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THE REPRODUCIBILITY AND VALIDITY OF USING THE DMAX
METHOD TO PREDICT PHYSICAL WORKING CAPACITY AT
FATIGUE THRESHOLD

by

JOSHUA JAMES RIFFE
B.S. Indiana University of Pennsylvania, 2014

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
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ABSTRACT

Although the original (ORG) physical working capacity at fatigue threshold (PWC_{FT}) assessment has demonstrated strong reliability and sensitivity to training and/or nutritional interventions, limitations may exist regarding the method of PWC_{FT} estimation. However, a relatively new mathematical method, called Dmax, has been shown to be objective and reliable when estimating metabolic and neuromuscular fatigue thresholds. To date, however, no study has examined whether the Dmax method for estimating the work rate at PWC_{FT} is similar to the ORG method. **PURPOSE:** The purpose of this study is to evaluate the reliability and validity of the Dmax-EMG method for estimating and tracking changes in PWC_{FT} . **METHODS:** In the reproducibility study, 11 men (age: 21.9 ± 1.37 years; height: 175.6 ± 8.65 cm; body mass: 82.1 ± 13.92 kg) completed two incremental exercise tests (GXT) to exhaustion on nonconsecutive days. In the validity study, 11 active men (age: 23.4 ± 3.0 years; height: 177.9 ± 7.8 cm; body mass: 80.9 ± 10.7 kg) and 10 active women (age: 22.3 ± 3.1 years; height: 166.6 ± 9.5 cm; body mass: 62.8 ± 8.7 kg) volunteered to perform 12 sessions of high-intensity interval training (HIIT) over 4-weeks along with a pre- and post-training GXT to compare the Dmax to the ORG method of estimating PWC_{FT} . **RESULTS:** The reproducibility study revealed no significant differences between the first (181.5 ± 29.2 W) and second (181.9 ± 26.18 W) GXT trials ($p = 0.87$). In addition, $ICC_{3,1}$ resulted in 0.949 with an SEM of 6.28 W and a MD of 17.41 W. In the cross-validation, the CE between actual and predicted PWC_{FT} was not statistically different at pre (-6.7 W; $p > 0.05$) or post (-7.2 W; $p > 0.05$). Pre ($r = 0.87$) and post ($r = 0.84$) validity coefficients were considered very strong and the pre and post TE PWC_{FT} values were 30.8 W and 32.5 W, respectively. Wide limits of agreement were calculated in the Bland-Altman analyses (pre: -68.36 to 54.93 W; post: -57.77 to 72.06 W) with no significant biases in both pre (-6.7 ± 30.8 W;

$p = 0.330$) and post (-7.2 ± 32.5 W; $p = 0.325$). In addition, 95.2% and 100% participants fell within ± 1.96 standard deviations of the mean difference for pre and post, respectively.

CONCLUSION: Results of the study suggest that the Dmax method is both a reproducible and a valid method to estimate PWC_{FT} when compared to the ORG method in young men.

This thesis is dedicated to my brother, Jacob, my mother, Beverly, and my father, Kenneth for their love and support.

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LIST OF ACRONYMS/ABBREVIATIONS

AT – Anaerobic threshold

CE – Constant error

D_{max} – Maximal perpendicular distance

EMG – Electromyography

FT – Fatigue threshold

GXT – Graded exercise test

HIIT – High-intensity interval training

MD – Minimal difference

ORG – Original method

P_{PEAK} – Peak power

PWC_{FT} – Physical working capacity at fatigue threshold

RMS – Root mean square

SEE – Standard error of the estimate

SEM – Standard error of the measurement

TE – Total Error

TF – Time of onset of fatigue

VT – Ventilatory threshold

CHAPTER I: INTRODUCTION

Several decades ago, deVries et al. (1982) developed a test using electromyographic (EMG) fatigue curves from supramaximal, discontinuous work rates during cycle ergometry to determine a power output at fatigue threshold (FT) also known as EMG_{FT} . EMG_{FT} showed a strong relationship ($r = 0.90$) with anaerobic threshold (AT), as determined by gas exchange parameters suggested by Davis et al. (1976), and deVries et al. (1982) concluded that FT evaluation with EMG values may provide an attractive alternative to existing methods. However, deVries et al. (1982; 1987) indicated that these supramaximal workloads used to determine EMG_{FT} may not be appropriate for all populations and suggested using a greater number of submaximal incremental power loadings to estimate FT. Thereafter, deVries et al. (1987) developed a test utilizing discontinuous, submaximal incremental workloads and EMG fatigue curves to identify the power output corresponding to the onset of fatigue, which they termed physical working capacity at FT (PWC_{FT}). In other words, the PWC_{FT} represents the highest power output that results in a nonsignificant increase in the electrical activity of the thigh muscles over time. Subsequently, deVries et al. (1987; 1989) reported that the new, discontinuous, submaximal PWC_{FT} protocol produced very high reliability ($ICC = 0.976$) and was sensitive to exercise training adaptations in elderly individuals.

The submaximal PWC_{FT} protocol involves an incremental series of two-minute work bouts with rest intervals that allow participants' heart rate values to return to within 10 beats per minute of pre-exercise levels. While the discontinuous PWC_{FT} assessment has demonstrated strong reliability, validity and sensitivity, the test requires a significant time commitment (1.5 to 2.0 hours). Thus, deVries et al. (1990) developed a continuous PWC_{FT} test (ORG) that examined consecutive 2-minute EMG fatigue curves, and compared it to the discontinuous protocol in

young men and women. The results illustrated that the PWC_{FT} values between the discontinuous and continuous protocols were not significantly different ($p > 0.05$), and demonstrated a strong relationship ($r = 0.856$). Due to the time required to complete the continuous protocol being much lower (~30-minutes), deVries et al. (1990) concluded that this ORG protocol might be preferable to the original, discontinuous PWC_{FT} test. Since modifying the discontinuous PWC_{FT} test, the ORG PWC_{FT} test has demonstrated strong reliability ($ICC = 0.94 - 0.976$), validity (deVries et al., 1990), and sensitivity to changes in fitness level and/or nutritional interventions (Miramonti et al., 2015; Stout et al., 2007; Camic et al., 2010; Stout et al., 2000; Stout et al., 2006) in young men and women.

The potential limitation of the ORG method of determining PWC_{FT} is that unusable data has been reported in approximately 10% of subjects (Camic et al., 2010; Jenkins et al., 2014; Housh et al., 1996; deVries et al., 1989). Previous studies have stated that instances where no significant increase in EMG over time occurred, or where multiple significant (“false positive”) slopes were recorded during the incremental work bouts, therefore making it difficult to objectively identify the PWC_{FT} (deVries et al., 1989; Housh et al., 1996; Jenkins et al., 2014). Interestingly, common methods used to detect AT (both lactate and ventilatory) have also demonstrated similar limitations (no breakpoint detected) because of the unreliable behavior of the physiological variables (Cheng et al., 1992). Cheng et al. (1992) proposed another mathematical method, called D_{max} , to identify fatigue thresholds. In principle, the D_{max} method examines the linear regression line between physiological variables (lactate, ventilatory, EMG) measured at the start and end of an incremental exercise test, as well as a third-order polynomial regression line that represents the physiological response during the test. The point at which the maximal distance (D_{max}) measured perpendicularly from the linear regression line to

the polynomial curve is defined as the fatigue threshold. The Dmax method has been shown to be reliable (ICC= 0.77 – 0.93) and valid when estimating lactate and ventilatory thresholds (VT) during incremental cycle ergometry in men (Zhou and Weston, 1997; Cheng et al., 1992).

Bergstrom et al. (2011) were the first to report the use of the Dmax method in young men and women to determine the power output at the onset of neuromuscular fatigue (Dmax-EMG) and suggested that it demarcates the moderate and heavy exercise intensity domains during an incremental cycle ergometer test. Furthermore, Bergstrom et al. (2011) reported that the oxygen consumption rate at VT (2.38 ± 0.63 L·min⁻¹) was not significantly different than the oxygen consumption rate at Dmax-EMG (2.21 ± 0.48 L·min⁻¹) and that the two thresholds demonstrated a strong relationship ($r = 0.83$).

Recently, Miramonti et al. (2015) used the Dmax-EMG method to estimate the power output at the onset of neuromuscular fatigue, demonstrating its reliability (ICC = 0.95) and sensitivity to HIIT. The authors termed the calculated threshold PWC_{FT}; however, this classification was premature as the Dmax-EMG method has yet to be validated against the ORG PWC_{FT} method (deVries et al., 1989; deVries et al., 1990). Therefore, the purpose of this study is to evaluate the validity of the Dmax-EMG method for estimating and tracking changes in PWC_{FT}.

Purpose

1. The purpose of this study is to evaluate the reproducibility and validity of the Dmax-EMG method for tracking changes on the onset of neuromuscular fatigue.

Research Questions

1. Can the Dmax method be used as a valid and reliable method to estimate PWC_{FT}?

2. Will the sensitivity to HIIT be similar when comparing the Dmax and ORG methods?

Hypotheses

1. The Dmax method will be a reliable and valid alternative to estimate PWC_{FT} .
2. The Dmax method will reveal similar sensitivity to changes following HIIT as the ORG method.

Operational Definitions

1. High-Intensity Interval Training (HIIT) – Training comprised of short intervals of high intensity exercise interspersed with rest periods.
2. Physical Working Capacity at Neuromuscular Fatigue Threshold (PWC_{FT}) – The power output at the onset of neuromuscular fatigue, determined using either the Dmax or ORG methods.
3. Maximal Perpendicular Distance (Dmax) – A method for determining the point of inflection on a polynomial regression by finding the maximal perpendicular distance between the regression curve and a line between the first and last points used in the regression.

Delimitations

1. Participants were healthy and free of disease and injury
2. Currently active (2-5 days of exercise per week)
3. No exercise 24-hours prior to either trial
4. Free from nutritional supplementation
5. Experience participating in a maximal exercise protocol
6. Participants were asked to maintain their normal diet while enrolled in the study
7. Approval from the New England Institutional Review Board

Assumptions

1. Participants answered all questionnaires truthfully.
2. Participants were not consuming nutritional supplements
3. A maximal effort was given during each graded exercise test.
4. No exercise occurred 24-hours before the pre- and post-training assessments.

Limitations

1. The sample was made up of volunteers, therefore, not meeting the underlying assumptions of random selection.
2. Participants were recruited from the University of Central Florida only.
3. The non-EMG leg may have overcompensated the EMG leg during assessment.
4. No dietary analysis to reveal differences in the participant's diets.

CHAPTER II: REVIEW OF LITERATURE

Theory of Neuromuscular Fatigue

deVries (1968)

Method for evaluation of muscle fatigue and endurance from electromyographic fatigue curves

One of the purposes of this study was to investigate the relationship between the work rate and electrical activity of muscle as a function of time in electromyography (EMG) fatigue curves from continuous isometric contractions at different percentages of maximal voluntary contractions (MVC). Fifteen college-age participants established their right elbow flexor MVC using a dynamometer gauge with an electrode placed over the belly of the biceps brachii that bisected the anterior axillary fold and the center of the cubital fossa. Appropriate percentages of each participant's MVC were then loaded (closest 5 pounds) as the subject attempted to support this weight continuously for their maximum endurance time. Integrals of electrical activity were recorded every 10-seconds. Participants were then tested once a week for three weeks at 60, 50, 40, and 30% MVC. Test-retest reliability of the method was found to be 0.934. The results of the study showed that EMG fatigue from the two different contractions increased EMG activity as time increased.

Effects of High-Intensity Interval Training

Helgerud, Høydal, Wang, Karlsen, Berg, Bjerkaas, Simonsen, Helgesen, Hjorth, Bach, and Hoff (2007)

Aerobic High-Intensity Intervals Improve $\dot{V}O_{2\max}$ More Than Moderate Training

The purpose of the study was to compare the effects of aerobic endurance training at different intensities and methods on stroke volume (SV), blood volume (BV), lactate threshold (LT), and running economy (RE). Forty healthy, nonsmoking, moderately-trained men were randomly assigned to one of four groups: 1) long slow distance (LSD) ran for 45-minutes at 70% max heart rate (MHR); 2) lactate threshold group ran for 24.25-minutes at 85% MHR; 3) an interval group that performed 47 trials of 15-seconds intervals at 90-95% MHR with 15-seconds of active rest of 70% MHR between trials; 4) a 4x4 interval running group where participants ran for 4-minutes at 90-95% MHR with 3-minutes of active rest (70% MHR) between each run. All groups were matched for total work and frequency that trained 3 days per week for 8-weeks. Results of the study showed both interval training groups significantly increased their $\dot{V}O_{2\max}$ while the other two groups did not with the 4x4 interval group improving slightly more than the 15-second interval group. These increases can be credited from a 10% increase in SV that increased cardiac output, which concludes that higher aerobic intensities leads to larger improvements in $\dot{V}O_{2\max}$. RE significantly improved in all groups with no differences between them illustrating that RE is not velocity specific when intensities are between 70 and 95% $\dot{V}O_{2\max}$. More importantly, SV significantly increased in the high-intensity interval (HIIT) groups. Thus, authors suggested that SV is vital when seeking cardiovascular gains via HIIT. Thus, the main finding is that HIIT is a more effective means of improving $\dot{V}O_{2\max}$ than training at the LT or continuous exercise.

Robinson IV, Stout, Miramonti, Fukuda, Wang, Townsend, Mangine, Fragala, and Hoffman (2014)

High-intensity interval training and β -hydroxy- β -methylbutyric free acid improves aerobic power and metabolic thresholds

The aim of this study was to study the effects of cycle ergometer high-intensity interval training (HIIT) and supplementation of 3 grams per day of HMB_{FA} on maximal oxygen consumption ($\dot{V}O_{2PEAK}$), ventilatory threshold (VT), respiratory compensation point (RCP), time to exhaustion (T_{MAX}), and peak power (P_{PEAK}). Thirty-four college-aged men and women were randomly assigned to control to one of the HIIT groups: placebo (PLA-HIIT) or the HMB_{FA} supplemented (HMB_{FA}-HIIT). Participants completed a baseline graded exercise test to determine $\dot{V}O_{2PEAK}$, VT, RCP, and T_{MAX} . The supplemented groups then completed 12 HIIT sessions consisting of 5-6 intervals at a 2:1 work to rest ratio. Intensities ranged from 85-120% of P_{PEAK} over the 4-week span and the control group was asked to maintain current diet and exercise habits. Both HIIT groups significantly increased in T_{MAX} and RCP compared to control. However, HMB_{FA}-HIIT saw a significant increase in $\dot{V}O_{2PEAK}$ and VT compared to the control and PLA-HIIT groups with no significant differences between training volumes between the HIIT groups. Results of the study demonstrate that HMB_{FA} may enhance some of the training adaptations related with HIIT. The authors suggest that these enhanced adaptations are derived from increases in mitochondrial biogenesis, fat oxidation, and improvements via metabolic processes that are regulated by adenosine monophosphate kinase and sirtuin activity in sarcomeres. To conclude, HIIT in conjunction with HMB_{FA} elicits greater changes in $\dot{V}O_{2PEAK}$ and VT than only HIIT.

Examining Neuromuscular Fatigue Thresholds

deVries, Tichy, Housh, Smyth, Tichy, and Housh (1987)

A method for estimating physical working capacity at the fatigue threshold (PWC_{FT})

The purpose of the study was to assess a submaximal protocol determine the highest power output that does not produce increases in EMG amplitude over time. This submaximal protocol was assessed in place of EMG_{FT}, which used supramaximal power outputs, but may not be suitable for certain populations. EMG signals were recorded for 10-second integrals every 20-seconds for each 2-minute stage before power output increased until a stage resulted in a significant increase in average RMS. Test-retest reliability was 0.947 with no significant difference between trials that showed the reliability of the submaximal protocol. In addition, the authors correlated PWC_{FT} with onset of blood lactate accumulation (OBLA), percent of heart rate range (%HRR), heart rate-workload relation (HR-WL), and critical power (CP). Results of the study demonstrate the PWC_{FT} test as a reliable as well as valid (0.67) alternative test to the supramaximal EMG_{FT} test, which suggests to have implications for populations that cannot sustain supramaximal intensity levels.

deVries, Housh, Johnson, Evans, Tharp, Housh, and Hughes (1990)

Factors affecting the estimation of physical working capacity at the fatigue threshold

The purpose of the study was to investigate several potential improvement in the methodology related with the physical working capacity at fatigue threshold (PWC_{FT}) test that encompassed: a continuous protocol in place of the original discontinuous protocol, use of a treadmill instead of a cycle ergometer, using a bipolar EMG lead system in place of the unipolar system, and the potential for residual fatigue from trials participated 24-hours apart. No significance differences were seen between results of the continuous and discontinuous procedures. The treadmill tested used a bipolar lead system to minimize the excess noise and

movement that are related to treadmill protocols. However, the bipolar system yielded smaller voltages when compared to the unipolar system at any given power output. There was a moderate correlation ($r = 0.60$) between the PWC_{FT} results of the unipolar and bipolar lead systems. When trials were completed 24-hours apart a correlation of $r = 0.812$ was seen, which indicates the PWC_{FT} test does not elicit residual fatigue. Authors of the study conclude that the continuous cycle ergometer test was reliable and valid to estimate PWC_{FT} , but further work was needed to validate the treadmill-based test.

Matsumoto, Ito, and Moritani (1991)

The relationship between anaerobic threshold and electromyographic fatigue threshold in college women

The purpose of the study was to validate the integrated electromyography (iEMG) estimation of neuromuscular fatigue threshold (EMG_{FT}) using the fatigue curves during several maximal 1 minute exercise periods on an electronically braked cycle ergometer and to determine the association between anaerobic threshold (AT) and EMG_{FT} in order to assess the highest exercise intensity that can be sustained without eliciting neuromuscular fatigue. Twenty young, female subjects volunteered to participate in the study whose fitness levels ranged from highly trained athletes to sedentary individuals. Bipolar surface electrodes were applied to the lateral area of the dominant quadriceps femoris muscle and the reference electrode was placed over the iliac crest. To assess EMG_{FT} , a 5-minute warmup of 50 W preceded the exercise tests that typically consisted of randomized power loads of 350, 300, 250, and 200 W, but some tests were altered based on subject fitness level. Rest between trials was no less than 15-minutes so allow heart rate to return to 5 bpm of resting values. To evaluate the relationship between AT and EMG_{FT} , participants used the same cycle ergometer as the EMG_{FT} test. After a 2-minute warmup

of 30 W, the $\dot{V}O_{2\text{MAX}}$ test begun with intensity being increased 30 W every minute until exhaustion was reached. The results of the study established a high correlation between AT and EMG_{FT} and that the magnitude of this correlation suggested approximately 67.7% of AT variance could be explained by EMG_{FT} , which suggests that iEMG can serve as a predictable estimation of EMG_{FT} .

Moritani, Takaishi, and Matsumoto (1993)

Determination of maximal power output at neuromuscular fatigue threshold

The purpose of this study was to determine the relationship between the anaerobic threshold (AT) and the maximal power output at neuromuscular fatigue threshold (EMG_{FT}), as estimated from EMG data from representative leg muscles during cycling. Twenty male and female college-aged students volunteered for the experiment. All subjects underwent a familiarization session of the equipment and experimental procedures before the first testing session. Subjects underwent a 5-minute warmup on a cycle ergometer at 50 W before starting the exercise tests. The exercise test intensities were randomized with loads usually 400, 350, 300, and 275 W for males and 350, 300, 250, and 200 W for females but were also subjective to change based on subject fitness levels. Each workload lasted 2-minutes to minimize error due to muscle temperature and to avoid accumulation of muscle fatigue. Rest periods of at least 25-minutes were given to allow heart rate to return within 5 beat of resting heart rate. Surface electrodes in a bipolar lead system were used on the lateral portion of the dominant quadriceps femoris muscle and spaced 4-cm apart to increase the sampling volume over the large muscle, while the reference electrode was placed over the iliac crest. EMG_{FT} values were determined by finding the rate of increase of the amplitude across each bout (slope), and plotting the slope coefficients against power output in order to estimate the y-intercept of a regression line derived

from the slope coefficients. The y-intercept of the slope signifies the power output that, in theory, results in no increase in EMG across an exercise bout, which reflects the AT. Authors reported that EMG_{FT} was highly correlated with AT ($r = 0.92$). The authors also reported that EMG_{FT} may be more associated with lactate steady state after finding that $\dot{V}O_2$ was significantly higher at EMG_{FT} compared to AT.

Pavlat, Housh, Johnson, Schmidt, and Eckerson (1993)

An examination of the electromyographic fatigue threshold

The purpose of this study was to compare times to exhaustion at various percentages of the EMG_{FT} . Eight male volunteers completed two initial EMG_{FT} tests on a cycle ergometer. After a 5-minute warmup of 50 W, participants performed four 1-minute, randomly ordered work bouts that were dependent on fitness level, which were determined by their physical activity history. Rest periods between each work bout were implemented to allow heart rate to return to within 5 beats of resting heart rate but did not last longer than 15-minutes. EMG_{FT} was calculated by averaging the power output of the highest non-significant slope and the power output of the lowest significant slope. Participants returned to the lab for five more visits on separate days to perform randomly ordered work bouts at power outputs equal to 85%, 100%, 115%, 130%, and 145% of EMG_{FT} to determine times to exhaustion at these various percentages. After a 5-minute warmup at 50 W and a 2-minute rest period, participants exercised at the selected power output at a pedal rate of 70 revolutions per minute (rpm). The test concluded once 60 rpm were unable to be maintained and time to exhaustion for each work bout was rounded to the nearest 0.1s via an electronic stopwatch. Surface electrodes were placed approximately 50 mm apart on the vastus lateralis muscle of the dominant leg while the reference electrode was placed on the iliac crest. Results of the study showed that EMG_{FT} power

curve analysis significantly over predicted the estimated power output of 30- and 60-minutes by 42% and 52%, respectively. Therefore, the authors were unable to validate a non-fatiguing power output by using the EMG_{FT} test, which coincides with findings from Housh et al. (1991).

Takaishi, Yasuda, and Moritani (1994)

Neuromuscular fatigue during prolonged pedaling exercise at different pedaling rates

The purpose of the study was to estimate the differences in neuromuscular fatigue among prolonged pedaling exercises performed at different pedaling rates at a given intensity that is determined by integrated electromyographic (iEMG) voltage-time relationship. Eight males who did not currently participate in cycling volunteered to perform a ramp protocol exercise test to exhaustion as well as five sessions of extended pedaling exercise. During the five sessions, subjects pedaled at a random cadence [40, 50, 60, 70, and 80 revolutions per minute (RPM)] for each session on separate days. However, three of the eight subjects exercised twice in one day with three hours of rest between each session. During the five sessions, iEMG electrodes were placed over the belly of the vastus lateralis muscle and a reference electrode over the anterior superior spine of the iliac crest in order to quantify the electrical activity of the muscle. Results of the study showed that neuromuscular fatigue estimated by the iEMG was coincident with higher pedaling rates of subjects. This may be due to that neuromuscular fatigue occurred sooner in the lower pedaling rates compared to the higher pedaling rates. Further, these results indicate that subjects preferred to pedal at higher percentages of $\dot{V}O_{2MAX}$ than those pedaling rates that produced fatigue sooner.

Housh, deVries, Johnson, and Evans (1995)

An evaluation of the physical working capacity at the fatigue threshold

The purpose of the presentation was to evaluate the time to exhaustion and integrated electromyographic (iEMG) patterns for continuous work bouts at power loadings below PWC_{FT} . Twelve adult males volunteered to perform a PWC_{FT} test and cycle at 80%, 85%, 90%, and 95% of PWC_{FT} for 30-minutes or until exhaustion occurred. Results showed that 3 subjects were unable to complete any of the power loadings for 30-minutes and 2 additional subjects failed to complete the 90% and 95% power loading trials. Thus, the PWC_{FT} test overestimated the resistance that could be maintained for 30-minutes with inducing exhaustion.

Pavlat, Housh, Johnson, and Eckerson (1995)

Electromyographic responses at the neuromuscular fatigue threshold

The purposes of this investigation were to examine the electromyographic (EMG) responses from the vastus lateralis during a continuous ride to exhaustion at the neuromuscular fatigue threshold (EMG_{FT}) and to determine what percentage of the power output at $\dot{V}O_{2MAX}$ the EMG_{FT} represents. Fifteen males volunteered to visit the laboratory on two occasions to determine EMG_{FT} and then to complete a continuous cycle ergometer assessment until exhaustion at a power output equal to EMG_{FT} . EMG electrodes were placed 5 cm apart on the lateral portion of the vastus lateralis muscle of the dominant leg. The reference electrode was placed on the iliac crest. Subjects performed four randomly ordered 1-minute workbouts at power outputs that ranged from 275 – 415 W that was dependent upon each subject's fitness level. Between workbouts, a rest period of at least 15-minutes to allow heart rate to return to 5 bpm of resting heart rate was given to allow sufficient recovery. Calculation of EMG_{FT} followed the mathematical model of deVries et al. (1987). Results of the study showed that EMG_{FT} could only be maintained for 3.75-minutes (mean), which is inconsistent with previous research that stated EMG_{FT} could be maintained for 20-minutes. In addition, a subsample of 10 subjects'

EMG_{FT} values represented approximately 105% of $\dot{V}O_{2MAX}$. Furthermore, the study showed that the EMG_{FT} assessment overestimates “the highest exercise intensity sustainable without electromyographic evidence of neuromuscular fatigue”.

Housh, deVries, Johnson, Evans, Housh, Stout, Bradway, and Evetovich (1996)

Neuromuscular fatigue thresholds of the vastus lateralis, vastus medialis and rectus femoris muscles

The purpose of the study was to compare power outputs at the onset of neuromuscular fatigue from the vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) during a physical working capacity at fatigue threshold (PWC_{FT}) test. Eleven adult males volunteered for the study with none of them being aerobically trained or regular bicyclists. Electromyographic (EMG) signals were recorded from a bipolar surface (2.54 cm center-to-center) electrode arrangement on the dominant thigh to measure voltages from the VL, VM and RF muscles. Electrodes for the VL were placed on the lateral portion of the thigh at the head of the greater trochanter and lateral condyle of the femur. VM electrodes were placed at 20% of the distance between the medial gap of the knee joint and the anterior superior spine of the pelvis. To ensure the electrodes for the RF did not overlap the sartorius muscle, they were placed 50% of the distance between the inguinal crease and the superior border of the patella. The center of the distal electrode was placed 2.54 cm below the center of the proximal electrode. After electrodes were placed at their respective locations, subjects completed a continuous, incremental PWC_{FT} assessment until volitional fatigue was reached. PWC_{FT} calculated for each muscle group. PWC_{FT} was calculated from the continuous protocol of deVries et al. (1990). Results of the study showed no differences in mean PWC_{FT} estimates for each muscle group. Although some

intersubjective differences were apparent, the superficial muscles of the quadriceps generally responded as a unit at the onset of neuromuscular fatigue.

Housh, Perry, Bull, Johnson, Ebersole, Housh, and deVries (2000)

Mechanomyographic and electromyographic responses during submaximal cycle ergometry

The intent of this investigation was to examine the mechanomyographic (MMG) and electromyographic (EMG) responses during continuous, cycle ergometer work bouts performed at constant power outputs. Eight adults volunteered to complete an incremental test to exhaustion while MMG and EMG signals were recorded in 30-second intervals with 15-seconds between recordings. EMG signals were recorded from a bipolar surface (7.62 cm center-to-center) electrode arrangement on the dominant thigh over the vastus lateralis and vastus medialis. The electrodes were placed halfway between the greater trochanter and the lateral condyle of the femur for the vastus lateralis while the electrodes for the vastus medialis were placed 20% of the distance between the medial gap of the knee joint and the anterior superior spine of the pelvis. A reference electrode was placed over the iliac crest. Results showed that EMG signals increased with time and with each power output. This may be related to peripheral, low-frequency fatigue, which is an increased activation that is required to achieve a given force in the presence of excitation-contraction coupling failure.

Perry, Housh, Weir, Johnson, Bull, and Ebersole (2001)

Mean power frequency and amplitude of the mechanomyographic and electromyographic signals during incremental cycle ergometry

The purpose of this study was to determine the relationships for mechanomyographic (MMG) amplitude, MMG mean power frequency (MPF), electromyographic (EMG) amplitude, and EMG MPF versus power output during incremental cycle ergometry. Seventeen adults (12 males, 4 females) participated and completed a continuous, incremental cycle ergometer test until exhaustion. The test began at 50 W and increased 30 W every 2-minutes while subjects kept a pedal cadence of 70 rpm. EMG and MMG samples were collected at the end of each 2-minute stage. EMG signals were identified via a bipolar surface electrode arrangement (7.62 center-to-center) on the vastus lateralis, midway between the greater trochanter and the lateral condyle of the femur while the reference electrode was placed over the iliac crest. This large distance between electrodes was chosen due to the piezoelectric crystal contact sensor, used to detect MMG, being placed between the two electrodes on the vastus lateralis. Results of the study showed a nonlinear increase in EMG amplitude whereas MMG amplitude was shown to increase linearly during the incremental test. This suggests that MMG may more closely reflect changes in power output compared to EMG that may be due to the recruitment of fast-twitch muscle fibers as power output increases, which have greater action potential amplitudes and higher firing rates compared to slow-twitch fibers.

Graef, Smith, Kendall, Walter, Moon, Lockwood, Beck, Cramer, and Stout (2008)

The relationships among endurance performance measures as estimated from $\dot{V}O_{2PEAK}$, ventilatory threshold, and electromyographic fatigue threshold: a relationship design

The purpose of the study was to investigate the metabolic relationship between $\dot{V}O_{2PEAK}$, ventilator threshold (VT), and the electromyographic fatigue threshold (EMG_{FT}) in addition to comparing the power output at $\dot{V}O_{2PEAK}$, VT, and EMG_{FT} . Participants included thirty-eight recreationally trained (1-5 hours/week), college-aged men. Participants performed a continuous

graded exercise test (GXT) on an electrically-braked cycle ergometer to determine $\dot{V}O_{2PEAK}$ and ventilatory threshold (VT). Electromyography (EMG) recordings were taken with a pre-gelled bipolar (2.54 cm center-to-center) surface electrodes that were placed over the lateral portion of the vastus lateralis muscle, halfway between the greater trochanter and the lateral condyle of the femur. Participants returned to the lab 24-48 hours later to perform the EMG_{FT} assessment. Each participant completed four 2-minute cycling bouts at incrementally ascending workloads (75 – 300 W). The initial bout began at the power output that VT ensued during the GXT. Rest between bouts was given so that heart rate returned to within 10 beats of their resting heart rate. EMG slopes for each stage were plotted over 120-seconds and the line of best fit was extrapolated to the y-axis to calculate EMG_{FT} . Results of the study showed that the EMG_{FT} suggests to be an attractive alternative when measuring VT from gas analysis due to the submaximal workloads as well as the elimination of participant motivation.

Camici, Housh, Johnson, Hendrix, Zuniga, Mielke, and Schmidt (2010)

An EMG frequency-based test for estimating the neuromuscular fatigue threshold during cycle ergometry

The purposes of this study were to determine if a mathematical model could be applied to the EMG amplitude domain in order to develop a new threshold called the mean power frequency fatigue threshold (MPF_{FT}) and to compare the power outputs associated with physical working capacity at fatigue threshold (PWC_{FT}), MPF_{FT} , ventilatory threshold (VT), and respiratory compensation point (RCP). Sixteen males volunteered to participate in the study and were untrained in aerobic exercise and participated in less than 4-hours per week of recreational physical activity. Measurements were obtained through two different tests: 1) a $\dot{V}O_{2MAX}$ test to attain VT and RCP using the V-slope method of Beaver et al. (1986) and 2) an incremental cycle

ergometer test (deVries et al., 1987) to determine PWC_{FT} and MPF_{FT} . Bipolar surface electrodes were recorded from the dominant leg over the vastus lateralis muscle. Results of the study indicate that the PWC_{FT} model could be applied to the frequency domain in order to calculate MPF_{FT} . In addition, the authors propose that PWC_{FT} may demarcate the moderate from heavy exercise domains while the MPF_{FT} demarcates heavy from severe exercise intensities.

Guffey, Gervasi, Maes, and Malek (2012)

Estimating electromyographic and heart rate fatigue thresholds from a single treadmill test

The purpose of this study was twofold; 1) to develop a fatigue threshold based electromyography (EMG) and heart rate (HR) responses for treadmill running from a single incremental test and 2) to propose a new fatigue threshold called the $RVEMG_{FT}$ and $RVHR_{FT}$. Eleven college-aged men volunteered for the study and were familiar with running on a treadmill. Each subject performed an incremental test on a motorized treadmill to exhaustion. Following a standardized warmup, the test started at 6.44 km/h and 0% grade with velocity increasing 1.61 km/h every 2-minutes to 14.49 km/h. At 14.49 km/h, exercise intensity was increased by 2% grade every 2-minutes. Two bipolar surface electrodes (20 mm center-to-center) were placed over the longitudinal axis of the vastus lateralis with the reference electrode being placed over the iliac crest. To determine $RVEMG_{FT}$ and $RVHR_{FT}$, six 10-second EMG amplitude segments were analyzed during each 2-minute stage and plotted vs time. A regression with then plotted with respect to the EMG amplitude across time to determine if the slope significantly increased. $RVEMG_{FT}$ and $RVHR_{FT}$ were defined as the average of the highest velocity that resulted in a nonsignificant slope coefficient and the lowest velocity that resulted in a significant positive slope coefficient. Results of the study showed that the fatigue threshold for EMG amplitude and heart rate can be determined from a single incremental treadmill test. These two

thresholds, however, provided significantly different velocities that may reflect the difference between peripheral and central fatigue.

Bergstrom, Housh, Cochrane, Kenkins, Lewis Jr, Traylor, Zuniga, Schmidt, Johnson, and Cramer (2013)

An examination of neuromuscular and metabolic fatigue thresholds

The purposes of this study were 1) to compare physical working capacity at the fatigue threshold (PWC_{FT}), gas exchange threshold (GET), respiratory compensation point (RCP), critical power (CP), power outputs associated with the gas exchange threshold (P_{GET}) and respiratory compensation point (PRCP), $\dot{V}O_{2PEAK}$, and peak power and 2) to propose potential mechanisms that trigger the onset of neuromuscular fatigue. Six men and four women, who were moderately trained (aerobic activity for a minimum of 30-minutes, 5 times per week), recreational athletes, volunteered for the study. Participants initially completed a graded exercise test until exhaustion in order to calculate gas exchange ratios, and PWC_{FT} . Each subject was fitted with bipolar surface EMG electrodes (30-mm interelectrode distance) on the vastus lateralis muscle of the right thigh while the reference electrode was placed over the iliac crest. CP was determined by a 3-minute all-out test of a cycle ergometer. Upon completing a 5-minute warmup of 50 W followed by 5-minutes of rest, the test began with no resistance at 70 revolutions per minute (rpm) for 3-minutes. Participants then increased pedal cadence to 110 rpm with resistance being determined by the linear mode of the electronically braked cycle ergometer (linear factor = power/preferred cadence²). 70 rpm was selected as the preferred cadence, as shown by Marsh and Martin (1997), of untrained cyclists. Linear factor was calculated as the power output halfway between $\dot{V}O_{2PEAK}$ and GET ($GET + 50\% \Delta$) divided by 70 rpm squared. CP was the average power output of the final 30-seconds of the test. Results of the study showed

that PWC_{FT} was 17% greater than $PGET$ but was highly correlated which coincides with Zugina et al. (2010), which indicates that PWC_{FT} overestimated the $PGET$ and, thus, the demarcation of the moderate and heavy exercise intensity domains. Differences in the physiological mechanisms as well as the protocol used to determine PWC_{FT} may be underlying reasons for the overestimated values. In addition, the changes in pH associated with VCO_2 and $PGET$ attenuated the effects of increased interstitial potassium on EMG amplitude that resulted in greater PWC_{FT} values compared to the $PGET$.

Nutritional/Training Interventions on Neuromuscular Fatigue

deVries, Brodowicz, Robertson, Svoboda, Schendel, Tichy, and Tichy (1989)

Estimating physical working capacity and training changes in the elderly at the fatigue threshold (PWC_{FT})

The purpose of this study was to determine the feasibility of the PWC_{FT} test in elderly populations, the sensitivity of the test to training adaptations, and the minimal intensity that is appropriate in prescribing exercise for the elderly. Forty-seven participants were recruited for the study, but 21 of the 47 provided unsatisfactory data in the pre- or post-test. Unsatisfactory data was defined by the authors as no EMG evidence of neuromuscular fatigue (no significantly slope coefficient) and those subjects that could not maintain the required pedal cadence. Therefore, twenty-seven healthy, elderly participants were used for data analysis. Subjects completed a PWC_{FT} pre- and post-test. Between assessments, participants completed either a low ($n = 10$) or high intensity ($n = 7$) exercise program three days per week for ten weeks on a stationary cycle ergometer. Data analysis of pre-test values determined no significances amongst the three groups, indicating that all groups appeared to be similar. Results showed 29.8% and 38.4%

improvements in PWC_{FT} values in the low- and high-intensity groups, respectively, and no significant difference between groups. The results of the study indicate the PWC_{FT} test as an appropriate fitness test in an elderly population.

Housh, deVries, Johnson, Evans, and McDowell (1991)

The effect of ammonium chloride and sodium bicarbonate ingestion on the physical working capacity at the fatigue threshold

The purpose of the study was to examine the effect of ammonium chloride (NH_4Cl) and sodium bicarbonate ($NaHCO_3$) ingestion on the physical working capacity at the fatigue threshold (PWC_{FT}). Two experiments were conducted that used nine adult males in each experiment. In the first experiment, subjects ingested capsules of NH_4Cl and $NaHCO_3$ that contained a total of 0.3 g·kg⁻¹ bodyweight with equal doses taken every 15-minutes over a 3-hour period and in a random order prior to exercise testing. Testing days were separated by 72-hours. After administration of the capsules, a discontinuous PWC_{FT} assessment was performed while electromyographic recording were taken from the vastus lateralis muscle with a unipolar lead arrangement. In experiment two, the subjects' ingestion protocol of NH_4Cl and $NaHCO_3$ were identical to experiment one. However, the substances were consumed in tablets for experiment two and used the continuous PWC_{FT} protocol. Results of the study indicate that NH_4Cl and $NaHCO_3$ had no effect on PWC_{FT} . Thus, the authors indicate that the manipulation of blood pH does not affect the threshold parameters from respiratory, blood lactate, or EMG measurements.

Stout, Eckerson, Ebersole, Moore, Perry, Housh, Bull, Cramer, and Batheja (2000)

Effect of creatine loading on neuromuscular fatigue threshold

The purpose of the study was to determine if creatine (Cr) loading effected on the onset of neuromuscular fatigue by monitoring electromyography curves from the vastus lateralis muscle using the physical working capacity at the fatigue threshold (PWC_{FT}) assessment in female athletes. Fifteen collegiate, female university crew members volunteered as subjects and were not taking Cr or any other dietary supplements for a minimum of 12-weeks prior to the investigation. During the study, subjects were asked to refrain from dietary supplements, nonprescription drugs, and caffeine. Participants completed two PWC_{FT} assessments, one prior to the supplementation period and one following. Following the initial PWC_{FT} test, participants were assigned to one of two groups using a double-blind design: 1) 20.0g of flavored dextrose powder as a placebo (Pl, n = 8) or 2) 5.0g of Cr monohydrate plus 20.0g of flavored dextrose in a flavored powder blend (Cr, n = 7). The powders were dissolved in the same amount of water and ingested four times per day for 5 consecutive days before post-testing. The PWC_{FT} post-testing assessment followed the same protocol as the pre-testing test. During both assessments, a bipolar electrode arrangement (2.54 cm center-to-center) was placed on the vastus lateralis of the right thigh, midway between the greater trochanter and lateral condyle of the femur with a reference electrode placed over the iliac crest. Results of the study showed Cr loading resulted in a significantly higher PWC_{FT} value when compared to the placebo group, demonstrating that Cr may delay the onset of neuromuscular fatigue in female athletes. Authors of the study attributed this finding to the possibility that augmented phosphocreatine levels in the muscle may have resulted in delaying anaerobic glycolysis.

Stout, Graves, Cramer, Goldstein, Costa, Smith, and Walter (2007)

Effects of creatine supplementation on the onset of neuromuscular fatigue threshold and muscle strength in elderly men and women (64 – 86 years)

The purpose of this study was to observe the effects of creatine (Cr) supplementation on the physical working capacity at the fatigue threshold (PWC_{FT}) and upper and lower body strength in men and women 64 to 86 years of age. Fifteen elderly, untrained men and women were assigned to the Cr group or the placebo group with each dose consisting of 5 grams. Each participant underwent two supplementation phases: 4 doses per day for 7 days, then two doses per day for 7 days with a 4-6 week washout period between phases. Subjects were asked to refrain from starting additional supplementation and to maintain their normal physical activity and diet patterns. Prior to and following the supplementation phases, subjects performed a discontinuous PWC_{FT} protocol on an electrically braked cycle ergometer, maximal isometric handgrip strength, and 30-second sit-to-stand assessments to evaluate upper and lower body strength. PWC_{FT} values were determined using EMG amplitude values from the vastus lateralis from the methods previously described by deVries et al. (1987). Results of the study showed that the Cr group saw a significant and large (15.6%) increase in PWC_{FT} values, which illustrates the delay that Cr supplementation has on the onset of neuromuscular fatigue that may be attributed to increased muscle creatine-phosphate (PCr). This increased muscle PCr has been previously purported to decrease reliance on anaerobic glycolysis, reduce the accumulation of metabolites associated with high intensity exercise (Volek et al., 1999; Volek and Kraemer, 1996; Bishop, Edge, and Goodman, 2004). The upper and lower body strength increases seen from this study may be due to the increase of fat-free mass or in increased rate of PCr resynthesized between trials. To conclude, it appears that 14 days of supplementing with Cr may increase upper-body grip strength and improve PWC_{FT} in elderly men and women. These implications suggest that Cr may be important for maintaining health and an independent lifestyle in the elderly population.

The effects of beta-alanine supplementation and high-intensity interval training on neuromuscular fatigue and muscle function

The purpose of this study was to examine the effects of beta-alanine supplementation in combination with high-intensity interval training (HIIT) on neuromuscular fatigue as measured by EMG_{FT} and electrical efficiency activity of the working muscle. Forty-six recreationally trained males volunteered to participate in the study. Subjects completed three EMG_{FT} tests as previously described by deVries et al. (1987) prior to, at the midpoint, and following two 3-week training periods. In addition to calculating EMG_{FT} from the incremental test, efficiency of electrical activity (EEA), which represents the functional state of a muscle and coincides with the change in electrical activity that accompanies an increase in workload, was analyzed using the bipolar surface electrodes. EEA was the average of the four slopes determined during the respective workloads used to quantify EMG_{FT} . During the EMG_{FT} tests, a bipolar electrode arrangement (2.54 center-to-center) was placed on the right thigh, midway between the greater trochanter and the lateral condyle of the femur while the reference electrode was placed over the spinous process of the 7th cervical vertebrae. Training procedures followed the procedures of Brown and Greenwood to prevent overtraining but still allowed for adequate progression. The first training period consisted of five 2-minute intervals with 1-minute rest periods between sets. The second 3-week session that began after midtesting, which followed a similar protocol that alternated repetitions during weeks 6 and 7 of training. Results of the study showed that HIIT training may significantly increase EMG_{FT} values, and therefore increasing power output that can be maintained for an extended period of time without exhaustion. In addition, HIIT was

shown to improve EEA, which reflects an improvement in lower-body skeletal muscle tissue function.

Camic, Housh, Zuniga, Hendrix, Mielke, Johnson, and Schmidt (2010)

Effects of arginine-based supplements on the physical working capacity at the fatigue threshold

The purpose of the study was to examine the effects of daily oral administration of arginine-based supplements for 4-weeks on the physical working capacity at the fatigue threshold (PWC_{FT}). Sixty-one men volunteered for the study, but only 50 provided valid PWC_{FT} values pre- and post-supplementation. Subjects were aerobically untrained and participated in no more than 4-hours of recreational activity per week, were in good health, did not report using nutritional supplements that could have affected the outcome of the study, and did not participate in another investigational product within 30 days of enrollment. Prior to supplementation, participants completed an incremental exercise test on a cycle ergometer to determine physical working capacity at fatigue threshold (PWC_{FT}). Subjects were assigned to one of three groups: 1) a placebo group ($n = 19$), 2) 1.5g of arginine ($n = 14$), or 3) 3.0g of arginine ($n = 17$). Ingestion instructions were to take one dose in a pill form every morning on an empty stomach after waking with 16 oz. of water for 28 days. After 28 days of supplementation, subjects returned to the lab for post-testing. During the visit, participants ingested 1 dose 60-minutes prior to the incremental test that used the same protocol as the pre-testing assessment. During both PWC_{FT} assessments, a bipolar (20-mm center-to-center) surface electrode arrangement was placed on the dominant leg over the vastus lateralis muscle in accordance with the SENIAM Project (Hermens et al., 1999). The method to determine PWC_{FT} for each subject was consistent with the protocol of deVries et al. (1990) that used the continuous approach to estimate PWC_{FT} . When analyzing

the PWC_{FT} results, authors reported 10.7% subjects reaching exhaustion before reaching a statistically significant slope from the EMG amplitude data during any 2-minute stage. Results of the study showed that showed arginine groups significantly improved PWC_{FT} while the placebo group did not. Authors credited this finding to reduced concentrations of metabolic byproducts, e.g., lactate and ammonia, or the improved blood flow associated with increased nitric oxide synthesis with arginine and grape seed extract.

McCormack, Stout, Emerson, Scanlon, Warren, Wells, Gonzalez, Mangine, Robinson IV, Fragala, and Hoffman (2013)

Oral nutritional supplement fortified with beta-alanine improves physical working capacity in older adults: A randomized, placebo-controlled study

The purpose of the study was to investigate the effects of an oral nutrition supplement (ONS) with two different doses of beta-alanine on body composition, muscle function, and physical capacity in older adults. Sixty older men (n = 27) and women (n = 33) volunteered for the double-blind, placebo-controlled study and were placed into one of three groups, all of which consumed the ONS that contained 230 kcal, 12 grams of protein, 31 grams of carbohydrate, and 6 grams of fat. Group one consumed only the ONS (n = 20), group two consumed the ONS and 800 mg of beta-alanine (ONS800; n = 19), and group three consumed the ONS and 1200 mg of beta-alanine (ONS1200; n = 21). Participants supplemented twice per day for twelve weeks. Pre- and post-supplementation performance testing included: dual-energy X-ray absorptiometry (DEXA) to estimate fat mass, total body lean soft tissue mass (TLSTM) and arm lean soft tissue mass (ALSTM), a discontinuous physical working capacity at fatigue threshold (PWC_{FT}) test used from a protocol previously described by deVries et al. (1987), a handgrip dynamometry test to assess muscular strength with the average and maximum values being recorded, and a 30-

second sit-to-stand test to measure low body functionality. EMG measurements were taken from bipolar (2.54 cm center-to-center) surface electrode arrangement taken from the right vastus lateralis muscular at approximately 60% of the distance from the lateral portion of the patella on a line with greater trochanter while the reference electrode was placed over the lateral epicondyle of the distal femur. Results of the study showed that both the ONS800 (17.8%) and ONS1200 (13.6%) groups significantly improved PWC_{FT} from pre to post with no significant differences between groups and also saw improvements muscle quality and function. Thus, the authors suggest that ONS fortified with beta-alanine may help maintain health and independent living in older adults.

Dmax Method and Fatigue Thresholds

Cheng, Kuipers, Snyder, Keizer, Jeukendrup, and Hesselink (1992)

A new approach for the determination of ventilatory and lactate thresholds

The purpose of this study was to develop a mathematical method that finds the point on a regression curve that is the maximal distance from a linear regression of the starting and ending points, known as the Dmax method. Eight male cyclists took part in an incremental exercise test to volitional fatigue with gas samples being collected every 30-seconds and venous blood samples being drawn during the last 30-seconds of each workload. To determine the Dmax, the data of each subject was plotted versus VO_2 so that a third-order polynomial regression could be used to form a best fit line for ventilation, breathing frequency, CO_2 output, and plasma lactic acid. After the linear regression was formed from the starting and ending points, the data point that yielded the furthest distance from the linear regression was deemed the threshold point. The reliability of all regressions were shown to be high by a variance analysis for regression. Results

of the study showed that similar values were obtained from the Dmax method when compared to traditional methods. The authors' conclusive findings were that the Dmax method is an objective and reproducible method to calculate ventilatory and metabolic thresholds with similar results and that breathing frequency can be used for detecting ventilatory threshold and stated that one of the advantageous of the Dmax method was that a threshold point can always be detected.

Zhou and Weston (1997)

Reliability of using the D-max method to define physiological responses to incremental exercise testing

The purpose of this study was to examine the reliability of using a mathematical method (Dmax) to define blood lactate kinetics in response to an incremental exercise test to compare the physiological responses corresponding to the workload at Dmax with those at the traditional 4 mmol lactate threshold. Ten healthy male cyclists and triathletes participated in the study. To establish reliability, participants performed two incremental exercise tests, in a fasted state, until exhaustion while controlling for their normal training activities for 4-weeks prior to the first trial then duplicated that training regimen prior to the second trial. All participants were asked to remain abstain from strenuous exercise for 48-hours and from alcohol and caffeine for 24-hours prior to both trials. A 10-minute standardized warmup exercise preceded 2-minutes of resting on the cycle ergometer which was followed by the exercise test that started at 150 W for 3-minutes and was increased by 25 W every 3-minutes until exhaustion. A third-order polynomial regression of plasma lactate concentration vs workload and a linear regression from the starting and ending points were obtained. The Dmax method identifies the point on the third-order polynomial regression that is the maximal distance from the linear regression as previously done by Cheng et al. (1992). Thus, the slope of the third-order polynomial regression was equal to that

of the linear regression, which signified the location of the Dmax. Results from the study showed that heart rate, $\dot{V}O_2$, and exercise time determined on the response of plasma lactate concentration to the incremental exercise test had a high test-retest reliability to be used as an alternative method to describe lactate kinetics as it has advantages of individuality and objectivity. In addition, the authors mention that 35% of subjects could not maintain exercise intensity 15 W above the Dmax point for 30-minutes but all subjects could exercise for 30-minutes at 15 W below the Dmax point, illustrating the sensitivity of responses to the given workloads for each individual.

Zugina, Housh, Camic, Bergstrom, Schmidt, and Johnson (2014)

The effect of different exercise protocols and regression-based algorithms on the assessment of the anaerobic threshold

The purpose of this study was to investigate the effect of ramp and step cycle ergometer protocols on the assessment of the anaerobic threshold (AT) using 3 computerized, regression-based algorithms. Thirteen healthy adults (men = 6, women = 7) volunteered for the experiment and exercised at least twice per week. Subjects visited the lab for testing sessions on two occasions; once to perform a step incremental cycle ergometer test and again to complete a ramp protocol, which were separated by 48-hours. Both procedures were completed until exhaustion and were randomized in terms or order of the tests. In the step test, the subject started pedaling at 30 W with resistance increasing 30 W every 2-minutes. In the ramp test, resistance started at 30 W and increased 15 W every minute (1 W every 4 seconds). Test-retest reliability was found to be 0.95 with no significant mean difference.

Three mathematical models were used to assess AT from gas exchange values collected from each protocol: breaking point, V-slope, and Dmax. The breaking point algorithm joins two regression equations, one before and one after the breaking point, into a single regression equation. The V-slope algorithm introduced by Beaver et al. (1986) divides the VCO_2 vs VO_2 relationship data points into two sequential segments. Each segment has a linear regression line and then calculates the intersection between the two regression lines. The Dmax method, as presented by Cheng et al. (1992), uses a third-order curvilinear regression to the VCO_2 vs VO_2 relationship while making a linear regression between the starting and ending points of the data. Therefore, the Dmax is the maximal distance the third-order curvilinear regression is from the linear regression. Results of the study showed no mean differences among the three computerized programs for VO_2 , HR, and power output at the AT. However, the step protocol resulted in significantly greater VO_2 and HR at the AT compared to the ramp protocol with no differences in power output.

Dmax Method and Physical Working Capacity at Neuromuscular Fatigue Threshold

Miramonti, Stout, Fukuda, Robinson IV, Wang, La Monica, and Hoffman (2015)

The effects of four weeks of high intensity interval training and β -hydroxy- β -methylbutyric free acid supplementation on the onset of neuromuscular fatigue

The purpose of this study was to investigate the effects of high-intensity interval training (HIIT) and β -hydroxy- β -methylbutyric free acid (HMB) supplementation on neuromuscular fatigue using the physical working capacity at fatigue threshold (PWC_{FT}) assessment. Thirty-seven participants (22 men, 15 women) completed a graded exercise test (GXT) as previously described by Robinson IV et al. (2014) prior to and following supplementation and HIIT. After

the initial trial, participants were randomly organized into one of three groups: control, placebo, or supplementation. The placebo and supplementation groups then completed 12 HIIT sessions while the control group was asked to maintain their regular diet and physical activity patterns. The intensity of each HIIT session ranged from 85-120% of peak power (P_{PEAK}) from the initial GXT on three non-consecutive days per week for 4-weeks. On exercise session days, each participant received 3 grams of HMB or placebo that was divided equally and consumed into three servings: 30-minutes prior to exercise, 60-minutes later, and 3-hours post-exercise. A bipolar electrode arrangement was placed over the vastus lateralis muscle of the right thigh (4.6 cm center-to-center) and placed via recommendations from the SENIAM project for EMG electrode placement. A Dmax method was used to calculate PWC_{FT} values as previously done by Bergstrom and colleagues (2011) that determines the point on a third-order polynomial regression curve that is the maximal distance from a linear regression line, which connects the start and end of the GXT. Results of the study showed that the onset of neuromuscular fatigue can be delayed via HIIT and further delayed from the combination of HIIT and HMB supplementation when assessed by PWC_{FT} . Authors attribute these findings to improved recovery, increased mitochondrial biogenesis, and increased capacity to transport lactate and H^+ out of skeletal muscle or into the mitochondria for oxidative metabolism. Test-retest reliability ($ICC_{2,1} = 0.95$) of the Dmax method to assess PWC_{FT} had no significant differences ($p = 0.91$) between the first (181.6 ± 29.2 W) and second (181.9 ± 26.3 W) trials, which were separated by 24 - 48 hours.

CHAPTER III: METHODS

Reproducibility Subjects

Eleven men (mean \pm SD; age: 21.9 ± 1.37 years; height: 175.6 ± 8.65 cm; body mass: 82.1 ± 13.92 kg) volunteered to participate in an investigation that examined the reliability of estimating the physical working capacity at fatigue threshold (PWC_{FT}) using the Dmax method. Study protocol, benefits, and risks of the study were explained prior to signing the informed consent. Health history, activity levels, and previous nutritional supplementation of participants were evaluated using a physical activity readiness questionnaire (PAR-Q) and a health and activity history questionnaire. The study was approved by the University Institutional Review Board. All participants signed an informed consent form prior to any data collection.

Reproducibility Study Protocol

All participants were required to visit the Human Performance Laboratory on two, nonconsecutive days for two exercise trials. Prior to trial 1, anthropometric measurements were obtained from all participants, comprised of age (years, y), height (cm), and body mass (kg). All participants then performed a graded exercise test (GXT) on a cycle ergometer to estimate PWC_{FT} using the Dmax method. Participants were asked to remain free from exercise and nutritional supplementation 24-hours prior to and between trials. All participants had prior experience in participating in a maximal GXT exercise protocol on the cycle ergometer.

Electromyography (EMG) Measurements

A bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick-Prep silver-silver chloride) arrangement was placed over the vastus lateralis (VL) muscle on the right thigh and a

reference electrode was placed over the lateral epicondyle. The EMG electrodes were placed on the VL, precisely two-thirds distance from the anterior superior iliac spine to the lateral patella, based on the recommendations from the SENIAM project for EMG electrode placement (Hermens et al., 1999). Prior to electrode placement, the skin at each site was shaved and cleaned with alcohol. Inter-electrode impedance was kept below 5,000 ohms with careful abrasion of the skin beneath the electrodes. The raw EMG signal was sampled at 1 kHz, differentially amplified (EMG 100c, bandwidth = 10-500Hz, gain: x2000; MP150 Biopac Systems, Inc., Santa Barbara, CA), and digitally bandpass filtered (zero-phase shift fourth-order Butterworth) at 10-500 Hz. The EMG signals were stored on a personal computer (Dell Latitude E6530, Dell Inc., Round Rock, TX) and expressed as root mean square (RMS) amplitude values (μVrms).

Dmax Method (Reproducibility Study)

PWC_{FT} was calculated using a Dmax method described previously (Bergstrom et al., 2011; Miramonti et al., 2015). The average EMG root mean square (RMS) values were calculated for each 10 second epoch during the GXT. To reduce variability, any three consecutive RMS value points were averaged and then plotted against the midpoint of each 30-second window (Figure 1) (Fox, 2000). Participant warmup values were excluded from analysis while the remaining data points were used to generate a third order polynomial regression demonstrating an increasing EMG amplitude in the time domain during the GXT.

The point of fatigue (T_{F}) (Figure 2) was the point on the third-order polynomial regression that was the maximal perpendicular distance from that linear regression of the starting and ending points of the GXT. T_{F} was used to estimate PWC_{FT} from an equation adapted from Berthon and Fellmann (2002) and Zuniga et al. (2013) that reads:

$$PWC_{FT} = P_o + a (n / N)$$

P_o is the power output that represents the stage T_F occurred; a is the increasing increment in power output between stages of the GXT (25 W); n , represented in seconds, is the difference between T_F and the beginning of the stage that T_F occurred, and N is the duration of each stage (120-seconds). Since the starting workload was 75 W, the equation is rearranged to:

$$PWC_{FT} = 75 + 25 (T_F / 120)$$

Statistical Analysis (Reproducibility Study)

A dependent t-test was used to compare PWC_{FT} values between the two trials. Statistical significance was set at an alpha level of 0.05. In addition, test-retest reliability was evaluated by calculation of intraclass correlation coefficient (ICC) model 3, 1 (Weir, 2005). Standard error of the measurement (SEM) was used to reflect the consistency within subjects. The SEM was used to calculate the minimal difference (MD) to be considered real.

Validity Subjects

Eleven active (currently exercising 2-5 times per week) men (age: 23.4 ± 3.0 years; height: 177.9 ± 7.8 cm; body mass: 80.9 ± 10.7 kg) and ten active women (age: 22.3 ± 3.1 years; height: 166.6 ± 9.5 cm; body mass: 62.8 ± 8.7 kg) volunteered to participate in the validity of D_{max} when estimating PWC_{FT} pre- and post-training, compared to the ORG method (deVries et al., 1987; deVries et al., 1990). Study protocol, benefits, and risks of the study were explained prior to signing the informed consent. Health history, activity levels, and previous nutritional supplementation of participants were evaluated using a physical activity readiness questionnaire (PAR-Q) and a health and activity history questionnaire. The training study was approved by the

University Institutional Review Board. All participants signed an informed consent form prior to any data collection.

Validity Study Protocol

On the first testing day (pre-training), anthropometric measures of age (years), height (cm), and body mass (kg) were collected from participants. To calculate PWC_{FT} , each participant then performed a GXT on a cycle ergometer until volitional fatigue. The peak wattage achieved (P_{PEAK}) was used to establish individual training intensity. Subjects then completed 12 HIIT sessions before post-testing. Sessions were scheduled three times per week for 4-weeks on nonconsecutive days and performed on an electrically braked cycle ergometer. Following the HIIT phase, subjects then returned to the lab for post-testing and repeated the same procedures as pre-training testing.

Training Protocol

Training sessions commenced with a five-minute warm up at a self-selected power output followed by five 2-minute bouts of work followed by 1-minute of rest. Training intensity of each HIIT session ranged from submaximal (85%) to supramaximal (120%) of P_{PEAK} (Miramonti et al., 2015). Participants performed HIIT sessions three times per week for 4-weeks on nonconsecutive days on an electronically braked cycle ergometer (Corival, Lode; Groningen, Netherlands). All training sessions took place under the supervision of a National Strength and Conditioning Association Certified Strength & Conditioning Specialist or an American College of Sports Medicine Health Fitness Specialist.

Original (ORG) Method (Validity Study)

The Dmax procedure was the same in the validity study as performed in the reproducibility study. PWC_{FT} values using the ORG method were measured during the GXT using procedures adapted from deVries et al. (1987; 1990). During each 2-minute interval, six 10-second EMG samples were recorded from the vastus lateralis. The average EMG amplitude values (RMS) were calculated for each 10-second epoch and were then plotted across time for each power output. The PWC_{FT} was defined as the average of the highest power output that resulted in a non-significant ($p > 0.05$; single-tailed t -test) slope coefficient for the EMG amplitude versus time relationship, and the lowest power output that resulted in a significant ($p < 0.05$) positive slope coefficient (Figure 3).

Statistical Analysis (Validity Study)

A two-way measures analysis of variance (ANOVA) was used to identify method \times time interaction for PWC_{FT}. If significant interaction occurred, then repeated Bonferroni adjusted independent t -test were run on pre and post PWC_{FT} values between groups. If significant main effect, then paired samples t -tests will be run examining the pre-training to post-training change scores for each method.

The cross-validation analysis of Dmax method used in this study at pre-training and post-training HIIT were based on the evaluation of the actual PWC_{FT} (ORG) versus the predicted PWC_{FT} (Dmax) via calculation of the constant error (CE = mean difference for actual PWC_{FT} – predicted PWC_{FT}), Pearson product-moment correlation (r), standard error of estimate [SEE = $SD_y\sqrt{(1-r^2)}$], and total error [TE = $\sqrt{\sum(\text{actual}_{\text{PWCFT}} - \text{predicted}_{\text{PWCFT}})^2/n}$]. In addition, Bland and Altman approach was used to assess the agreement between methods (Bland & Altman, 1986)

for pre-training and post-training estimates of PWC_{FT} . Statistical significance was set at an alpha level of 0.05. Data were analyzed via SPSS (Version 23.0, SPSS Inc., Chicago, IL).

CHAPTER IV: RESULTS

Reproducibility Study

There was no significant difference ($p = 0.870$) for the first (181.5 ± 29.2 W) and second (181.9 ± 26.18 W) trials. Reliability yielded an $ICC_{3,1}$ of 0.949 with an SEM of 6.28 W and MD of 17.41 W.

Validity Study

HIIT improved PWC_{FT} from pre to post when determined by Dmax (Pre: 177.4 ± 36.2 W; Post: 198.3 ± 38.3 W; $p = 0.000$) and ORG (Pre: 170.7 ± 56.9 W; Post: 191.1 ± 57.1 W; $p = 0.008$). The repeated measures analysis of variance (ANOVA) revealed no significant method \times time interaction ($p = 0.957$) and no main effect for method ($p = 0.235$) but, a significant main effect of time was observed ($p < 0.001$).

Table 1 presents the results of the cross-validation analyses. The mean difference (CE) between the actual and predicted PWC_{FT} at pre (-6.7 W; $p > 0.05$) and post (-7.2 W; $p > 0.05$) between ORG and Dmax were not significantly different. Further, the validity coefficients for Pre ($r = 0.87$) and Post ($r = 0.84$) were considered very strong (Evans, 1996). The TE, which accounts for the errors associated with both the CE and SEE, were 30.8 and 32.5 for pre and post PWC_{FT} values, respectively.

As shown in Figure 3, the 95% limits of agreement (LOA) ranged from -68.36 to 54.93 W with a non-significant bias of -6.7 ± 30.8 W ($p = 0.330$), which suggests that the Dmax method calculated, on average, 6.7 W higher than the ORG. The regression line, though, shows the Dmax method overestimated PWC_{FT} at low power outputs and underestimated at high power

outputs compared to the ORG method with a weak, negative correlation ($r = -0.435$; $p = 0.049$). However, 20 of the 21 participants fell within ± 1.96 standard deviations of the mean difference.

In the post-training analyses, LOA values ranged from -57.77 to 72.06 W that included a non-significant bias of -7.2 ± 32.5 W ($p = 0.325$), illustrating that Dmax calculated PWC_{FT}, on average, 7.2 W higher than ORG. In addition, the regression line in Figure 4 shows the Dmax method overestimated PWC_{FT} at low power outputs and underestimated at high power outputs compared to the ORG method with a moderate, negative correlation ($r = -0.604$; $p = 0.004$). Nonetheless, all participants fell within ± 1.96 standard deviations of the mean difference.

CHAPTER V: DISCUSSION

Previous studies (deVries et al., 1990, Stout et al., 2006, Stout et al., 2007, Jenkins et al., 2014) have shown high reliability ($ICC = 0.85 - 0.975$) and low standard error of the measurement ($SEM \pm 6$ to 11 W) when estimating PWC_{FT} in men and women using the ORG method proposed by deVries et al. (1987, 1990). The current study is the first to demonstrate similar test-retest reliability ($ICC_{3,1} = 0.949$) for estimating PWC_{FT} using the Dmax method, with a low standard error of the measurement ($SEM = 6.28$ W). However, previous studies have reported that the ORG method may result in unsatisfactory data in approximately 10% of subjects and therefore dropped from the study (Camic et al., 2010; Jenkins et al., 2014; Housh et al., 1996; deVries et al., 1989). For example, when using the ORG method, unsatisfactory data included tests in which there was no EMG evidence of muscular fatigue (significant slope coefficient) and therefore, unable to determine PWC_{FT} (deVries et al., 1989; Housh et al., 1996; Jenkins et al., 2014). Unlike the ORG method, an estimated PWC_{FT} was found in all of the subjects in the current study using the Dmax method. While both methods provided high reliability, the Dmax method may overcome the shortcomings of the ORG method when estimating PWC_{FT} .

The cross-validation analyses were based on the following criteria (Lohman, 1981; Sinning et al., 1985): the mean values for actual and predicted PWC_{FT} should be comparable at pre and post. In addition, the TE should be determined because it reflects the true difference between the actual and predicted PWC_{FT} , while the SEE reflects only the error associated with the regression between the variables. Further, there should be close similarity between TE and SEE because it reflects the relationship between the regression lines for actual versus the predicted PWC_{FT} .

The findings of the current study indicated that the work rate at PWC_{FT} estimated by D_{max} and the ORG methods were not significantly different and presented a strong correlation for pre ($r = 0.87$) and post ($r = 0.84$) HIIT (Table 2). In addition, there was a significant increase in PWC_{FT} for ORG (+10.7%) and D_{max} (+10.5%) but no significant difference in the change values between the measures (Table 1). Furthermore, the SEE values for pre (28.4 W) and post (31.8 W) training were similar. When the SEE values were expressed as a percentage of the mean of the ORG PWC_{FT} (%SEE), the values for pre- and post-training were 16.5%. It has been suggested that SEE values ranging between 10 and 20% are typical of field methods used to estimate $\dot{V}O_{2MAX}$ and ventilatory threshold (VT) measures (McArdle, 2001; Malek et al., 2007). The %SEE values observed for PWC_{FT} in the present study were similar to cross validation estimates of VT reported by Malek et al. (18 to 20%).

While the SEE provides important information regarding error associated with the regression between ORG versus D_{max} , the TE is the best criterion for determining the accuracy of the prediction because it combines the errors associated with CE and SEE (Lohman, 1992). Furthermore, Lohman (1992) has suggested that valid predictions will exhibit similar values for SEE and TE. In the present study, the TE and %TE for Pre (30.8 W; 18%) and Post (32.5 W; 17%) were very similar to SEE values (Table 2), which suggests the estimated PWC_{FT} from the D_{max} method is valid when compared to ORG before and after HIIT. The Bland-Altman Plots (Figures 4-5) revealed that there was no systematic bias between ORG and D_{max} for pre (-6.7 ± 30.8 W; $p = 0.330$) and post (-7.2 ± 32.5 W; $p = 0.325$) training. In addition, 95.2% and 100% of participants fell within ± 1.96 standard deviations of the mean the difference for pre and post, respectively. In conclusion, this study suggests that the D_{max} method is a valid, sensitive and reliable method used to estimate PWC_{FT} in young men. Future studies are needed to validate the

Dmax method for estimating PWC_{FT} in other populations such as youth and older men and women.

APPENDIX A: FIGURES

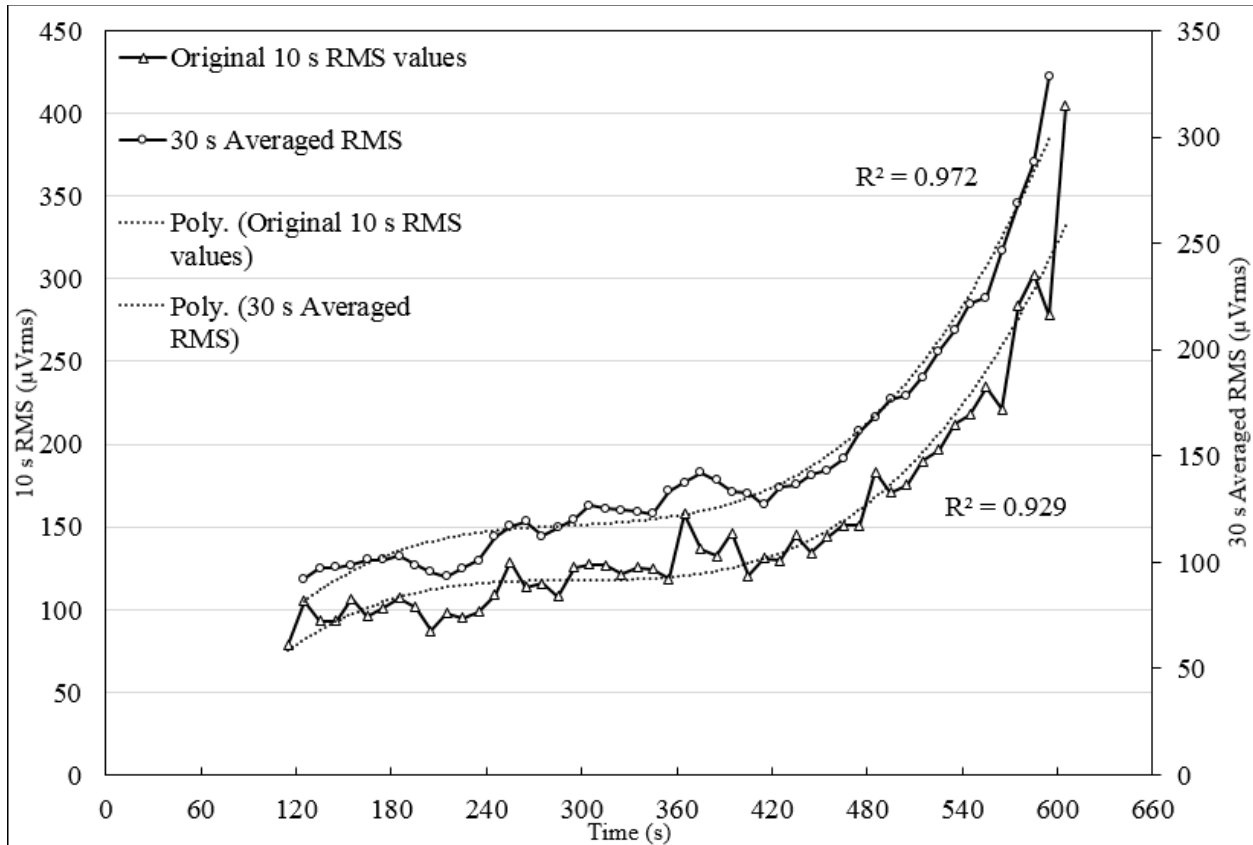


Figure 1

Averaging 30-second RMS values.

Averaging 30-second RMS values. The triangles represent the 10-s epoch RMS values and the bottom dotted line represents the third-order polynomial regression of the original data ($R^2 = 0.929$). The circles represent moving averages of each possible set of three consecutive 10-s values with the dotted line representing the third-order polynomial regression of the smoothed data ($R^2 = 0.972$). The graph is not to scale to prevent overlap. RMS = root mean square (μV_{rms}).

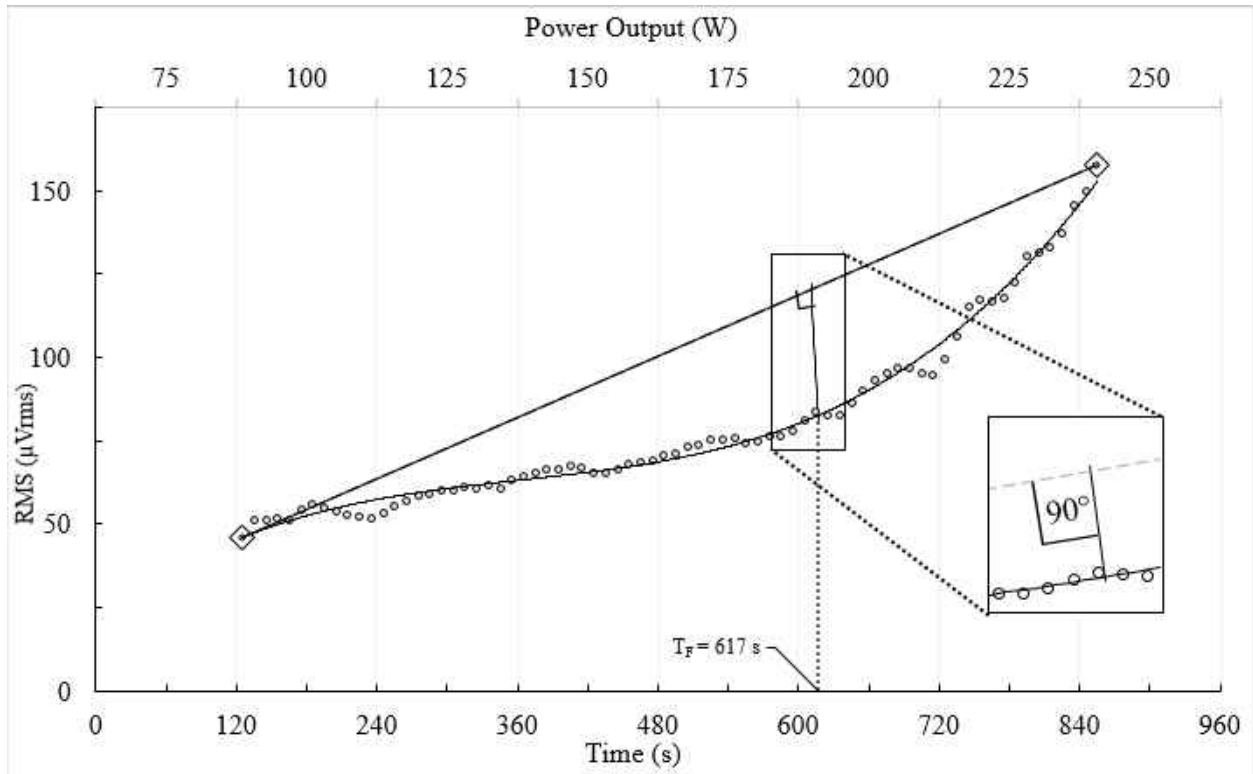


Figure 2

Dmax method to determine T_F .

Dmax method to determine T_F . The circles represent the 30-second averages of the original RMS values while the solid black line represents the third-order polynomial regression of the data. The diamonds represents the first and last data points in the analysis with the dashed line as the linear regression between those two points. The line connecting the linear regression and the third-order polynomial regression is the maximal perpendicular distance (D_{max}). The dotted line that continues under the maximal perpendicular distance line shows the T_F from the third-order polynomial regression. RMS = root mean square (μV_{rms}).

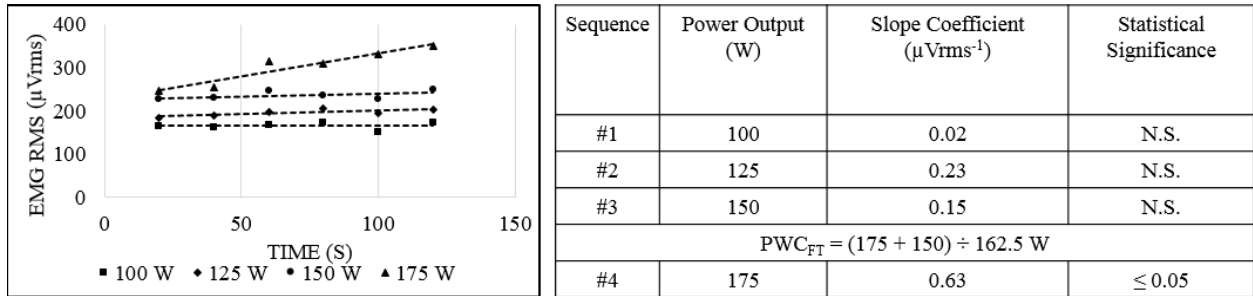


Figure 3

Illustration of the ORG method to estimate PWC_{FT} .

Illustration of the method used to determine the physical working capacity at fatigue threshold (PWC_{FT}). The different shapes represent a 10-s epoc for that power output stage. EMG μVrms : electromyographic amplitude in root mean squared microvolts; N.S.: not significant.

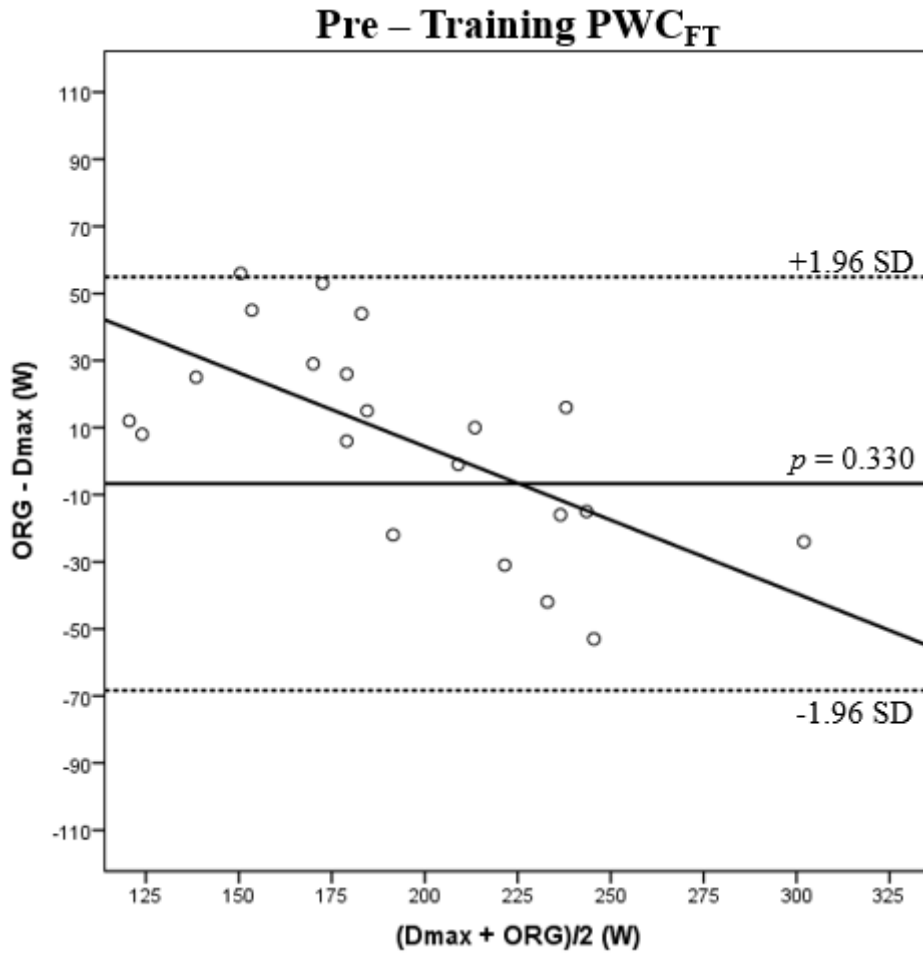


Figure 4

Pre-training PWC_{FT} analysis of agreement between ORG and Dmax methods.

Pre – training PWC_{FT} analysis of agreement between the ORG and Dmax methods. The middle solid line represents the mean of the differences between the methods (bias). The upper and lower dashed lines represent the bias \pm 1.96 SD (95% Limits of Agreement). $R^2 = 0.497$.

*Statistically significant from 0.

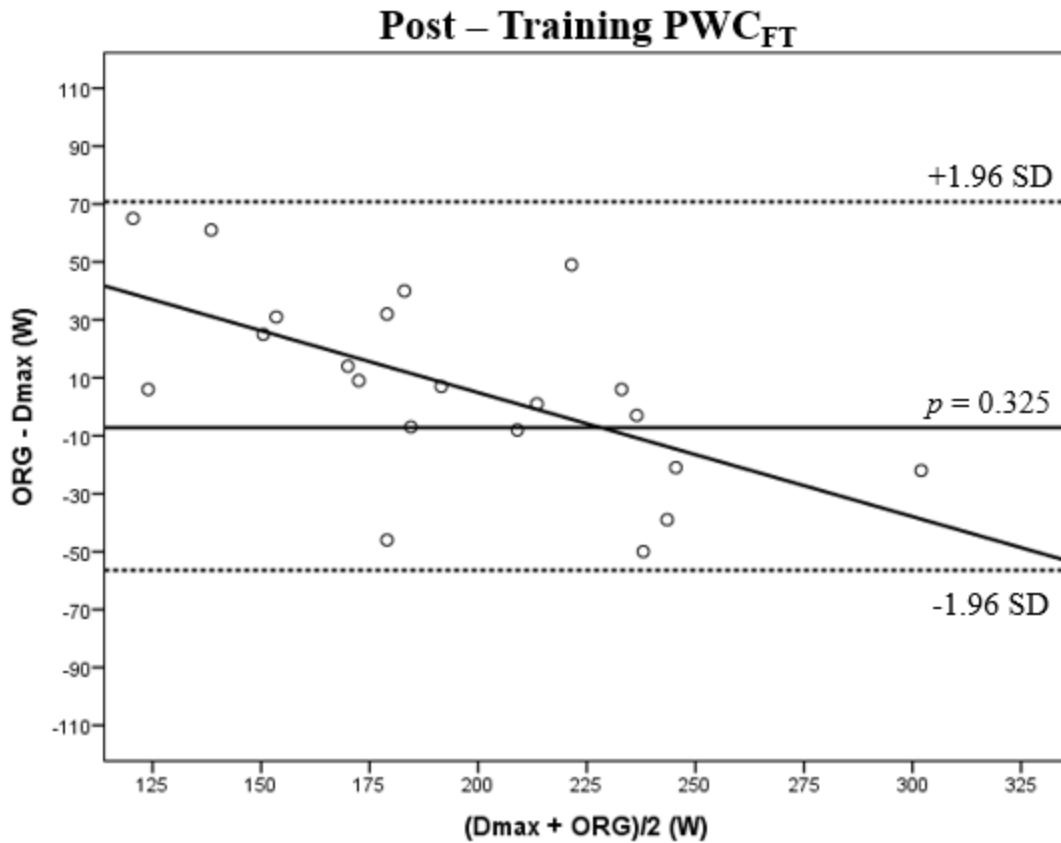


Figure 5

Post-training PWC_{FT} analysis of agreement between the ORG and D_{max} methods.

Post – training PWC_{FT} analysis of agreement between the ORG and D_{max} methods. The middle solid line represents the mean of the differences between the methods (bias). The upper and lower dashed lines represent the bias ± 1.96 SD (95% Limits of Agreement). $R^2 = 0.365$.

*Statistically significant from 0.

APPENDIX B: TABLES

Table 1

Baseline values and change in PWC_{FT} following HIIT.

Method	Variable	Pre (W)	Post (W)
ORG	PWC _{FT}	170.7 ± 56.9	191.1 ± 57.1*
Dmax	PWC _{FT}	177.4 ± 36.2	198.3 ± 38.3*

*Significant ($p < 0.05$) Pre to Post change.

Table 2

Cross-validation of PWC_{FT} pre- and post-training.

Method	PWC _{FT} (W) Mean±SD	CE	r	SEE (W)	SEE%	TE (W)	TE%
Dmax Pre	177.4 ± 36.2	-6.7	0.87	28.4	16.6	30.8	18.0%
ORG Pre	170.7 ± 56.9						
Dmax Post	198.1 ± 38.3	-7.2	0.84	31.8	16.6	32.5	17.0%
ORG Post	191.1 ± 57.1						

SEE% calculated as SEE/mean of actual_{PWCFT}; TE% calculated as TE/mean of actual_{PWCFT}.

APPENDIX C: UCF IRB LETTER



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

Notice that UCF will Rely Upon Other IRB for Review and Approval

From : **UCF Institutional Review Board**
FWA00000351, IRB00001138

To : **Edward H. Robinson IV**

Date : **August 19, 2013**

IRB Number: **SBE-13-09475**

Study Title: **The effects of β -Hydroxy- β -methylbutyrate Free Acid Gel and High-Intensity Interval Training on Quadriceps Muscle Architecture and Quality, Neuromuscular Economy, and Metabolic Performance in Recreationally Trained Individuals**

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida IRB Chair designated Reviewer on August 19, 2013. The UCF IRB accepts the New England Institutional Review Board's review and approval of this study for the protection of human subjects in research. **The expiration date will be the date assigned by the New England Institutional Review Board and the consent process will be the process approved by that IRB.**

This project may move forward as described in the protocol. It is understood that the New England IRB is the IRB of Record for this study, but local issues involving the UCF population should be brought to the attention of the UCF IRB as well for local oversight, if needed.

All data, including signed consent forms if applicable, must be retained in a locked file cabinet for a minimum of five years (six if HIPAA applies) past the completion of this research. 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.

Failure to provide a continuing review report for renewal of the study to the New England IRB could lead to study suspension, a loss of funding and/or publication possibilities, or a report of noncompliance to sponsors or funding agencies. If this study is funded by any branch of the Department of Health and Human Services (DHHS), an Office for Human Research Protections (OHRP) IRB Authorization form must be signed by the signatory officials of both institutions and a copy of the form must be kept on file at the IRB office of both institutions.

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

Signature applied by Patria Davis on 08/19/2013 10:41:19 AM EDT

IRB Coordinator

APPENDIX D: NEW ENGLAND IRB APPROVAL



August 13, 2013

Edward H. Robinson, IV
University of Central Florida
12494 University Boulevard
Orlando, FL 32816

Re: (IRB# 13-257): SBE-13-09475: "The Effects of β -Hydroxy- β -Methylbutyrate Free Acid Gel and High-Intensity Interval Training on Quadriceps Muscle Architecture and Quality, Neuromuscular Economy and Metabolic Performance in Recreationally Trained Individuals"

This is to inform you that New England Institutional Review Board (NEIRB), via expedited review (Thursday Board), has approved the above-referenced research protocol and the participation of the above-referenced investigative site in the research. The approval period is **8/13/2013 to 7/28/2014**. **Your study number is 13-257. Please be sure to reference either this number or the name of the principal investigator in any correspondence with NEIRB.**

Continued approval is conditional upon your compliance with the following requirements:

- A copy of the **Informed Consent Document**, NEIRB version 1.0, approved on 8/13/2013 is enclosed. Only NEIRB-approved informed consent documents should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.
- The following must be promptly reported to NEIRB: changes to the study site, and all unanticipated problems that may involve risks or affect the safety or welfare of subjects or others, or that may affect the integrity of the research.
- Approval is valid for enrollment of the number of subjects indicated on your submission form. If you anticipate enrolling more than this number of subjects, NEIRB approval must be obtained prior to exceeding the approved enrollment number.
- All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB except where necessary to eliminate apparent immediate hazards to the study subjects.
- Compliance with all federal and state laws pertaining to this research, and with NEIRB's SOPs.
- The enclosed subject materials (*PAR-Q and You Questionnaire and QConfidential Medical and Activity History questionnaire*) have been approved. The enclosed recruitment advertisement (*Print Ad*) has been conditionally approved. Please make the indicated revisions and re-submit it to NEIRB for final approval. Advertisements, letters, internet postings and any other media for subject recruitment must be submitted to NEIRB and approved prior to use. Please refer to *NEIRB Guidelines for Recruitment and Advertising*, available at www.neirb.com.
- All deaths, life-threatening problems or serious or unexpected adverse events, *whether related to the study article or not*, must be reported to the IRB. The Serious Adverse Event Form is available at www.neirb.com.
- Any and all necessary FDA approvals must be received prior to your initiation of the trial. If this study is being conducted under an IDE, a copy of the FDA IDE approval letter must be submitted to NEIRB.
- The study cannot continue after 7/28/2014 until re-approved by NEIRB. A Study Renewal Report must be completed and returned to NEIRB prior to the expiration of the approval period.



85 Wells Avenue · Suite 107 · Newton, MA 02459 Phone: 617-243-3924 · Fax: 617-969-1310 www.neirb.com



- When the study is completed, terminated, or if it is not being renewed - complete and submit a Study Completion Report to NEIRB. The Study Completion Report can be accessed via the NEIRB website at www.neirb.com.

Shana R. Ross

Shana R. Ross, MCJ, CIM, CIP
Lead Administrator

Copy: NEIRB Chair
Enclosures



85 Wells Avenue · Suite 107 · Newton, MA 02459 Phone: 617-243-3924 · Fax: 617-969-1310 www.neirb.com

APPENDIX E: INFORMED CONSENT



The effects of β -Hydroxy- β -methylbutyrate Free Acid Gel and High-Intensity Interval Training on Quadriceps Muscle Architecture and Quality, Neuromuscular Economy, and Metabolic Performance in Recreationally Trained Individuals

Informed Consent

Principal Investigator(s): Edward H. Robinson IV, M.A./M.S.
Jeffrey R. Stout, Ph.D.

Sponsor: Metabolic Technologies Inc.

Investigational Site(s): University of Central Florida
College of Education and Human Performance
Sport and Exercise Science

Introduction: Researchers at the University of Central Florida (UCF) study many topics. To do this we need the help of people who agree to take part in a research study. You are being asked to take part in a research study that will include 40 men and women at UCF. You have been asked to take part in this research study because you are an active young adult who routinely participates in recreationally training. You must be between 18 and 35 years of age to be included in this research study.

The principle investigators conducting the research are Edward H. Robinson IV, and Dr. Jeffrey R. Stout. They will be supported by Dr. Jay R. Hoffman, Dr. Maren S. Fragala (Sport and Exercise Science in the College of Education), and Dr. Leonardo Oliveira (Sports Medicine Physician at UCF and medical monitor of the study).

What you should know about a research study:

- Someone will explain this research study to you.
- A research study is something you volunteer for.
- Whether or not you take part is up to you.
- You should take part in this study only because you want to.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

1. Purpose of the research study:

We will examine two factors in this study:

- 1) How exercise that involves repeated short-to-long bouts of high-intensity exercise interspersed with recovery periods, also known as, high intensity interval training (HIIT) effects cardiovascular and muscular adaptations to this form of endurance training.
- 2) How supplementation with the free acid form β -Hydroxy- β -methylbutyrate (HMB-FA)—a chemical found naturally in the body and in some of the foods that we eat—effects the cardiovascular and muscular adaptations to this form of endurance training.

Testing location and time requirements:

All testing will be conducted in the Human Performance Lab (HPL) in the College of Education and Human Performance building at the University of Central Florida. All measures and tests are conducted for research purposes only. The results will not be used to diagnose any illness or disease, and will not provide any meaningful information to your physician.

Time requirements: We expect that you will be in this research study for approximately 6 weeks and will consist of 17 visits to the HPL. The first visit will last approximately an hour, the second and third visits about an hour and a half, and the training visits, 3 per week for 4 weeks, will last less than 30 minutes, and the final two testing visits will last approximately an hour and a half.

What you will be asked to do in the study:

Upon being admitted to the study you will be assigned a subject number. Each subject number will be associated with one of three groups: a control group (CTL), an HIIT only group (HIIT) or a group which will take the amino acid metabolite HMB and perform HIIT (HMB-HIIT). Determination of the group associated with each subject

number will occur by randomization (similar to flipping a coin). Of the 40 subjects that will be recruited for the study, 10 subjects will be assigned to CTL and 15 to each of the training groups. You will be unable to change your assigned study group to a different study group.

Individuals assigned to CTL will undergo testing on visits 2 and 3. They will then be asked to continue their normal exercise routine for 4 weeks and will undergo post-testing (visits 16 and 17) after this time period. Participants in the HIIT and HMB-HIIT groups will be asked on training days, to consume 1 gram HMB-FA or placebo 30 min prior to training, 1gram HMB-FA or placebo 1 hour post training, and 1gram HMB-FA or placebo 3 hours post training. On non-training days, individuals will consume HMB-FA or placebo 3 times per day (8am, 12pm and 4pm).

Preliminary Visits (3):

Visit 1: You will be asked to read and sign this consent form before any study-related procedures are performed. During this first visit, the following will be done:

Complete the Physical Activity Readiness Questionnaire (PAR-Q)

Complete the self-reported medical and activity history questionnaire

Your age, race and gender will be collected

Your body measurements (height, weight) will be measured

You will be given a 3-day food log to complete prior to visit 3. The dietary intake on this food log will be considered your pre-testing diet and you will be asked to maintain this style of diet during all experimental trials.

Visit 2: The second visit will take place at least 24 hours following visit 1. On this visit:

You will have an ultrasound performed on the quadriceps in your leg. For this, you will be asked to lie flat on your back on an examination table with your legs extended. A lubricated probe will be placed over your thigh to collect information about your muscle (cross-sectional area, fascicle length, echo intensity, muscle thickness). These images will provide the ability to rate the quality of your muscle and how the muscle quality may change after the training intervention.

You will be outfitted with surface electrodes over the vastus lateralis muscles in your quadriceps to measure electromyography (EMG). You will also be asked to perform a maximal leg extension to record a maximal EMG signal. The EMG signal will also be collected during the VO₂peak testing.

You will also be asked to perform a VO₂max test, which will include pedaling on the cycle ergometer at increasing resistance until you can no longer continue. Expired gases will be collected via a mask to determine oxygen uptake, respiratory quotient, energy expenditure and ventilatory threshold.

Visit 3: The third visit will take place no sooner than 48hrs following visit 2. On this visit:

You will have a blood sample taken. The total volume of blood that will be obtained during this study will be <25 ml. To put the total volume of blood being drawn in proper perspective, one pint (475 ml) of blood is typically drawn when donating blood. All blood draws will be conducted under sterile conditions. As an additional

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Approved by NEIRB on 8/13/2013
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safeguard in preventing contamination new disposable gloves will be used for all blood draws. The discomforts associated with the blood drawing procedures are minimal, but sometimes bruising and infection may occur, and your arm might become sore. This soreness usually resolves in a few days. If it persists, contact your doctor.

All blood samples collected will be frozen until analysis. However, blood samples obtained will only be used for this specific study and any leftover blood will be discarded following analysis.

You will have a dual energy x-ray absorptiometry (DEXA) scan performed to assess total and regional body composition. The DEXA machine consists of a padded table with a mechanical arm that uses low dose x-ray to measure muscle, adipose and bone mass. You will be asked to lie flat on your back, with your arms at your sides, legs extended and feet together. The mechanical arm of the DEXA will then pass slowly over your body, without contact. The full body scan will last about 15 minutes. You will perform a 3-minute critical power test. After a self-selected warm-up, you will begin with 60 seconds of unloaded cycling at 90 rpm, followed by an all-out three-minute effort with resistance being set as a function of pedaling rate. The resistance will be adjusted during the all-out effort using the linear mode on the cycle ergometer that sets the power output at 50% of the difference between the ventilator threshold and peak power output assessed during the graded exercise test. EMG assessment will also be conducted during this test, electrode placement will be the same as previously described.

Training Visits (12):

If you are in the HIIT or HMB-HIIT group, you will complete 4 weeks of high intensity interval training (HIIT). The training will occur in the Human Performance Lab 3 times per week for 4 weeks with alternating training sessions of sub maximal and supramaximal workloads. Your training load will be determined as a percentage of the peak power output from the graded exercise test. Each training session with a 5-minute warm up at 50 W, followed by a protocol of 5 or 6 2-minute exercise bouts (total time 15-17 minutes) at a predetermined percentage of VO_{2peak} . There will be 1 minute of complete rest in between exercise bouts during which you will be asked about your perceived readiness to continue exercise.

Post-training Testing visits (2):

These visits will mirror visit 2 and visit 3.

Funding for this study: This research study is being funded by Metabolic Technologies Inc.

Risks:

The risks involved with this study are minimal, but may include musculoskeletal injuries occurring during the training protocol. These injuries include muscle strains and pulls. However, the interval training portion of the study is similar to a hard

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training session that experienced recreationally trained individuals have previously performed during training. The risks associated with the blood draw include some momentary pain at the time of the draw, but other discomfort should be minimal. It is also possible for a bruise to develop at the site or for individuals to report dizziness and 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 at the site of the blood draw will be cleaned and prepared with a disinfectant wipe before the hypodermic is inserted. In addition, the blood draw will occur while the participant is lying supine. There are no risks or discomforts associated with any of the ultrasound measures. Procedures such as DEXA used during this research study involve X-rays. However, the cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you. Additionally all testing and training will be overseen by individuals certified in CPR and AED. An AED is located in the building where testing and training will occur.

You should report any discomforts or injuries to one of the principle investigators Edward Robinson, 407-823-2367, ned.robinson@ucf.edu or Dr. Jeff Stout, 407-823-2367, jeffrey.stout@ucf.edu.

Benefits

There are no direct benefits to participants.

Compensation or payment:

Upon completion of the study, you will receive a \$100 payment for participation. No compensation will be provided if you are unable to complete the study.

Confidentiality: 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 questionnaire or performance tests will be held in strict confidence. Individual results will remain confidential and only be relayed to the subject 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 5 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 and the ID number will be removed upon disposal. Participant names and I.D. numbers will be stored apart from the blood samples; the identifiers will be removed from the samples and destroyed when the samples are disposed.

Study contact for questions about the study or to report a problem: If you have questions, concerns, or complaints, or think the research has hurt you, please contact Ned Robinson or Dr. Jeff Stout, Human Performance Laboratory, Sport and Exercise Science (407) 823-2367 or by email at ned.robinson@ucf.edu or jeffrey.stout@ucf.edu.

IRB contact about your rights in the study or to report a complaint: Research at the University of Central Florida involving human participants is carried out under the oversight of the New England Institutional Review Board (NEIRB). For information about the rights of people who take part in research, please contact: New England Institutional Review Board, at 1-800-232-9570. You may also talk to them for any of the following:

Your questions, concerns, or complaints are not being answered by the research team.
You cannot reach the research team.
You want to talk to someone besides the research team.
You want to get information or provide input about this research.

Withdrawing from the study:

You have the right to discontinue participation without penalty, regardless of the status of the study. Your participation in the study may also be terminated at any time by the researchers in charge of the project. This could be based upon your refusal to follow study instructions or follow the study protocol. Depending upon when you withdraw, you may be able to receive compensation for the time that you did participate. Please refer back to the "Compensation or Payment" section on the top of this page.

VOLUNTEER'S STATEMENT:

I have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I may contact Edward Robinson if I have any more questions about taking part in this study. Edward Robinson or the company he/she is employed by is being paid by the sponsor for my participation in this study.

I understand that my participation in this research project is voluntary. I know that I may quit the study at any time without harming my future medical care or losing any benefits to which I might be entitled. I also understand that the investigator in charge of this study may decide at any time that I should no longer participate in this study.

If I have any questions about my rights as a research subject in this study I may contact:

New England Institutional Review Board
Telephone: 1-800-232-9570

By signing this form, I have not waived any of my legal rights.

I have read and understand the above information. I agree to participate in this study. I understand that I will be given a copy of this signed and dated form for my own records.

Study Participant (signature)

Date

Print Participant's Name

Person who explained this study (signature)

Date

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