, 1993

Correlation Between Inflection of Heart Rate / Work Performance Curve and Myocardial Function in Exhausting Cycle Ergometer Exercise

Pokan and colleages (1993) conducted a study investigating the relationship between myocardial function and heart rate deflection point during incremental intensity exercise. Myocardial function was examined as left ventricular ejection fraction, or the volume of blood pumped per contraction. Fifteen participants (23±3 years) completed a modified Conconi test on a cycle ergometer. Exercise intensity began at an intensity of 40 Watts, and increased incrementally by 20 Watts every 90 seconds until exhaustion. Heart rate and blood lactate levels were recorded throughout each test, as well as immediately after the test and three minutes after the test. Left ventricular ejection fraction was analyzed via radionuclide ventricular scintigraphy.

Linear regression was used to determine the relationship between left ventricular ejection fraction and heart rate behavior, including heart rate deflection point, from the "work rate / heart rate" relationship. Researchers found a strong relationship between heart rate deflection point and left ventricular ejection fraction, (r = -0.672, p < 0.01). Pokan et al. (1993) also found that the highest level of left ventricular ejection fraction occurred at the same breakpoint as heart rate deflection point, and once this breakpoint was reached, heart rate and left ventricular ejection fraction simultaneously began to stabilize. From these findings, one of the potential causes of heart rate deflection point could be function of the heart, or more specifically left ventricular ejection fraction. However, not much research has been done on the causes behind heart rate deflection point. Future research should take into consideration left ventricular ejection fraction as well as other possible underlying causes, such as blood lactate levels, potassium levels, parasympathetic drive, epinephrine levels, and norepinephrine levels (Pessenhofer et al., 1991; Pokan et al., 1995; Pokan et al., 1998; Tulppo et al., 1998; Hofmann et al., 1999). *Lucia, Carvajal, Boraita, Serratosa, Hoyos, and Chicharro, 1999*

Heart Dimensions May Influence the Occurrence of the Heart Rate Deflection Point in Highly Trained Cyclists

An interesting variable to keep in mind when assessing heart rate deflection point is the size of the heart muscle itself, and how heart rate deflection point may be related to heart dimensions. Lucia et al. (1999) recruited twenty one highly trained cyclists (25±3.0 years) who completed an echocardiographic evaluation of the left ventricular end diastolic internal diameter, left ventricular posterior wall thickness at end diastole, interventricular septal wall thickness at end diastole, left ventricular mass index, left atrial dimension, longitudinal left atrial dimension, right atrial dimension, and the ratio of early to late diastolic flow velocity.

Participants performed a graded exercise test on the cycle ergometer. The protocol consisted of an increase in intensity of 25 Watts per minute until exhaustion, with a constant cadence of 70-90 revolutions per minute. Gases and heart rates were measured throughout each trial, and ventilatory thresholds were determined by visual inspection using two experienced researchers. Heart rate deflection point was found after plotting an "intensity / heart rate"

relationship curve through use of a computer-based linear regression model. This deflection point was found at the point of change from the linear phase of the "intensity / heart rate" relationship to the curvilinear phase. Researchers were only able to find these deflection points in approximately 66.7% of participants. In examining heart dimensions of the participants, all subjects showed hypertrophied left ventricular internal dimensions with proportional increase in wall thickness, which was to be expected in highly trained endurance athletes.

The subjects who did show a heart rate deflection point were found to also be the subjects with the thickest wall dimensions, versus heart volume. Because of this finding, researchers suggest that heart rate deflection point may be due to more efficient cardiac function during high intensity exercise in athletes with a thicker myocardial wall, but future research should look into diastolic and systolic ventricular function during exercise to gain a better understanding of this. It has also been noted that stroke volume does not plateau at high exercise intensities in well-trained endurance athletes, which might be an indicator of a true heart rate deflection point in the 66.7% of the participants that showed one (Gledhill, Cox, & Jamnik, 1994).

Tocco, Sanna, Mulliri, Magnani, Todde, Mura, Ghiani, Concu, Melis, and Crisafulli, 2015

Heart Rate Unreliability during Interval Training Recovery in Middle Distance Runners

Tocco et al. (2015) examined a potential reason why heart rate deflection point may not be a reliable measure to estimate anaerobic and ventilatory thresholds to determine training intensities. Cardiovascular drift, or cardiac drift, is a known phenomenon where the heart rate begins to change, or "drift", after approximately ten minutes of exercise, regardless of testing protocol. This may be due to a reduction in stroke volume because of a reduction in blood volume and ventricular filling, which could be due to the increase in heart rate (Coyle, 1998). This means that a heart rate deflection point could be seen around the time of a cardiac drift, which is a completely time-dependent measure. Because of this, it is still unknown if a heart rate deflection occurs because of a switch in energy system requirements (aerobic to anaerobic), or if it is due to this cardiac drift phenomenon.

Researchers in this study took a look at thirteen runners (females, n = 2, 25.0±2.8 years; males, n = 11, 33.3±9.1 years) who completed two interval training sessions on a motorized treadmill. Throughout each test, researchers consistently measured heart rate, oxygen uptake, carbon dioxide production, pulmonary ventilation, carbon dioxide production excess, and respiratory exchange ratio. Participants began running at a speed of 10 kilometers per hour, followed by a 1 kilometer per hour increase every two minutes, until exhaustion. From this test, researchers were able to determine a second break point, which they considered the ventilatory threshold. The interval training trials were then performed at 80% and 120% of the participants' ventilatory threshold. Researchers found that using these fixed heart rates for interval training was not a reliable and accurate way to establish proper training level and recovery, and may be a cause of under training. More research should be done using fixed heart rates (or a percentage of the second break point in linearity) while taking cardiac drift into account, which may occur around the same time as the second break point.

Age and Gender Differences

Ferreira, Coelho, de Souza, Costa, Osiecki, and de Oliveira, 2015

Influence of Gender on Heart Rate Curves during a Progressive Test in Young Runners

All of the previously mentioned research studies examining heart rate deflection points have used adult participants for their data. The purpose of this study, however, was to examine the heart rate deflection points of a younger population, as well as investigate the potential gender differences in heart rate deflection point intensities that may be seen. This was the first known study that looked at heart rate deflection point in young runners. When compared to adults, it has been shown that a lower stroke volume and left ventricular ejection fraction affect cardiovascular responses to exercise in young athletes (Turley & Wilmore, 1997; Vinet, Nottin, Lecoq, & Obert, 2002). Within the youth population, differences can also be seen between trained and untrained children. In an adult population, differences have been seen between men and women when examining cardiac morphological differences and heart rate deflection points, due to different systolic ventricular volumes. In the young population, however, gender does not appear to affect systolic ventricular function, which may mean that gender in children may not affect heart rate deflection point.

Nineteen young runners were recruited for this study (n = 11 boys, 13.7 ± 1.6 years; n = 8 girls, 12.8 ± 1.3 years) and tested for body composition measurements. Participants completed a graded exercise test on a track with a starting speed of 8 kilometers per hour, with speed increases of 1 kilometer per hour every two minutes until exhaustion. Heart rates were measured via heart rate monitor, and a cutoff point of 140 beats per minute was used. Heart rate deflection point was determined after plotting the "speed / heart rate" relationship curve. A third-degree polynomial fit was used, followed by a linear fit of the first and last points. The maximum perpendicular distance between the two lines was considered the heart rate deflection point (D-max).

Researchers were able to determine a heart rate deflection point for all subjects involved. Peak velocity was found to be significantly lower in girls versus boys (p = 0.05), and heart rate peak was similar between the two genders. No significant differences were found between genders for heart rate deflection point with corresponding variables of velocity, heart rate, and percent of heart rate peak (p > 0.05). The finding that there was no difference between genders

for intensity at heart rate deflection point may be because of the possible theory that boys have a lower running economy than girls which forces them to shift to a lower intensity (Armstrong, Welsman, & Kirby, 1999). A significant difference was found, however, between genders for percent peak velocity at heart rate deflection point (p < 0.05). Past research has shown that peak oxygen consumption values were significantly higher for boys when compared to girls of the same age, which could explain the difference in peak velocity among genders, as well (Armstrong, Welsman, & Kirby, 1999, Eisenmann, Pivarnik, & Malina, 2001). Also, because boys may shift to a lower intensity during the trial due to lower running economy, peak velocity at the end of the test may be indicative of a higher anaerobic capacity in boys which resulted in a higher power output at the end of the test. Overall, researchers concluded that the heart rate deflection point is an easy and applicable way to test young runners, regardless of gender.

CHAPTER III: METHODOLOGY

Participants

Twenty-three recreationally active individuals between the ages of 18 and 35 were recruited for this study (men, n = 10; women, n = 13). Two female participants were removed due to health reasons not associated with the study, and one for non-compliance. One male participant was removed due to inability to determine a heart rate deflection point. Therefore, data for 9 males (age 25.56 ± 3.17 years; height 1.77 ± 0.05 meters; body mass 83.52 ± 6.77 kilograms) and 10 females (age 22.78 ± 2.11 years; height 1.64 ± 0.07 meters; body mass 62.28 ± 6.20 kilograms) were included in the final analysis.

Research Design

Participants reported to the University of Central Florida's Human Performance Laboratory (HPL) for an initial screening visit, a pre-testing trial which included a graded exercise test to exhaustion (GXT) on the treadmill, and a 5,000-meter treadmill time trial (TT). On the initial visit, anthropometrics were collected and participants were familiarized with the testing protocol. On the second visit, each participant performed a GXT to determine VO₂peak, time to exhaustion, and ventilatory threshold (VT). Following pre-screening and pre-testing, participants completed the TT. Every visit was separated by a minimum of 48 hours, and for each visit to the HPL, participants were required to arrive two hours post-prandial and to have not exercised for at least 24 hours prior to the testing session. In addition, participants were asked to replicate their dietary habits, assessed via dietary food logs completed for the day before and day of each trial, and to avoid caffeine consumption on the day of the trial (Appendix G).

Variables

When examining the comparison of methods to determine the dependent (HRDP), the independent variables were: (a) 2SEG, (b) D-max, and (c) RCP. When examining the relationship to the dependent variable (HRDP), the independent variables were: (a) time to complete the 5,000-meter time trial (5K_{time}) and (b) RCP.

Instrumentation

- A motorized treadmill (Woodway 4Front[™], Waukesha, Wisconsin, United States) was used to complete GXTs and TT.
- Open-circuit spirometry and a metabolic cart (True One 2400® Metabolic Measurement System, Parvo Medics, Inc., Sandy, Utah, United States) were used to analyze oxygen and carbon dioxide parameters in order to estimate volume of consumed oxygen (VO₂) (ml·kg⁻¹·min⁻¹) by sampling and analyzing breath-by-breath expired gases.
- A wireless heart rate monitor (Polar[®] RS800CX, Kempele, Finland) was used to measure HR throughout each assessment.
- A calibrated physician's scale (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA) was used to measure participants' body weight and height prior to each testing session.

Initial Screening and Testing Methods

Initial Screening

Participants were recruited by word-of-mouth from the University of Central Florida. Following an explanation of all procedures, risks and benefits, each participant provided his/her informed consent to participate in the study (Appendix D). The Institutional Review Board of the University of Central Florida approved the research protocol (Appendix C).

Participants also completed a Physical Activity Readiness Questionnaire (PAR-Q; Appendix E) in order to screen subjects for any risk factors associated with participating in the study. Individuals self-reported to be free of musculoskeletal injury as determined by the PAR-Q. Participants were excluded if they had any history of cardiovascular disease, metabolic, renal, hepatic, or musculoskeletal disorders or were taking any other medication as determined by a medical history questionnaire (Appendix F). For inclusion in the study, participants were also required to have a VO₂peak greater than 35 ml·kg⁻¹·min⁻¹.

Graded Exercise Test (GXT)

The first visit included a GXT on a motorized treadmill (Woodway 4Front[™], Waukesha, Wisconsin, United States), which was used to determine VO₂peak. Participants completed a fiveminute warm-up on the treadmill at a self-selected speed prior to testing. Each participant was fitted with a heart rate monitor (Polar[®] RS800CX, Kempele, Finland) to record HR and body weight was measured on a calibrated physician's scale (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). The GXT protocol was individualized, depending on each participant's estimated one-mile running time. Subjects completed a two-minute warm-up phase, which was excluded from data analysis. Immediately after the two-minute warm-up, the treadmill speed was increased by one mile per hour (mph) every two minutes, for six minutes. For the remainder of the test, speed did not increase. Instead, treadmill incline (or grade) increased by 1.0% every 60 seconds until the participant could no longer continue. During this test, participants' HRs were monitored, as well as respiratory measures via metabolic cart (Appendix H). Participants were not able to see their speed, distance, or time during this treadmill test.

5,000-meter Treadmill Time Trial

Each participant was fitted with a heart rate monitor (Polar[®] RS800CX, Kempele, Finland) to record HR and body weight was measured on a calibrated physician's scale (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). Participants performed a 5-minute warm-up at a self-selected intensity on a motorized treadmill (Woodway 4FrontTM, Waukesha, Wisconsin, United States). Following the warm-up, participants completed a TT, which participants were encouraged to complete as quickly as possible. Ratings of perceived exertion (RPE; ranging from "0 = nothing" to "20 = very, very hard") were recorded every 5 minutes during the TT.

Prior to each TT, a metabolic cart (True One 2400® Metabolic Measurement System, Parvo Medics, Inc., Sandy, Utah, United States) and open-circuit spirometry was used to estimate VO_2 (ml·kg⁻¹·min⁻¹) by sampling and analyzing breath-by-breath expired gases. During the trial, participants' HRs and respiratory measures were monitored. Participants were not able to see their speed or time during the TT but were able to monitor their distance. Total time to completion (5K_{time}) was recorded (Appendix H).

Gas Exchange Analysis

The metabolic cart (True One 2400® Metabolic Measurement System, Parvo Medics, Inc., Sandy, Utah, United States) was calibrated with room air and gases of known concentration. Gas analyzers were calibrated approximately 30 minutes prior to testing. Flowmeter calibration was also performed prior to exercise to determine accuracy of flow volume while collecting data. Participants wore a head unit and breathing mask that stabilized a one-way valve around their mouth. Oxygen and carbon dioxide were analyzed through a sampling line after the gases passed through a heated pneumotach and mixing chamber. Respiratory gases (VO₂, VCO₂, VE, and

respiratory exchange ratio (RER)) were monitored continuously and expressed as 30-second averages.

VO₂peak was determined to be the highest 30-second VO₂ value during the test and coincided with at least two of the following three criteria: (a) 90% of age-predicted maximum HR; (b) RER > 1.1; and/or (c) a plateau of VO₂ (less than 150 mL per minute increase in VO₂ during the last 60 seconds of the test) (Howley, Bassett, & Welch, 1995). Previous work in our lab has shown the test-retest reliability for VO₂peak to be ICC = 0.96 (SEM 1.4 ml·kg·min⁻¹).

Analysis of the Heart Rate Deflection Point

HRDP values were determined using two methods: (1) the D-max method using an exponential-plus-constant regression model (HRDP_{D-max}, Figure 2) and (2) the bi-segmental linear regression method (HRDP_{2SEG}, Figure 3). For each method, HR values were analyzed using a cutoff point starting at VT, determined to be 80% of the participants' maximum achieved HR during the GXT.

HRDP_{D-max} was considered to be the point at which the slope of the exponential plus constant regression curve was equal to the slope of the linear regression line connecting the first and last HR points. Alternatively, this deflection point denotes the maximum perpendicular distance between the linear and nonlinear regression lines. The exponential-plus-constant model was used to determine HRDP from HR and time (t), using the following equation (Da Silva, Peserico, & Machado, 2014):

$$HR(t) = a + (b \times e^{c \times t})$$

The coefficients a, b, and c, as well as the coefficient of determination (r^2) , were calculated through use of a computerized graphing program (Origin, OriginLab Corporation, Northampton,

Massachusetts, Figure 4). The following formula was then used to determine the HRDP in Microsoft Excel (Figure 5):

$$HRDP = \ln((e^{c \times \max t}) - e^{c \times \min t})) / ((c \times \max t) - (c \times \min t))) / c$$

In order to find HRDP_{2SEG}, the "HR / time" relationship curve was divided into two linear regression segments, with the HRDP denoting the intersection of the two segments. A computerized data analysis and graphing program (Origin, OriginLab Corporation, Northampton, Massachusetts) was used for this method (Figure 6). A piecewise fitting function (3.2.2.24 Fitting with a Piecewise Linear Function, n.d.) was defined consisting of two linear segments, expressed as:

$$Y = \begin{cases} \frac{y1(x3-x)+y3(x-x1)}{x3-x1} & , \text{ if } x < x3\\ \frac{y3(x2-x)+y2(x-x3)}{x2-x3} & , \text{ if } x \ge x3 \end{cases}$$

After fitting the data, $HRDP_{2SEG}$ were calculated by defining the bisection of the two linear segments from the fitting result.

Determination of Respiratory Compensation Point

RCP values were also determined via 2SEG. VCO₂ and VE were analyzed using a cutoff point starting at VT as previously described. The VCO₂-VE relationship curve was divided into two linear regression segments, with the RCP denoting the intersection of the two segments. A computerized data analysis and graphing program (Origin, OriginLab Corporation, Northampton, Massachusetts) was used for this method. A piecewise fitting function (3.2.2.24 Fitting with a Piecewise Linear Function, n.d.) was defined consisting of two linear segments, expressed as:

$$Y = \begin{cases} \frac{y1(x3-x)+y3(x-x1)}{x3-x1} & , \text{ if } x < x3\\ \frac{y3(x2-x)+y2(x-x3)}{x2-x3} & , \text{ if } x \ge x3 \end{cases}$$

After fitting the data, RCP were calculated by defining the bisection of the two linear segments from the fitting result.

Statistical Analyses

All data were analyzed to provide descriptive statistics for HRDP_{2SEG}, HRDP_{D-max}, RCP, and 5K_{time}. Statistically significant differences and comparisons were analyzed between HRDP_{2SEG}, HRDP_{D-max}, and RCP. Possible relationships to HRDP with RCP and 5K_{time} were also examined. The Shapiro-Wilk test was used to test data for normality. Statistical analysis was conducted through use of SPSS (Version 21.0).

One-way repeated measures analysis of variance (ANOVA) was used to compare HRDP_{2SEG}, HRDP_{D-max}, and RCP. Pearson product moment correlations were used to examine the relationship between the HRDP estimation methods and both RCP and 5K_{time} performance.

Steiger's Z-tests were used to compare the correlation coefficients of HRDP_{2SEG} and HRDP_{D-max} with the correlations of RCP and $5K_{time}$, through use of correlation matrices, in order to determine if the different methods of estimating HRDP were correlated equally with RCP and $5K_{time}$ (Steiger, 1980). Fisher's z transformations were used to convert correlation coefficients to z-scores, followed by the implantation of specific equations proposed by Steiger (1980).

Bland Altman plots were created to evaluate the levels of agreement (regardless of the strength of the relationship) between HRDP_{2SEG}, HRDP_{D-max}, and RCP, as well as identify outliers. The differences and means of two compared variables were computed, which were used to plot the y- and x-axis, respectively. A one-sample t-test on the differences of the two variables was performed. The standard deviations (SD) and mean of the differences were used to find the upper limit [(SD \cdot 1.96) + mean] and lower limit [mean – (SD \cdot 1.96)], which were included in each Bland Altman plot. The mean difference was represented as the proportional bias in each

plot, and, through linear regression, this bias was considered either significant or non-significant, based on an alpha level of $p \le 0.05$. Non-significant proportional bias is an indication of a high level of agreement between methods.

CHAPTER IV: RESULTS

Nineteen participants performed a GXT (average VO₂peak for males: 48.98 ± 7.37 ml·kg·min⁻¹; average VO₂peak for females: 42.32 ± 4.13 ml·kg·min⁻¹), lasting an average of 10.45 ± 1.27 minutes for males and 11.15 ± 1.95 minutes for females, and a TT (average 5K_{time} for males: 26.82 ± 3.15 minutes; average 5K_{time} females: 30.61 ± 4.51 minutes). Shapiro-Wilk tests for normality revealed non-significance for all variables, indicating samples that were normally distributed for HRDP_{2SEG} (p = 0.653); HRDP_{D-max} (p = 0.491); RCP (p = 0.559), and 5K_{time} (p = 0.262). The descriptive statistics for all participants are listed in Table 1. Individual and mean (\pm 95% confidence interval) HRDP_{2SEG}, HRDP_{D-max}, and RCP values are shown in Figure 1.

Averages were calculated for HRDP_{2SEG} (176.70±9.40 bpm), HRDP_{D-max} (178.18±6.85 bpm), RCP (176.92±6.63 bpm), and 5K_{time} (28.81±4.29 minutes). Table 2 displays the minimums, maximums, and means ± standard deviations for all variables. When comparing HRDP_{2SEG}, HRDP_{D-max}, and RCP, Mauchly's test of Sphericity indicated non-significance ($X^2 = 2.364$, p = 0.307). Within-subjects effects, using Sphericity Assumed estimates, revealed no significant differences between HRDP_{2SEG}, HRDP_{D-max}, and RCP (F_{2,36} = 3.739, p = 0.533, $\eta^2 = 0.034$).

When comparing the two methods of determining HRDP, a strong positive correlation (r = 0.831, p < 0.0001, Figure 10) was found between the HRDP_{2SEG} and HRDP_{D-max}. When comparing HRDP_{2SEG} and HRDP_{D-max} to RCP, strong positive correlations were seen between RCP and HRDP_{2SEG} (r = 0.650, p = 0.003, Figure 11) and between RCP and HRDP_{D-max} (r = 0.619, p = 0.005, Figure 12). Table 3 displays the correlations between HRDP_{2SEG}, HRDP_{D-max}, and RCP.

When examining the relationship between HRDP and TT, non-significant moderate positive correlations were found between HRDP_{2SEG} and $5K_{time}$ (r = 0.419, p = 0.074, Figure 14) and between HRDP_{D-max} and $5K_{time}$ (r = 0.241, p = 0.321, Figure 15). When examining the relationship between RCP and $5K_{time}$, non-significant weak positive correlations (r = 0.193, p = 0.429, Figure 13) were found between RCP and $5K_{time}$. Table 4 displays the relationship of $5K_{time}$ to HRDP_{2SEG}, HRDP_{D-max}, and RCP.

The Steiger's Z-test was used to compare the correlations of $HRDP_{2SEG}$ and $HRDP_{D-max}$ with the correlations of RCP and $5K_{time}$. When comparing the $HRDP_{2SEG}$ and $HRDP_{D-max}$ with the RCP (p = 0.142, z = 1.469) and $5K_{time}$ (p = 0.024, z = 2.258), no statistically significant differences were found between the correlation coefficients of HRDP estimates and RCP, but were found between coefficients of HRDP estimates and $5K_{time}$.

Bland-Altman plots were created to evaluate the level of agreement between (1) HRDP_{2SEG} and HRDP_{D-max} (mean difference = -1.484 ± 5.319 , upper limit = 8.941, lower limit = -11.909; Figure 7), (2) HRDP_{2SEG} and RCP (mean difference = -0.2211 ± 7.161 , upper limit = 13.815, lower limit = -14.258, Figure 8), and (3) HRDP_{D-max} and RCP (mean difference = 1.263 ± 5.884 , upper limit = 12.796, lower limit = -10.269, Figure 9). Similar limits of agreement were found for all comparisons (HRDP_{2SEG} and RCP, p = 0.070; HRDP_{D-max} and RCP, p = 0.868; HRDP_{2SEG} and HRDP_{D-max}, p = 0.029), however, systematic bias was found between HRDP_{2SEG} and HRDP_{D-max}.

CHAPTER V: DISCUSSION

This study aimed to examine the relationship between different HRDP estimates and a potentially corresponding performance measure (5K_{time}), as well as a metabolic threshold determined using gas exchange analysis (RCP). No differences were found between the two methods used to determine HRDP or between HRDP estimates and RCP. Strong relationships were found between HRDP_{2SEG} and HRDP_{D-max}, HRDP_{2SEG} and RCP, and HRDP_{D-max} and RCP. However, there was no relationship found between 5K_{time} and estimates of HRDP or RCP. Thus, the examined methods used to determine HRDP, as well as the use of RCP, may be accurate in measuring anaerobic threshold, but not in estimating 5,000m treadmill running performance.

Methods of Determining Heart Rate Deflection Point

Previous research has independently established D-max and 2SEG to be valid methods of non-invasively determining HRDP to estimate performance variables when compared to a more invasive measure of obtaining blood lactate levels (Pereira et al., 2015; Higa et al., 2007). In a study conducted by Pereira et al. (2015), researchers investigated the relationship between HRDP_{D-max} and maximal lactate steady state in active college-aged males. Following a 3,000m time trial on a 400m track to establish mean running velocity, subjects performed a GXT on a motorized treadmill. Each GXT began at 65% of each subject's 3,000m mean running velocity, and speed was increased slowly by 0.5 kph every 3 minutes until exhaustion, with a constant treadmill incline of 1%. The researchers found no significant difference between velocity at the HRDP_{D-max} and the velocity at maximal lactate steady state (p > 0.05) (Pereira et al., 2015), demonstrating that the D-max method of determining HRDP may be an accurate measure to estimate running velocity at maximal lactate steady state.

For the current study, the specific method of D-max used was based on a study conducted by Da Silva, Peserico, & Machado (2014) in middle-aged recreationally-active women who completed a GXT featuring slow, steady (increase of 1 kph every 3 minutes) increases in speed with a constant treadmill incline of 1% and an outdoor 10,000m time trial. The researchers found that using an exponential-plus-constant regression curve model provided a higher correlation between HRDP_{D-max} and 10,000m running performance (r = 0.96) than the third-order polynomial regression curve model.

In addition to using D-max to determine HRDP, researchers have also examined the 2SEG method and its accuracy for estimating anaerobic threshold. (Higa et al. 2007). Researchers recruited recreationally active females in the same age range as those in the current study, as well as a group of recreationally active females in a higher age range. Each GXT was performed on a cycle ergometer, and utilized increases in work load by 10-20 Watts per minute until exhaustion. Higa et al. (2007) found a strong relationship between HRDP_{2SEG} and anaerobic threshold determined from 2SEG (r = 0.75, p < 0.05). These results, in combination with others, support the use of 2SEG as an acceptable method of determining HRDP (Davis et al., 1983; Aunola & Rusko, 1992; Kara et al., 1996; Carey, 2002; Buchheit, Solano, & Millet, 2007). To the best of the authors' knowledge, the direct comparison of the D-max and 2SEG methods of estimating HRDP in the current study support is unique and, due to the similar and related values, provides support for the use of either approach in recreationally-trained individuals.

Heart Rate Deflection Point and Running Performance

The lack of a relationship between HRDP and running performance in the current study differs from prior research, which indicated HRDP to be a valid method of assessing running performance for distances between 3,000m and 10,000m (Zacharogiannis & Farrally, 1993;

Petit, Nelson, & Rhodes, 1997; Da Silva, Peserico, & Machado, 2014). Inconsistencies between findings examining the relationship between HRDP and running performance may be due to GXT protocols. Rather than using small increments, as proposed by Conconi, Ferrari, Ziglio, Droghetti, & Codeca (1982), the current study's GXT protocol consisted of larger increases in intensity. Conconi and colleagues (1982) suggested that the use of larger increments of increasing intensity during the GXT may increase the amount of large spikes or jumps in heart rate, which may provide inaccurate or nonexistent HRDPs in participants.

In a study by Zacharogiannis and Farrally (1993), male and female participants in the same approximate age range as the current study completed a GXT protocol involving slow increases in treadmill running speed. A 3,000m time trial was completed following the GXT, which took place on an outdoor track, unlike the current study which conducted a 5,000m time trial on a motorized treadmill. It has been shown that using a treadmill in a laboratory setting compared to using an over-ground track or a surface outdoors creates inconsistencies in the mechanics and energy cost of running, which may affect the relationship between HRDP and running performance (Jones, & Doust, 1996; Schache et al., 2001; Kong, Koh, Tan, & Wang, 2012; Sinclair et al., 2013). Researchers have previously used adjustment equations in order to more accurately measure oxygen requirements, or VO₂, of treadmill running (Leger & Mercier, 1984; Bassett et al., 1985). Other researchers have utilized adjusted treadmill protocols, which included using a constant 1% treadmill incline (Jones & Doust, 1996; Heck et al., 1985) or a constant 2% treadmill incline (Tegtbur, Busse, & Braumann, 1993), in order to account for the air resistance experienced outdoors. However, Zacharogiannis and Farrally (1993) did not use a constant treadmill incline during the GXTs and were still able to find a strong relationship between HRDP and 3,000m outdoor running performance (r = 0.940).

Conconi and colleagues (1982) have suggested that the use of small, steady increments in increasing intensity allow there to be a clear break point in linearity in the "speed / heart rate" relationship, which could explain the findings by Zacharogiannis and Farrally (1993). A later study, involving male and female moderately-trained to highly-trained runners in the same approximate age range as the current study, investigated the use of a modified Conconi test on a motorized treadmill (Petit, Nelson, & Rhodes, 1997). As used in the previously mentioned study by Zacharogiannis and Farrally (1993), the increases in speed used by Petit and colleagues (1997) were kept small, which agreed with the Conconi test's recommendations. Petit, Nelson, & Rhodes (1997) used a mathematical model to find HRDP, which allowed them to calculate a prediction time for 10,000m running performance. Between this predicted performance time through use of HRDP and actual performance time, researchers were able to find a strong relationship (r = 0.980). In another study by Da Silva, Peserico, & Machado (2014), recreationally-trained runners in a slightly higher age range than the current study completed a GXT, which utilized small, slow increases in treadmill speed, similar to the protocol implemented by Zacharogiannis and Farrally (1993). An important commonality between the GXTs of these studies is the use of small, steady speed increases. Possibly due to this, a relationship was able to be found between HRDP and running performance in all of the previously mentioned studies. This could provide a potential explanation of why a relationship was not found between HRDP and 5K_{time} in the current study which utilized quicker increases in intensity during the GXTs.

However, more research has since been done on this topic and researchers have been successful in determining HRDPs while still utilizing faster increases in intensity. One of the more recent studies conducted by Sentija, Vucetic, & Markovic (2007) investigated the validity

of a modified Conconi test, which utilized a shorter, faster treadmill protocol. Researchers used male runners of varying abilities, in the same approximate age range as the current study, and compared the use of slow, gradual increases in intensity (originally proposed by the Conconi test) to the application of quick, larger increases in speed (Sentija, Vucetic, & Markovic, 2007). In order to better control running speed, GXTs were also done on a motorized treadmill, instead of the originally proposed outdoor track. It has been reported that when speed is increased too quickly, the heart rate deflection point is underestimated (Conconi et al., 1996). Contrary to this theory, Sentija and researchers (2007) found that the HRDPs found during the "fast treadmill protocol", indicating that the runners were utilizing a higher percentage of their maximum heart rate during the "fast treadmill protocol". Researchers also found a very strong relationship between HRDPs recorded during the "slow" and "fast treadmill protocols", as well as found no differences between the protocols (Sentija and researchers, 2007). Therefore, research seems to support that fast or slow treadmill protocols may be valid methods for determining the HRDP.

Respiratory Compensation Point

In examining the relationships between HRDP estimates and RCP in recreationally trained runners, the current study found strong correlations between HRs at RCP and HRDP_{2SEG}, and HRs at RCP and HRDP_{D-max}. In coordination with these findings, Buchheit and colleagues (2007) found moderate correlations between HRDP and RCP (r = 0.61, p < 0.001). The treadmill GXT protocol that these researchers used included quicker increases in running speed, of 1 kph every one minute with a constant 1% treadmill incline (Buchheit, Solano, & Millet, 2007). Similar to Buchheit and colleagues (2007), Marques-Neto and researchers (2012) also found a strong correlation between the second HRDP and RCP. However, the treadmill GXT protocol

utilized slower increases in speed and incline (Marques-Neto, Maior, Neto, & Santos, 2012). Moderately strong correlations between HRDP and RCP were also found by Redkva et al. (2013). As with Marques-Neto and colleagues (2012), Redkva et al. (2013) created a GXT protocol which used slower increases in running speed. Some of the previously mentioned research studies have utilized slow increases in treadmill speed with a constant treadmill incline of 1%, whereas the current study used quicker increases in speed and increased treadmill incline by 1% every minute (Buchheit, Solano, & Millet, 2007; Marques-Neto, Maior, Neto, & Santos, 2012; Redkva et al., 2013). However, the previously mentioned studies and the current study all found a moderate to strong relationship between HRDP and RCP, indicating that, regardless of the specific GXT protocol, the HRDP may be an accurate method to measure this metabolic threshold.

Additionally, the current study aimed to investigate the relationship between RCP and $5K_{time}$. In addition to not finding a relationship between HRDP estimates and $5K_{time}$, the current study was also unable to find a relationship between RCP and $5K_{time}$. No difference was found between HRDP estimates and RCP, and strong relationships were found between the two thresholds, as well. Because of the lack of differences and strong relationship between HRDP and $5K_{time}$, it may be acceptable to say that there is no significant relationship between RCP and $5K_{time}$, either.

Conclusion

In conclusion, the current study found that there was no difference in HRs at the HRDP when using the D-max and 2SEG methods of HRDP determination and that these methods could possibly be used interchangeably. However, there may be systematic bias between these two methods, possibly due to 2SEG overestimating HRDP. This study also found that there were no

differences between RCPs and HRDPs of either method, and strong relationships were found between RCP, HRDP_{2SEG}, and HRDP_{D-max}. These findings suggest that RCP and HRDP may provide similar estimates of the anaerobic threshold.

Conversely, no relationship was seen between treadmill running performance and either estimate of HRDP or RCP. This could have possibly been due to the training statuses of the participants, the comfortability of the participants running on a treadmill, the design of the treadmill protocol, or an unknown factor. Further research should be conducted examining anaerobic threshold, through estimation of HRDP and/or RCP, and its relation to running performance in various populations in order to examine differences in HRDP between various age groups, genders, and training statuses.

APPENDIX A: FIGURES

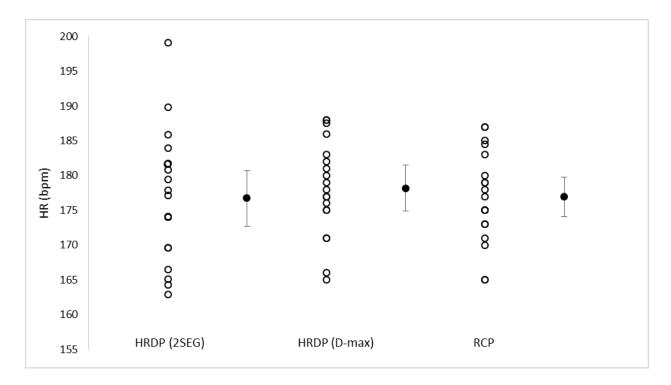


Figure 1. Individual and mean (\pm 95% CI) HRDP_{2SEG}, HRDP_{D-max}, and RCP values.

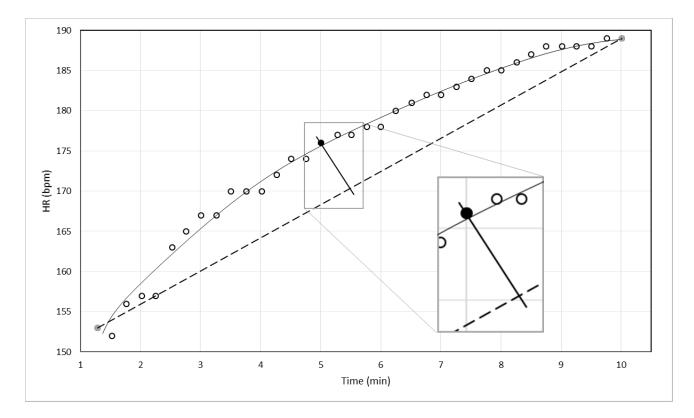


Figure 2. Example of a participant's $HRDP_{D-max}$ on the "time / HR" relationship graph.

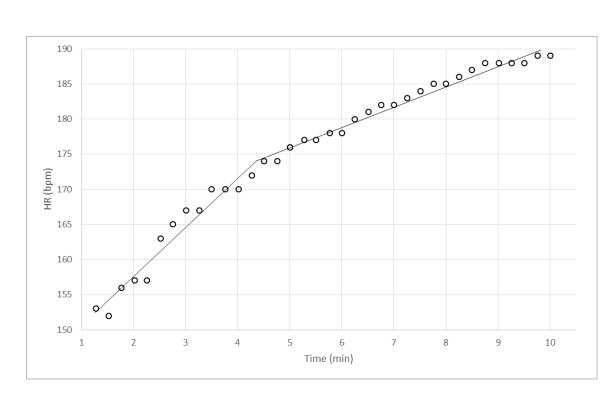


Figure 3. Example of a participant's HRDP_{2SEG} on the "time / HR" relationship graph.

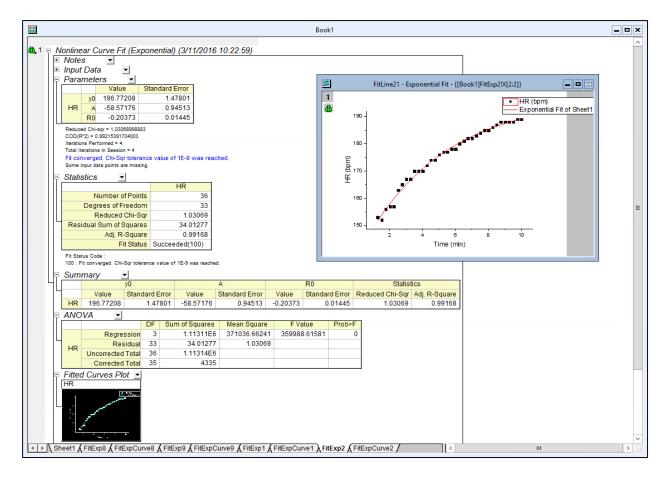


Figure 4. HRDP_{D-max} calculations in OriginLab©.

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Figure 5. HRDP_{D-max} calculations in Microsoft Excel.

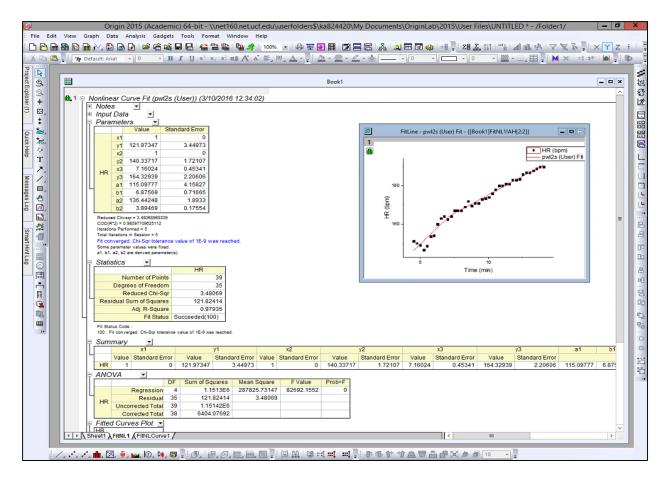


Figure 6. HRDP_{2SEG} calculations in OriginLab©.

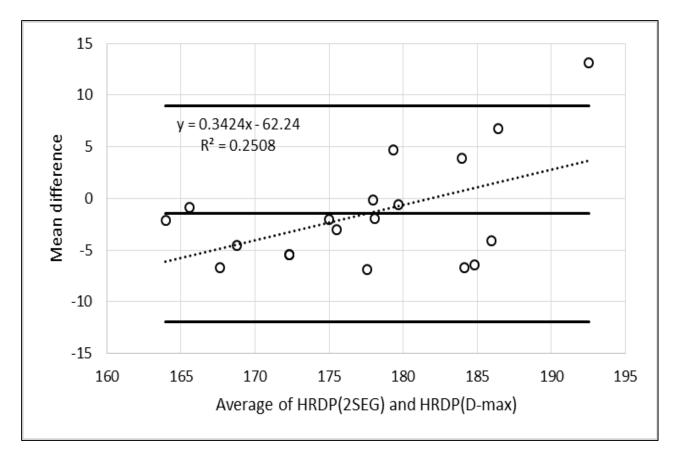


Figure 7. Bland Altman Plot: HRDP_{2SEG} and HRDP_{D-max}.

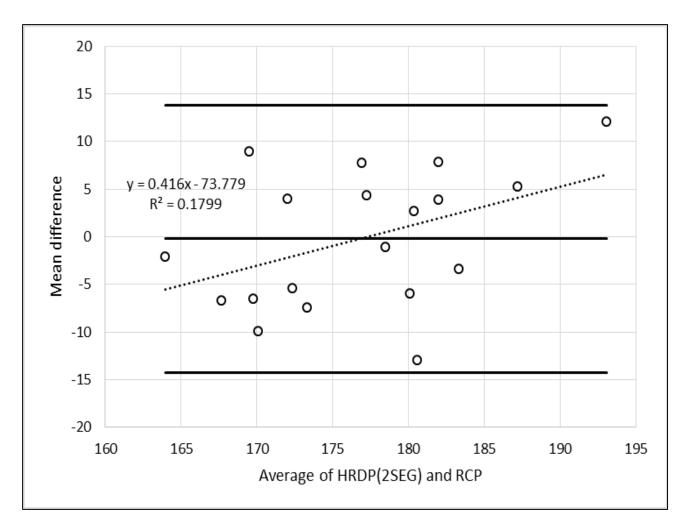


Figure 8. Bland Altman Plot: HRDP_{2SEG} and RCP.

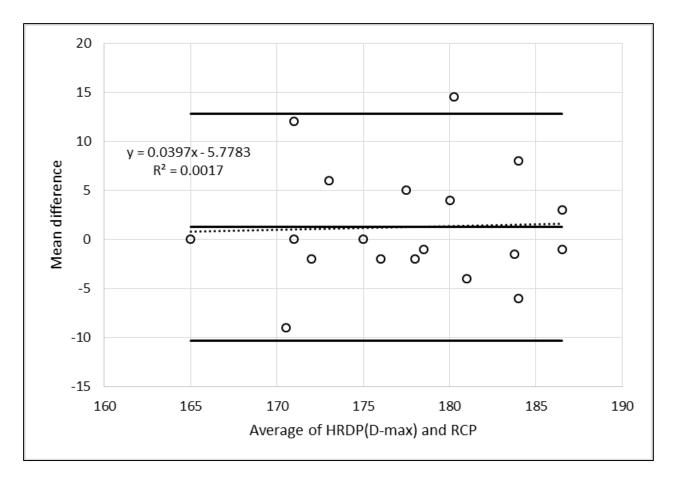


Figure 9. Bland Altman Plot: HRDP_{D-max} and RCP.

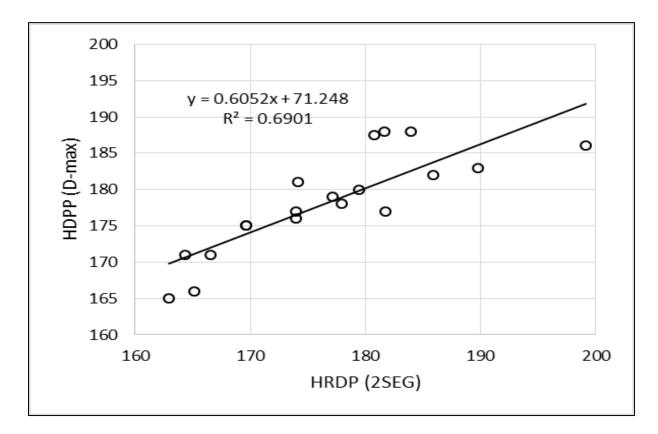


Figure 10. Relationship between HRDP_{D-max} and HRDP_{2SEG}.

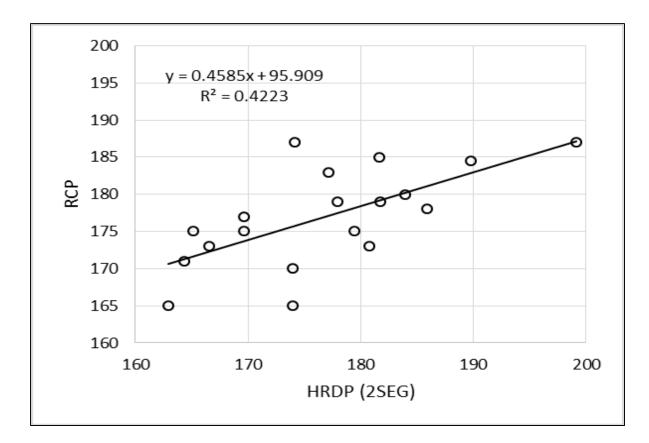


Figure 11. Relationship between $HRDP_{2SEG}$ and RCP.

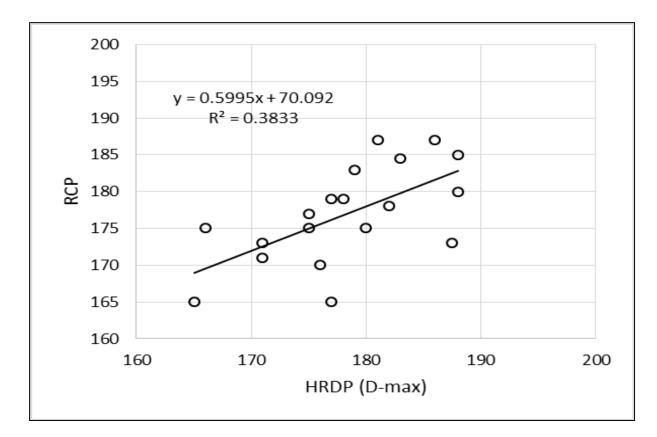


Figure 12. Relationship between $HRDP_{D-max}$ and RCP.

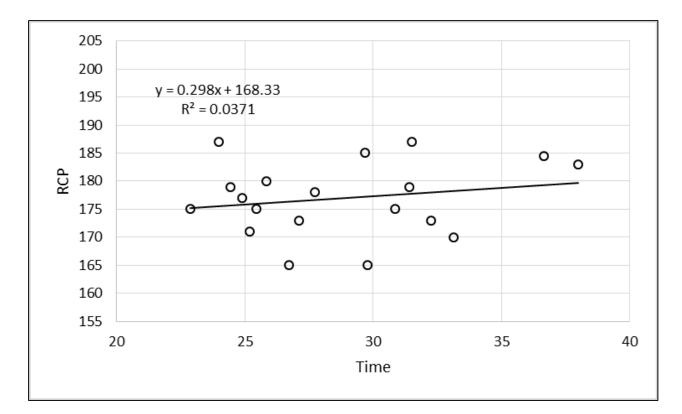


Figure 13. Relationship between RCP and 5K_{time}.

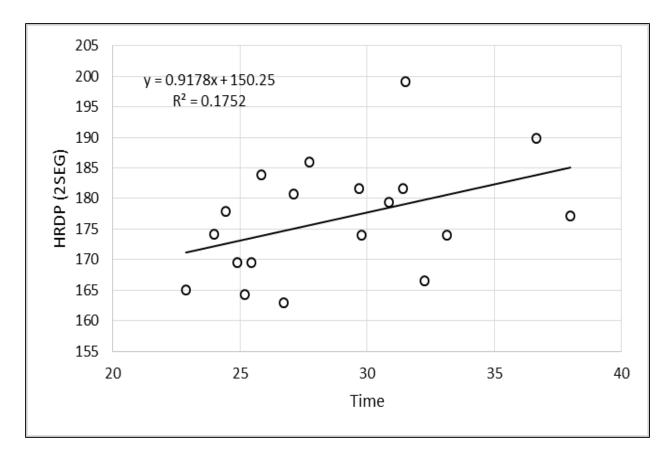


Figure 14. Relationship between $HRDP_{2SEG}$ and $5K_{time}$.

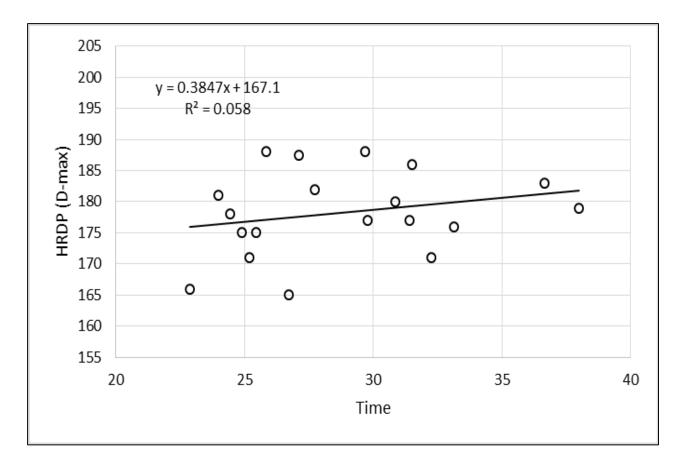


Figure 15. Relationship between $HRDP_{D-max}$ and $5K_{time}$.

APPENDIX B: TABLES

	N	Age (yrs)	Height (m)	Weight (kg)	VO2peak (ml·kg·min ⁻¹)
Males	9	25.56 ± 3.17	1.77 ± 0.05	83.52 ± 6.77	48.98 ± 7.37
Females	10	22.78 ± 2.11	1.64 ± 0.07	62.28 ± 6.20	42.32 ± 4.13

Table 1. Participant characteristics expressed as mean \pm standard deviation.

Ν		Minimum	Maximum	Mean	SD	
HRDP _{2SEG}	19	162.90 bpm	199.10 bpm	176.70 bpm	9.40 bpm	
HRDP _{D-max}	19	165.00 bpm	188.00 bpm	178.18 bpm	6.85 bpm	
RCP	19	165.00 bpm	187.00 bpm	176.92 bpm	6.63 bpm	
5Ktime	19	22.87 min	37.98 min	28.62 min	4.26 min	

Table 2. Performance statistics: minimum, maximum, and mean \pm standard deviation of all variables.

	р	r
HRDP _{2SEG} vs. HRDP _{D-max}	<0.0001*	0.831
HRDP _{2SEG} vs. RCP	0.003*	0.650
HRDP _{D-max} vs. RCP	0.005*	0.619

Table 3. Correlations between $HRDP_{2SEG}$, $HRDP_{D-max}$, and RCP.

* = significant difference

	р	r
HRDP _{2SEG}	0.074	0.419
HRDP _{D-max}	0.321	0.241
RCP	0.429	0.193

Table 4. HRDP and RCP in comparison to $5K_{time.}$

APPENDIX C: IRB APPROVAL



University of Central Florida Institutional Review Board Office of Research & Commercialization 12201 Research Parkway, Suite 501 Orlando, Florida 32826-3246 Telephone: 407-823-2901 or 407-882-2276 www.research.ucf.edu/compliance/irb.html

Approval of Human Research

From: UCF Institutional Review Board #1 FWA00000351, IRB00001138

To: Jay R. Hoffman and Co-PIs: David Church, Jeffrey Ray Stout

Date: February 13, 2015

Dear Researcher:

On 2/13/2015 the IRB approved the following human participant research until 2/12/2016 inclusive:

Type of Review:	Submission Response for UCF Initial Review Submission
	Form Expedited Review
Project Title:	Effect of Turkish Coffee Consumption on 5K Time Trial
	Running Performance and Reaction Time
Investigator:	Jay R. Hoffman
IRB Number:	SBE-15-10986
Funding Agency:	Strauss Group Inc.(Strauss Group)
Grant Title:	
Research ID:	N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form **cannot** be used to extend the approval period of a study. All forms may be completed and submitted online at https://iris.research.ucf.edu.

If continuing review approval is not granted before the expiration date of 02/12/2016, approval of this research expires on that date. When you have completed your research, please submit a Study Closure request in iRIS so that IRB records will be accurate.

<u>Use of the approved, stamped consent document(s) is required.</u> The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a signed and dated copy of the consent form(s). Participants or their representatives must receive a copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the <u>Investigator Manual</u>.

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Patria Davis on 02/13/2015 10:37:24 AM EST

IRB Coordinator

APPENDIX D: IRB FORM



Institute of Exercise Physiology and Wellness University of Central Florida 4000 Central Florida Blvd Orlando, Florida 32816-1250

Effect of Turkish Coffee Consumption on 5K Time Trial Running Performance and Reaction Time

Dr. Jay. R. Hoffman, Ph.D. Dr. Jeffrey R. Stout, Ph.D. David Church, M.S.

Sport and Exercise Science College of Education University of Central Florida

Study Proposal

1) Protocol Title

• Effect of Turkish Coffee Consumption on 5K Time Trial Running Performance and Reaction Time

2) Study Sponsor

• Strauss Group, Inc., Petach Tikva, Israel

3) Study Objectives

• To determine the effects of the ingestion of "Elite Turkish Coffee," on metabolic, cardiovascular, subjective, blood, and performance measures.

4) Background

Coffee is a popular drink throughout the world, both for performance and nonperformance reasons. A primary component of coffee is caffeine, which is a widely used mild central nervous system stimulant that has been shown to stimulate bronchodilation of alveoli, vasodilation of blood vessels, neural activation of muscle contraction, blood filtration in the kidneys, catecholamine secretion, and lipolysis (Sokmen et al., 2008). Caffeine is absorbed by the stomach and small intestine within 45 minutes of ingestion and is then distributed throughout all tissues of the body (Liguori et al., 1997). Although varying among individuals, its half-life is approximately 5 hours for healthy adults (Meyer et al., 1991). The principal physiological mode of action of caffeine is as an antagonist of adenosine receptors in the brain. By increasing plasma caffeine concentrations, the caffeine is able to bind to the receptors more rapidly than adenosine acting as a competitive inhibitor (Daly et al., 1981). The reduction of adenosine activity leads to increased secretion of excitatory neurotransmitters, dopamine and glutamate (Kalmar & Cafarelli, 1999).

Studies have shown that acute ingestion of caffeine can increase mental focus and lower pain perceptions (Motl et al., 2003; Motl et al., 2006; Doherty et al., 2004), in addition to improving cognitive function and reaction time (Adan & Serra-Grabulos, 2010; Haskell et al., 2005). Caffeine ingestion has been demonstrated to be beneficial for endurance athletes (Bridge & Jones, 2006; Ganio et al., 2009; Graham, 2001; Graham & Spriet, 1991). However, the methods of delivery (i.e. coffee, capsule, powder) vary, and no data exist in relation to endurance performance and the Turkish method of preparation of coffee.

Graham, Hibbert, and Sathasivam (1998) suggest that the effects of coffee may not be directly attributed to caffeine. Additional compounds are present in coffee that may play a role in the metabolic effects of coffee. Studies have shown no significant difference between carbohydrate utilization (Casal & Leon, 1985; Costill et al., 1978), and performance time, glucose, glycerol, and lactate during a "voluntary," exhaustion run between caffeinated and decaffeinated beverages(Graham, 1998). This raises the importance of brew style as Turkish coffee has been reported to have higher concentrations of tocopherols than other preparation methods (Alves et al., 2010), phenol and thus high anti-oxidant capacity in-vitro (Gunduc & El, 2003), and higher caffine content than American and instant brewing methods. Turkish coffee has been demonstrated to lower heart rate and blood pressure one hour after consumption which may be beneficial to performance (Awaad et al., 2011). These factors

combine warrant the investigation of the effect preparing Turkish coffee has on mental and physical performance.

5) Setting of the Human Research

• The study site is the Human Performance Lab (HPL) in the College of Education building at the University of Central Florida.

6) Resource available to conduct the Human Research

- Many of the lab members are teachers within the university and recruiting by word-of-mouth. This large population should be sufficient to recruit enough suitable subjects in the given amount of time.
- 20 subjects are being recruited for the study, with each subject taking ~8 total hours to complete the study. Thus, it is estimated to take a total of 160 hours of time with subjects in the lab to complete the study.
- All equipment required for the study is available in the Sport and Exercise Science's Human Performance Laboratory. Our lab has an extensive research history, and publications demonstrating their ability to perform all assigned task that is required for this study.
- All individuals will be read and described the protocol by the primary investigators before any testing begins. Pilot testing will be performed to ensure that researchers are aware of the duties related to this research study.
- Although unlikely, our lab has a first aid kit, staff trained in CPR and AED within the facility.

7) Study Design

- Randomized, double-blind, counter-balanced, placebo controlled, and will be conducted following the International Conference on Harmonisation (ICH) Guidelines and in accordance with the Declaration of Helsinki. Institutional Review Board (IRB) approval (University of Central Florida IRB) will be acquired prior to conducting any protocol-specific procedures. All participants will be screen via a PAR-Q, if any of the seven questions are answer with a "yes," then clearance by a doctor will be needed for an individual to participate in the study.
- Randomization will be performed by a random number generator. An individual not involved with the study will place the two types of coffee in separate containers which will be labelled "A," and "B." The key will be sealed in an envelope and only opened at the end of the study or in the unlikely event of an adverse event.
- Recruitment Methods
 - Approximately 20 subjects (both men and women) who are regular caffeine consumers (1 cup of coffee per day) between the ages of 18-39 y will be recruited to participate in this study. Word-of-mouth throughout the University of Central Florida will be used to recruit participants.
- Inclusion Criteria
 - Between the ages of 18 and 39.

- Regular coffee consumers (1 cup of coffee per day)
- Healthy as assessed by Pre-exercise Testing Health Status Questionnaire
- Subject provides written and dated informed consent to participate in the study
- Be able to complete a 5k

• Exclusion Criteria

- Having a history of medical or surgical events that may significantly affect the study outcome, including cardiovascular disease, metabolic, renal, hepatic, or musculoskeletal disorders
- Use of medicine that may significantly affect the study outcome
- Use of nutritional supplements (such as ribose, protein drinks, creatine, vitamins) in the 3 months prior to the start of the study
- Subject is participating in another clinical trial or has received an investigational product within thirty days prior to screening/enrollment
- Pregnancy

• Study Endpoints

• Participants will make three visits to the HPL during the duration of the study. The first will be a VO₂max and familiarization trial. The second and third will be the 5k time trial visit with either the decaffeinated or caffeinated Elite Turkish Coffee. During the 5k times trial visits, subjects will provide four blood samples. After the collection of the last blood sample on the last visit the study will be concluded.

• Study Timelines

- Participation in this study will take approximately three weeks (3 different visits).
- Participant recruitment will occur during the Spring 2015 semester.
- Enrollment will be completed during the Spring 2015 semester.

8) Procedures involved in the Human research

• Time Trial Performance

During each testing session subjects will perform a 5 km time trial on a Woodway Treadmill (Waukesha, WI). All participants will be instructed to complete the 5 km as quickly as they can. During exercise oxygen consumption, minute ventilation, respiratory exchange ratio, heart rate and blood pressure will be measured continuously. During testing a researcher trained in CPR will be present, and in the event the use of an AED is required, our facility contains one within 100 feet of the testing area.

• Metabolic Measures

Metabolic measures during exercise will be performed with a ParvoMedics TrueOne Metabolic System OUSW 4.3.4. Measurements of VO₂ (ml·kg·min⁻¹), VCO₂ (ml·min⁻¹), ventilation (VE), and respiratory quotient (RQ), will be recorded. Machine calibration will be performed prior to each testing session.

• Cardiovascular Measures

Average heart rate and blood pressure will be measured at each assessment time point using a wireless heart rate monitor (Polar[®] RS800CX, Kempele, Finland) and a digital blood pressure monitor (Omron Healthcare, Inc, HEM-712C, Vernon Hills, Illinois). Heart rate and blood pressure will be measured and recorded every 5 minutes during exercise.

• Subjective Measures

Participants will be instructed to assess their subjective feelings of energy, alertness, and focus using a 15-cm visual analog scale (VAS) before coffee consumption, 30-minutes following coffee consumption and immediately following exercise. The scale will be anchored by the words "Lowest" and "Highest" to represent extreme ratings where the greater measured value represents the greater feeling. Questions will be structured as "My level of energy is", "My level of alertness is", and "My level of focus is?"

• Upper Body Reaction Measurements

Measurement of upper body reaction time will be performed on the Dynavision D2 Visuomotor Training Device (D2; Dynavision International LLC, West Chester, OH). The D2 is a light-training reaction device, developed to train sensory motor integration through the visual system (Wells et al., 2014). It consists of a board (4 foot x 4 foot) that can be raised or lowered relative to the height of the participant. It contains 64 target buttons (lights) arranged into five concentric circles surrounding a center screen that can be illuminated to serve as a stimulus for the participant. Participants will be required to assume a comfortable athletic stance and stand at a distance from the board where they can easily reach all of the lights. The board height will be kept consistent for all testing trials and will be adjusted per participant so the center screen is located just below eye level. A total of three different reaction tests will be conducted.

The first assessment will measure the participant's ability to react to a stimulus (light) as it changes position on the board. An initial stimulus (light) will be presented on the D2 in a random location. The stimulus (light) will remain lit until it will be touched by the participant. A stimulus (light) will then appear at another random location. The participant will then be instructed to successfully identify and touch as many stimuli (lights) as possible within 60 seconds. The number of successful "hits" will be recorded for each trial.

The second assessment will be similar to the previous measure in that participants will also be required to react to a visual stimulus (light) as it changes position on the board. However, during this trial the stimulus (light) will remain lit for 1 second before it changed to another random location and the participant will have to verbally recite a five digit number that will be presented on the center screen of the D2 every 5 seconds. The appearance of the digits places a cognitive demand on the information processing resources of the participant. The participant will be instructed to successfully identify and touch each stimulus before it changes position and score as many touches as possible within 60 seconds. The number of successful "hits" will be recorded for each trial.

The third assessment will measure the participant's visual, motor, and physical reaction times to a visual stimulus with the dominant hand. The test will be initiated when the participant places and holds his/her hand on an illuminated "home" button. At this point, a stimulus (light) will be presented randomly in one of five locations, parallel to the home button. Visual reaction time will be measured as the amount of time it takes to identify the stimulus (light) and initiate a reaction by taking their hand off the home button. Motor response time will be measured as the amount of time it takes to physically touch the stimulus (light) with their hand following the initial visual reaction and will be measured as the amount of time between the hand leaving the home button and touching the stimulus (light). Physical reaction time is a measurement of the total elapsed time from the introduction of the target stimulus (light) to the physical completion of the task (returning to the home button after touching the stimulus). All measures will be recorded to the 1/100's of a second. Participants will perform this assessment ten times. The average time for all ten assessments will be recorded.

Lower Body Reaction Measurements

Lower body reaction time will be measured using a 20-second reaction test on the Quick BoardTM (The Quick Board, LLC, Memphis, TN) reaction timer. Participants will stand on a board of five circles in a 2 x 1 x 2 pattern. Participants will straddle the middle circle and react to a visual stimulus located on a display box that depicts one of five potential lights corresponding with the circles on the board. Upon illumination of a light, the participant will attempt to move the dominant foot to the circle that corresponds to the visual stimulus. Upon a successful "hit" with the foot, the next stimulus appears. The total number of successful attempts during the 20-second test and the average time between the activation of the light and the response to the corresponding circle will be recorded.

• Multiple Object Tracking Assessments

Multiple object tracking will be assessed using a Cave Automatic Virtual Environment (CAVE) system. The CAVE consists of a 7 ft \times 7 ft \times 7 ft room that includes a canvas projection screen on the front wall which served as the surface for image projection. During each session, the participant will wear three dimensional glasses. A three-dimensional image of 8 tennis balls will be projected onto the front screen. The participant will track 4 of the 8 balls that move in three-dimensions. At the beginning of each trial, the 8 balls appear frozen on the screen for 2 seconds while half of them turned grey indicating the balls the participant is to track. After the 2 seconds, the balls all become the same color again and will begin to move in three dimensions. At the conclusion of the trial (8 seconds), the balls will freeze and a number will appear on each ball. The participant will call out the numbers of the four balls he/she was supposed to be tracking. Velocity of movement begins at a slow tracking speed and increases or decreases depending on whether the participant correctly identifies the 4 correct balls. Each participant will perform 20 trials per session. The velocity of movement that was most successful will be recorded.

Blood Measures

During each experimental trial, all blood samples will be drawn from one of the subjects forearm veins using a Teflon[™] cannula by personnel trained in phlebotomy with extensive experience in both research and clinical settings. A cannula is a hollow tube, which can be inserted into the opening of a vein and serve as a channel for the transport of fluid. The cannula prevents the need for multiple needle pricks from being performed. The risks associated with the placement of the cannula are not any different than that experienced by a normal blood draw using a needle and syringe. The cannula will be kept open following each blood draw with an infusion of a saline solution. This solution contains salt that is similar to the osmolarity of the blood and acts to minimize potential blood clotting within the cannula that may occur with prolonged use. The cannula placement will not interfere with the ability to perform the exercise routine. The first blood draw occurred at baseline (BL) prior to coffee consumption. Following coffee ingestion an additional blood draw will occur 30-min post-consumption (T30) and a third and fourth blood draw will occur before and following the 5K time trial (IP), respectively. Each participant's blood samples will be obtained at the same time of day during each session.

The total amount of blood drawn during each visit will not exceed 16ml. To put the volume of blood being drawn in proper perspective, one pint (475 ml) of blood is typically drawn when donating blood. To reduce the risk of dizziness and fainting from the blood draws, all blood draws will occur while participants are in a supine position. All blood draws will be conducted under sterile conditions and disposed appropriately. As an additional safeguard in preventing contamination new disposable gloves will be required for all blood draws. The discomforts associated with the blood drawing procedures are minimal, but sometimes bruising and infection may occur, and the arm might become sore. This soreness usually resolves in a few days. If it persists, the participant will be instructed to contact their personal physician.

All blood samples will be collected into a Vacutainer[®] tube containing sodium heparin. The sodium heparin tube will be kept chilled prior to each blood draw. The tube will then be subsequently centrifuged at $3,000 \times g$ for 15 min. The resulting plasma will be placed into a 1.8-mL microcentrifuge tubes and frozen at -80° C for later analysis. The resulting plasma will be aliquoted and stored at -80° C until analysis. Samples will be thawed only once for biochemical analysis at a later date. Blood test that will be run on subject's blood to determine the bioavailability of caffeine. Lactate and glucose levels will be measured to determine if Turkish coffee can alter substrate utilization during the 5k time trial.

• Data and specimen management

The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any person or party. All information attained from the medical and activity questionnaires or performance tests will be held in strict confidence. Individual results will remain confidential and only be relayed to the participant upon request. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. All information will be destroyed five years from the end of the study and not used for other research purposes. Participant folders and blood storage tubes will be marked with an I.D. number to protect against a breach of confidentiality. Participant names and I.D. numbers will be stored apart from the blood samples. Identifying information will be removed from the samples and destroyed when the samples are disposed of.

9) Withdrawal of Participants

Participants have the right to discontinue participation without penalty, regardless of the status of the study. Participants will also be instructed that their participation in the study may be terminated at any time by the researchers in charge of the project. This could be based on the participant's inability to follow supplementation protocol. This includes:

- Failure to adhere to requirements
- Failure to complete all 3 visits to the human performance lab
- The investigator or University of Central Florida IRB can also stop participation in this study at any time.

10) Risk to Participants

The risks associated with the blood draw may include some momentary pain at the time the needle is inserted into the vein, but other discomfort should be minimal. It is also possible for a bruise to develop at the site that the needle entered the skin or for individuals to report dizziness and possibly faint after the blood is drawn. It is also rare, but possible to develop minor infections and pain after the blood draw. To minimize the risks, the skin area where the needle or cannula is inserted will be cleaned and prepared with a disinfectant wipe before the needle or cannula is inserted. Needles and cannulas are sterile, gloves are worn by the person trained in obtaining blood. Following the blood draw the puncture site will be covered with a bandage. A cannula will be used to minimize the number of needle sticks. Subject may experience normal soreness that often accompanies a 5 km time trial.

11) Potential direct benefits to participation

There are no direct benefits to participation.

12) Adverse Events

In the event that an adverse event should occur, the University of Central Florida IRB will be informed immediately. Any necessary medical treatment will be handled as described below.

13) Provisions to protect the privacy interests of participants

Participant's privacy will be protected at all times. All phlebotomy procedures will be conducted in a private room. Participants will remain clothed during all blood draws.

14) Provisions to maintain the confidentiality of data

The results of this study will be published as part of a scientific publication. No individual results will be published or shared with any person or party. All information attained from the medical and activity questionnaires or performance tests will be held in strict confidence.

Individual test results will remain confidential and <u>only</u> be shared with the participant upon their request. Individual test results will not be shared with participants on a consistent basis. However, if blood tests are recognized as abnormal, the individual concerned will be referred to his primary physician for follow up. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. All information will be destroyed five years from the end of the study and not used for other research purposes. Plasma specimens will be stored and locked in the Human Performance Laboratory. Specimens will be stored until analysis. Participant folders and specimen storage tubes will be marked with an I.D. number to protect against a breach of confidentiality, and the ID number will be removed upon disposal.

15) Cost to participants

N/A

16) Consent process

A consent form will be read and verbal consent will be required for participation and enrollment. The consent process will be before the participant completes the PAR-Q and Confidential Medical and Activity questionnaire. The participant will be provided with the time necessary to read all documents. There will be an investigator available to explain the study's protocol and answer any questions that each potential participant may have.

17) Process to document consent in writing

Participants must understand and provide verbal consent prior to being considered enrolled in the study. Participants will receive a copy of the consent form for their records.

18) Vulnerable populations

N/A

19) Drugs or Devices

N/A

20) Multi-site Human Research

N/A

21) Sharing of results with participants

Individual test results will be shared with the participant <u>only</u> at their request. Test results will not be shared with participants on a consistent basis. In the event a blood test is recognized as abnormal, the individual will be informed and referred to their primary physician.

22) References

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APPENDIX E: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)



(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	YES NO 1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?						
		2.	Do you feel pain in your chest when you do physical a	activity?			
		3.	In the past month, have you had chest pain when you	were not doing physical activity?			
		4.	Do you lose your balance because of dizziness or do	you ever lose consciousness?			
		5.	Do you have a bone or joint problem (for example, ba change in your physical activity?	ick, knee or hip) that could be made worse by a			
		6.	ls your doctor currently prescribing drugs (for examp dition?	le, water pills) for your blood pressure or heart con-			
		7.	Do you know of <u>any other reason</u> why you should not	do physical activity?			
lf you	Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell						
answe	 You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice. Find out which community programs are safe and helpful for you. 						
If you ans • start b safest • take pa	 NO to all questions If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can: start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go. take part in a fitness appraisal – this is an excellent way to determine your basic fitness so 						
that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active. PLEASE NOTE: If your health changes so that you then answer YES t any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.							
	nformed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing his questionnaire, consult your doctor prior to physical activity.						

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME	
SIGNATURE	DATE
SIGNATURE OF PARENT	WITNESS

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Canadian Society for Exercise Physiology www.csep.ca/forms

APPENDIX F: MEDICAL AND ACTIVITY HISTORY QUESTIONNAIRE

Confidential Medical and Activity History Questionnaire

Pa	rticipant #		
W	hen was your last physical exa	mination?	
1.	List any medications, herba month:	ls or supplements you currently take or have taken th	e
	Medication	Reason for medication	

last

- 2. Are you allergic to any medications? If yes, please list medications and reaction.
- 3. Please list any allergies, including food allergies that you may have?

4. Have you ever been hospitalized? If yes, please explain.

Year of hospitalization	Reason

5. Illnesses and other Health Issues

List any chronic (long-term) illnesses that have caused you to seek medical care.

Have you ever had (or do you have now) any of the following. Please circle questions that you do not know the answer to.

Sickle cell anemia	yes	no
Cystic fibrosis	yes	no
Water retention problems	yes	no
Heart pacemaker	yes	no
Epilepsy	yes	no
Convulsions	yes	no
Dizziness/fainting/unconsciousness	yes	no
Asthma	yes	no
Shortness of breath	yes	no
Chronic respiratory disorder	yes	no
Chronic headaches	yes	no
Chronic cough	yes	no
Chronic sinus problem	yes	no
High blood pressure	yes	no
Heart murmur	yes	no
Heart attack	yes	no
High cholesterol	yes	no
Diabetes mellitus or insipidus	yes	no
Rheumatic fever	yes	no
Emphysema	yes	no
Bronchitis	yes	no
Hepatitis	yes	no
Kidney disease	yes	no
Bladder problems	yes	no
Tuberculosis (positive skin test)	yes	no
Yellow jaundice	yes	no
Auto immune deficiency	yes	no
Anemia	yes	no
Endotoxemia	yes	no
Thyroid problems	yes	no
Hyperprolactinemia	yes	no
Anorexia nervosa	yes	no
Bulimia	yes	no
Stomach/intestinal problems	yes	no
Arthritis	yes	no
Back pain	yes	no
Gout	yes	no
Hepatic encephalopathy	yes	no
Mania	yes	no
Hypermania	yes	no
Monosodium glutamate hypersensitivity	yes	no
Seizure disorders	yes	no

Any others (specify):_____

products?	yes	no
Do you have a history of drug or alcohol		
dependency?	yes	no
Do you ever have any pain in your chest?	yes	no
Are you ever bothered by racing of your heart?	yes	no
Do you ever notice abnormal or skipped heartbeat	s? yes	no
Do you ever have any arm or jaw discomfort, naus	sea,	
Or vomiting associated with cardiac symptoms?	yes	no
Do you ever have difficulty breathing?	yes	no
Do you ever experience shortness of breath?	yes	no
Do you ever become dizzy during exercise?	yes	no
Are you pregnant?	yes	no
Is there a chance that you may be pregnant?	yes	no
Have you ever had any tingling or numbness in		
your arms or legs?	yes	no
Has a member of your family or close relative		
died of heart problems or sudden death before		
the age of 50?	yes	no
Has a health care practitioner ever denied or		
restricted your participation in sports for any		
problem	yes	no
If yes, please explain:		

Are you presently taking any nutritional supplements or ergogenic aids? (if yes, please detail.

APPENDIX G: DIETARY RECALL

Dietary Recall Instructions

1. Use the forms provided to record everything you eat or drink on the days instructed.

2. Indicate the name of the FOOD ITEM, the AMOUNT eaten, how it was PREPARED (fried, boiled, etc.), and the TIME the food was eaten. If the item was a brand name product, please include the name. Try to be accurate about the amounts eaten. Measuring with measuring cups and spoons is best, but if you must make estimates, use the following guidelines:

- A fist is about 1 cup
- Tip of your thumb is about 1 teaspoon
- A thumb represents about 1 ounce of cheese
- A golf ball represents about 2 tablespoons
- The palm is about 3 ounces of meat (roughly the size of a deck of cards)

3. Try to maintain your normal diet, and be honest about what you eat. The information you provide is confidential.

4. Follow the specific instructions below when reporting foods:

• MILK – indicate % fat, source (e.g., cow, almond, coconut), and flavoring (if any).

• FRUITS & VEGETABLES – an average serving size of cooked or canned fruits and vegetables is ½ cup. Fresh, whole fruits and vegetables should be listed as small, medium, or large. Be sure to indicate sugar or syrup is added to fruit and list if any margarine, butter, cheese sauce, or cream sauce is added to vegetables. When recording salad, list items comprising the salad separately and be sure to include salad dressing used.

• EGGS – indicate whole or whites only, method of preparation (e.g., scrambled, fried, poached), and number eaten.

• MEAT/POULTRY/FISH – indicate approximate size or weight, in ounces, of the serving. Be sure to include any gravy, sauce, or breading added and preparation method.

• CHEESE – indicate kind, number of ounces or slices, and whether it is made from whole milk, part skim, or is low calorie.

• CEREAL – specify kind, brand, whether cooked or dry, and measure in terms of cups or ounces. *Consuming 8 oz. of cereal is not the same as consuming 1 cup of cereal. Be sure to include any milk consumed with cereal (see MILK).

• BREADS – specify kind (e.g., whole wheat, enriched wheat, white) and number of slices.

• BEVERAGES – include everything drink, excluding water. Be sure to record cream and sugar used in tea and coffee, whether juices are sweetened or unsweetened, and whether soft drinks are diet or regular.

• FATS - record any butter, margarine, oil, or other fats used in cooking or on food.

• PREPARED DISHES/CASSEROLES – list the main ingredients, approximate amount of each ingredient to the best of your ability, and brand (if applicable).

5. Express approximate measures in cups (c), tablespoons (T), teaspoons (t), grams (g), ounces (oz), pieces, etc.

6. If you are unsure of how to report any food items you consume, please take pictures of the packaging and Nutrition Facts panel, when possible.

Subject #:	
9	

Day 1

Meal	Time	FOOD/BEVERAGE DESCRIPTION	AMOUNT	Total kcal (from label)
Breakfast				
Bre				
ch				
Lunch				
Dinner				
Di				
Snacks				
S				

Subject #:	
0	

Day 2

Meal	Time	FOOD/BEVERAGE DESCRIPTION	AMOUNT	Total kcal (from label)
Breakfast				
Bree				
Lunch				
ır				
Dinner				
S				
Snacks				

APPENDIX H: DATA COLLECTION SHEET

VO2max Testing Worksheet

 SUBJECT #:
 DATE:

 AGE:
 WEIGHT
 (kg)

 HEIGHT
 (cm) Treadmill

Stage Speed/Incline	VO2	RER	HR	RPE
	102			M L

5K Time Trial Worksheet

SUBJECT #:	DATE:

AGE: _____ WEIGHT _____ (kg) HEIGHT _____ (cm) Treadmill _____

5K Time Trial & Metabolic Cart							
Time	HR	RPE	RER	VO2	VE		
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							

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