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THE EFFECTS OF CURCUMIN AND FENUGREEK SOLUBLE FIBER ON THE PHYSICAL WORKING CAPACITY AT THE FATIGUE THRESHOLD, PEAK OXYGEN CONSUMPTION, AND TIME TO EXHAUSTION

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THE EFFECTS OF CURCUMIN AND FENUGREEK SOLUBLE FIBER ON THE
PHYSICAL WORKING CAPACITY AT THE FATIGUE THRESHOLD, PEAK
OXYGEN CONSUMPTION, AND TIME TO EXHAUSTION

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Education
at the University of Kentucky

By

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Lexington, Kentucky

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2019

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ABSTRACT OF THESIS

THE EFFECTS OF CURCUMIN AND FENUGREEK SOLUBLE FIBER ON THE PHYSICAL WORKING CAPACITY AT THE FATIGUE THRESHOLD, PEAK OXYGEN CONSUMPTION, AND TIME TO EXHAUSTION

Curcumin, a polyphenol, has been suggested to improve metabolic byproduct clearance and increase nitric oxide production in working muscle. These purported effects may delay neuromuscular fatigue. Therefore, the purpose of this study was to examine the effects of curcumin in combination with fenugreek (CUR) or fenugreek soluble fiber alone (FEN) on the neuromuscular fatigue threshold (PWC_{FT}), time to exhaustion (T_{lim}) on a graded exercise test (GXT), and $\dot{V}O_{2peak}$ in untrained subjects. Forty-seven, college-aged, aerobically untrained individuals were randomly assigned to one of three supplementation groups; placebo (PLA, $n=15$), curcumin + fenugreek, CurQfen® (CUR, $n=18$), or fenugreek soluble fiber (FEN, $n=14$). All subjects performed a maximal GXT on a cycle ergometer to determine the PWC_{FT} , T_{lim} , and $\dot{V}O_{2peak}$ before (PRE-test) and after (POST-test) 28 days of daily supplementation. Statistical analyses included 3 separate, one-way ANCOVAs to determine if there were any differences among the groups (PLA, CUR, FEN) for adjusted post-test scores for the PWC_{FT} , $\dot{V}O_{2peak}$, and T_{lim} . The respective pre-test score was used as the covariate. In addition, reliability analyses (PRE- to POST-test) for the PLA group were used to calculate the minimal difference needed to be real (MD). The adjusted POST PWC_{FT} values showed no statistical differences between groups ($F=3.141$, $p=0.053$); however pairwise LSD

comparisons indicated a significant difference between the CUR and PLA groups ($p=0.016$), but not between the CUR and FEN groups. Therefore, separate one-way ANCOVAs were used to examine the adjusted PWC_{FT} means for the PLA vs. CUR ($F = 4.906$, $p=0.035$) and the PLA vs. FEN ($F = 2.969$, $p = 0.097$). The one-way ANCOVA for $\dot{V}O_2$ peak ($F= 0.612$ $p= 0.547$) and T_{lim} ($F = 0.688$, $p = 0.508$) values showed no statistical difference among the groups. Individual responses in each group showed ~20% of subjects in the CUR group, ~7% in the FEN group, and ~6% in the PLA group had values greater than the MD for the PWC_{FT} , but none of the subjects in the PLA, FEN, or CUR groups exceeded the MD for $\dot{V}O_2$ peak or T_{lim} . These findings indicated CurQfen® supplementation increased the PWC_{FT} compared to a placebo, but not compared to fenugreek soluble fiber alone. However, there were no effects of CurQfen® on $\dot{V}O_2$ peak or T_{lim} . The mechanisms responsible for delaying time to neuromuscular fatigue may include increased NO production and increased blood flow to remove metabolic byproducts; however, the cellular changes which could lead to increases in T_{lim} and $\dot{V}O_2$ peak may not have been sensitive to the GXT protocol or the given dosage of curcumin supplementation. Considering individual responses, CurQfen® supplementation resulted in a real change in the PWC_{FT} for a small portion of the subjects (~20%). These findings suggested that CurQfen® supplementation without exercise training may help to improve time to neuromuscular fatigue in certain individuals, but the group mean analyses were not necessarily reflective of the responses for a majority of the subjects.

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11/21/19
Date

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DEDICATION

To my parents Stephanie and David Herrick, and Grandpa Bob.

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CHAPTER 1. INTRODUCTION

Curcumin is an active ingredient of polyphenolic curcuminoids, which are found in the spice turmeric (Boonla et al. 2014). Curcumin has been well studied in clinical investigations over the last few decades and has been shown to have anti-inflammatory, anti-cancer, anti-diabetic, anti-hypertensive, anti-dementia and antioxidant properties (Boonla et al. 2014). Many of the purported effects of curcumin are related to reducing oxidative stress as well as improving markers of vascular function and cardiac performance in animal models (Boonla et al. 2014). Recent studies, however, have also demonstrated that curcumin supplementation may increase blood antioxidant capacity and reduce exercise-induced oxidative stress in humans (Takahashi et al. 2013). Specifically, curcumin supplementation has been shown to offset muscle damage and functional deficits associated with the eccentric nature of downhill running (Davis et al. 2007). Previous investigators have reported decreased markers of muscle damage, including inflammatory cytokines and creatine kinase after curcumin supplementation compared with a placebo (Davis et al. 2007). These decreases in inflammatory markers may have offset some of the performance deficits associated with eccentric exercise-induced muscle damage (Davis et al. 2007).

Recently, curcumin has been shown to decrease the accumulation of metabolic byproducts such as lactate and ammonia (Sahin et al. 2016) during acute exercise bouts. Specifically, it was reported (Sahin et al. 2016) that decreased levels of lactate and muscle malondialdehyde were associated with increased run times to exhaustion in rodents supplemented with curcumin compared to a placebo. It was suggested that the

increased clearance of metabolic byproducts with curcumin supplementation might potentially be linked to increased production of nitric oxide (NO), a potent vasodilator, and the subsequent vasodilatory response (Boonla et al 2014). Thus, previous research (Boonla et al. 2014, Sahin et al. 2016, Takahashi et al. 2013) has shown the potential for curcumin to have a wide spectrum of bioactivities that may promote health as well as improve endurance performance. There are currently limited data, however, regarding the effects of curcumin supplementation on submaximal and maximal exercise performance in humans (Boonla et al. 2014).

The physical working capacity at the fatigue threshold (PWC_{FT}) is an electromyographic (EMG) technique that theoretically estimates the highest power output that can be maintained for an extended period of time without evidence of neuromuscular fatigue (deVries et al 1987, 1990). Specifically, during a continuous incremental test, the PWC_{FT} is defined as the average of the highest power output that results in no significant change in EMG amplitude (a nonsignificant slope coefficient) and the lowest power output that results in a significant increase in EMG amplitude (a significant positive slope coefficient) (deVries et al 1987, 1990). An important contributing factor to the etiology of neuromuscular fatigue is the accumulation of various metabolic byproducts and/or ions (i.e., inorganic phosphate, hydrogen ions, ammonia and potassium) (Camic et al 2010, Zuniga et al 2010, Bergstrom et al 2013) that interfere with skeletal muscle contractile mechanism and propagation of the action potential along the sarcolemma (Clausen 2013, Enoka and Stuart 1992, Gladden 2004, MacLaren et al 1989, Robergs et al 2004, Westerblad et al 2002). Previously, investigators (Camic et al. 2010) examined the effects of daily oral administration of arginine-based supplements for 4 weeks on the PWC_{FT} in

aerobically untrained men. Like curcumin, one of the physiological actions of arginine is to up regulate nitric oxide (NO) production and increase endothelium-dependent vasodilation (Boonla 2014). The authors (Camic et al. 2010) reported significant increases in the PWC_{FT} after supplementation with arginine compared to a placebo. Thus, it is possible that curcumin supplementation may work similarly to arginine to delay the onset of neuromuscular fatigue and the PWC_{FT} through NO-mediated vasodilation (Boonla et al. 2014) and improved metabolic byproduct clearance (Sahin et al 2016).

Previous investigators (Sahin et al. 2016) have reported increased run times to exhaustion in rodents supplemented with curcumin compared to a placebo. Consistent with the findings by Sahin et al. (2016), Huang et al. (2015) reported that curcumin supplementation dose-dependently increased grip strength and endurance performance compared to the vehicle treatment (Huang et al 2015). Furthermore, previous investigators have shown that curcumin supplementation, in combination with endurance training, has the potential to accelerate mitochondrial biogenesis in skeletal muscle by increasing cAMP levels (Hamidie et al 2015). Peripheral cellular changes, such as increased mitochondrial density, have been shown to improve submaximal performance, (Basset 2000). Thus, it is possible the cellular effects of curcumin to improve markers of aerobic respiration may also delay time to fatigue and improve maximal oxygen consumption ($\dot{V}O_2$) during incremental exercise.

Curcumin has poor absorption into the intestines and consequently low bioavailability in the blood, and thus has been combined with other compounds to improve absorption and increased blood concentration levels (Krishnakumar et al 2012). One ingredient that has been studied in combination with curcumin is the naturally

growing plant, fenugreek soluble fiber (Krishnakumar et al 2012). The galactomannan component of fenugreek has been combined with curcumin (CurQfen®) to increase absorption from the small intestine (Krishnakumar et al 2012). Galactomannan is a soluble fiber guar gum, and a potential active component of fenugreek seeds (Srichamroen et al 2008). Specifically, galactomannans from fenugreek have been shown to have increased effects on glycemic and lipidemic status in rats (Srichamroen et al 2008). Thus, it is possible the fenugreek component in CurQfen® may increase FFA release and decrease insulin levels, supporting improvements in submaximal exercise performance markers. No previous studies, however, have examined the effects of supplementation with curcumin in combination with fenugreek soluble fiber or fenugreek soluble fiber alone on submaximal or maximal indices of endurance performance. Therefore, the purpose of this study was to examine the effects of curcumin in combination with fenugreek (CUR) or fenugreek soluble fiber alone (FEN) on the neuromuscular fatigue threshold (PWC_{FT}), time to exhaustion (T_{lim}) on a graded exercise test (GXT), and $\dot{V}O_{2peak}$ in untrained subjects. Based on the results of previous studies (Boonla et al. 2014, Sahin et al. 2016, Takahashi et al. 2013), it was hypothesized that curcumin supplementation would delay the onset of neuromuscular fatigue and increase the PWC_{FT} as well as increase T_{lim} and $\dot{V}O_{2peak}$ compared to a placebo.

CHAPTER 2. REVIEW OF LITERATURE

2.1 Curcumin: Mechanisms of Action

Boonla et al. (2014)

Previous studies have shown that increased production of oxidants, reduced nitric oxide (NO) bioavailability, and reduced activity of antioxidants in the vascular system are involved in hypertension and cardiovascular disease. Research has found that polyphenols play an important role in the maintenance of health and prevention of diseases. Curcumin is an active ingredient of polyphenolic curcuminoids extracted from the rhizomes of the spice turmeric. Curcumin is well known for its anti-inflammatory, anti-cancer, anti-diabetic, anti-hypertensive, anti-dementia and antioxidant properties and recent studies have shown that curcumin reduces oxidative stress in rodent models. However, there have been no reports about the activity of curcumin in 2kidney-1clip (2K-1C)-induced hypertension-induced endothelial dysfunction and vascular remodeling. Therefore, the present study was designed to investigate the effects of curcumin on 2K-1C - induced hypertension, endothelial dysfunction, vascular remodeling and oxidative stress in male rats. The experimenters randomly placed the rats into the sham operated groups or 2K-1C groups. Hypertension was induced by clipping the left renal artery with a silver clip in groups 3-5. Group 1 rats were sham operated and received the control, propylene glycol, and group 2 sham operated rats received 100 mg/kg/day of curcumin. Groups 3–5 were clipped rats that received propylene glycol alone, 50 mg/kg/day of curcumin and 100 mg/kg/day, respectively. After 6 weeks of treatment, the authors found that curcumin ameliorated hemodynamic performance in 2K-1C hypertensive rats, by reducing blood pressure, increasing hindlimb blood flow and decreasing hindlimb

vascular resistance. The results also showed that curcumin attenuated hypertension-induced oxidative stress and vascular structural modifications. Overall, the authors concluded that the mechanisms responsible for the antihypertensive action of curcumin in 2K-1C hypertension-induced endothelial dysfunction and vascular remodeling involve the improvement of nitric oxide bioavailability and a reduction in oxidative stress.

Sahin et al. (2016)

The purpose of this study was to investigate the effects of curcumin supplementation on oxidative stress markers, exercise endurance, performance, and changes in serum and muscle proteins in rats following a test to exhaustion. This study used 28 male rats, assigned to one of four different treatment groups 1) control, 2) no exercise + curcumin supplement, 3) exercise only 4) exercise + curcumin supplementation. Supplementation was administered every day for 6 weeks in the form of 100 mg·kg⁻¹ CurcuWin oral tablets. The rodents completed a 6-week exercise period in which they exercised on a motor driven rodent treadmill 5 days a week for 45 minutes at a rate of 25 m·min⁻¹. Following the 6-week exercise protocol, the rats were sacrificed and blood and muscle samples were analyzed for muscle markers, oxidative stress, and antioxidant markers. The results showed there were decreased levels of lactate and muscle malondialdehyde in the treatment groups which received the curcumin supplement. The run time to exhaustion also improved in the supplement groups and muscle nuclear factor and heat shock protein levels were significantly lower in group 4 (exercise + curcumin). The results of this study suggested that curcumin may have the ability to help prevent muscle damage during exercise by regulating certain proteins, which can lead to increased endurance and exercise performance.

Takahashi et al. (2013)

The purpose of this study was to investigate the effects of curcumin supplementation on exercise-induced oxidative stress (reactive oxygen species [ROS]) in humans. Ten untrained male subjects (23-30 years of age) were included in this study. Prior to supplementation, the subjects performed a baseline test to determine the $\text{VO}_{2\text{max}}$. Each subject then participated in 3 laboratory-based trials in a randomized order: (1) placebo (control), (2) single curcumin supplementation (90 mg only before exercise) and (3) double curcumin supplementation (90 mg before and immediately after exercise). A washout period of at least one week was included between each condition trial. For each trial, the subjects exercised at 65% of their $\text{VO}_{2\text{max}}$ for 60 minutes. Blood samples were taken pre-exercise, immediately after, and 2 hours post exercise. The results showed that the curcumin supplementation trials had decreased exercise-induced serum concentrations of d-ROMs and TRX-1 and increased serum BAP concentrations and plasma GSH after exercise. There were no changes in heart rate or RPE among the three trials. These findings suggested that curcumin supplementation may increase blood antioxidant capacity, and, therefore help to decrease exercise-induced oxidative stress.

Summary

Curcumin, an active ingredient of polyphenolic curcuminoids extracted from the rhizomes of the spice turmeric is well known for its an anti-inflammatory, anti-cancer, anti-diabetic, anti-hypertensive, anti-dementia and antioxidant properties (Boonla et al 2014). Recently, curcumin has also been shown to decrease hypertension-induced oxidative stress and vascular structural modifications (Boonla et al. 2014). Curcumin has also shown to reduce blood pressure, increase hindlimb blood flow, and decreasing

hindlimb vascular resistance in mice. In addition, curcumin supplementation has been shown to improve NO bioavailability and reduce oxidative stress (Boonla et al. 2014). A study by Takahashi et al. (2013) showed that curcumin also has physiological effects on human subjects as well, including decreased exercise-induced oxidative stress. These findings extended those of the rodent study by Boonla et al. (2014) and suggested curcumin may have the potential to improve endurance performance in humans (Takahashi et al. 2013).

2.2 Physiological and Performance Effects of Curcumin supplementation

Davis et al. (2007)

The purpose of this study was to investigate the effects of curcumin supplementation on muscle damage and functional deficits associated with downhill running in rodents. The heavy eccentric muscle contraction in downhill running has been linked to increased muscle fiber damage, inflammation, and delayed onset muscle soreness (DOMs) as well as several other functional deficits. The mice used in this study were randomly assigned to one of four groups, 1) downhill running + placebo, 2) downhill running + curcumin, 3) uphill running + placebo, or 4) uphill running + curcumin. The “control” in this study was the uphill running group, since it has been shown to produce little to no muscle damage and inflammation. Previous studies have shown that voluntary and involuntary running in rodents was significantly decreased following a downhill run for up to 4 days. The suggested reasoning behind this decrease in performance is due to inflammation including increases in brain IL-1 associated with downhill running. The authors hypothesized that the anti-inflammatory activity of

curcumin would decrease recovery time following a downhill run and increase running performance due to a reduction in inflammation-induced deficits in regeneration of muscle fibers, soreness, and central nervous system fatigue. Curcumin supplementation was administered to the mice in the form of a food pellet containing 10 mg of curcumin powder (Down-Cur and Up-Cur), and the placebo groups were fed a food pellet without curcumin (Down-Plac and Up-Plac)] every day for 3 days prior to the up/downhill run. Following supplementation, the mice performed their initial uphill/downhill run tests. Two groups of mice (Down-Plac and Down- Cur) ran on a rodent treadmill at -14% grade and the other two groups (Up- Plac and Up- Cur) ran at a +14% grade at a rate of 22m/min for 150 minutes. Following the uphill/downhill runs, a subset of mice were used to assess recovery of running performance. These mice ran to fatigue on a motorized treadmill at 36 m/min and 8% grade at either 48 or 72 h post-up/downhill run. A second subset of mice were used to assess recovery of voluntary activity following the up/downhill run on the rodent treadmill. Voluntary wheel running activity during the “active” dark period was measured automatically for 7 consecutive days. The results showed that downhill running was in fact associated with an increase in inflammatory cytokines and creatine kinase, however, the increase in these metabolic byproducts were offset in the group that received the curcumin supplement. The groups who received the curcumin supplement also showed increased voluntary run times as compared to those who received the placebo and the groups who participated in the involuntary run. The importance of these results suggest that the anti-inflammatory phytochemical curcumin can decrease recovery time following high intensity eccentric exercise, known to produce muscle damage and DOMS. Curcumin also appears to be capable of enhancing a

behavioral response (spontaneous running), which implied an important central nervous system component (e.g., drive, motivation) that may be related to less pain and reduced inflammatory cytokines.

Huang et al. (2015)

The aim of this study was to evaluate the potential benefits of curcumin supplementation in a mouse model on physical performance test and exhaustive swimming. The authors hypothesized that curcumin supplementation may mediate exercise-induced metabolites, energy distribution, and even improve physical performance. This experiment used male mice, which were randomly placed into one of four groups to receive treatment by oral gavage: placebo control (water), curcumin supplement at $12.3 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, $24.6 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, and $61.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. The supplementation period lasted a total of 28 days. Each mouse subject was tested for its forelimb grip strength, and completed an exhaustive swimming test to evaluate performance and time to fatigue. The physical fatigue-associated biomarkers included, serum lactate, ammonia, blood urea nitrogen (BUN), and glucose and tissue damage markers such as aspartate transaminase (AST), alanine transaminase (ALT), and creatine kinase (CK). The results of this study showed a dose-dependent response for curcumin supplementation on increased grip strength and endurance performance and significantly decreased lactate, ammonia, BUN, AST, ALT, and CK levels post exercise. Muscular glycogen content, an important energy source for exercise, was also significantly increased in the groups who received the curcumin supplement as compared to the placebo. From these results, the authors concluded that curcumin supplementation may

have a wide spectrum of bioactivities for promoting health, improving exercise performance, and preventing fatigue.

Summary:

Recent studies have observed the physiological and performance effects of curcumin supplementation, particularly in animal subjects. Davis et al. (2007) reported increased voluntary run times for those mice receiving the curcumin supplement compared to those who received the placebo. These results suggested that the anti-inflammatory phytochemical curcumin can decrease recovery time following high intensity eccentric exercise, known to produce the most muscle damage and DOMS. Interestingly, due to the increased voluntary run times, curcumin appears to be capable of enhancing a behavioral response, which implies an important central nervous system component (e.g., drive, motivation) that may be related to less pain and perhaps reduced inflammatory cytokines (Davis et al. 2007). Curcumin supplementation also has an effect on lactate, ammonia, BUN, AST, ALT, and CK (physical fatigue-associated biomarkers) levels post exercise. Huang et al. (2015) found a significant decrease in the levels of these metabolites (lactate, ammonia, etc.) post exercise in the subjects given curcumin supplementation compared with a placebo. The authors (Huang et al. 2015) also reported increased grip strength and endurance performance among the supplemented groups. Muscular glycogen content, an important energy source for exercise, was also significantly increased in the groups who received the curcumin supplement as compared to the placebo (Huang et al. 2015). From the results of these studies (Davis et al. 2007, Huang et al. 2015), it appears that curcumin supplementation may have a wide spectrum

of bioactivities for promoting health, improving exercise performance, preventing fatigue, and even a potential CNS component.

2.3 Timing and Dosage of Curcumin Supplementation

Krishnakumar et al. (2012)

Curcumin has been shown to have many positive physiological actions and has shown not to be toxic even at very high doses of 8–12 g/day, however, despite the safety, efficacy, and well-tracked mechanisms of action, curcumin has poor systematic bioavailability upon oral administration due to inadequate intestinal absorption, rapid metabolism and short half-life. The low bioavailability of curcumin limits its use as a therapeutic agent, therefore the purpose of this study was to investigate the bioavailability of curcumin combined with the spice fenugreek as a vehicle for administration in animals and humans. 8 human male subjects participated in this study. Each subject was tested with the unformulated and formulated curcumin supplements. For the first test, each subject was first given two capsules of 500 mg of unformulated curcumin and 2 ml blood sample were withdrawn at 0, 0.5, 1, 3, 5, 8 and 24 h post-dose for analysis. After 1 week of curcumin feeding, the subjects were given three capsules of 500 mg containing newly formulated curcumin supplement (containing curcumin and fenugreek) and the same protocol was repeated (the dosage of the three formulated capsules was equivalent to 600 mg of curcumin). The same was repeated again after 1 week with 250 mg formulated supplement and the blood samples were withdrawn for analysis (the 250mg dosage was equivalent to 100mg of unformulated curcumin). The results of this study found that in humans, the absorption of curcumin + fenugreek supplement was 15.8 times higher in

oral dosages of 1500 mg (equivalent to 600 mg curcumin), 12.9 times higher at 250 mg (equivalent to 100 mg curcumin) of CGM as compared to 1000 mg of unformulated curcumin. These results indicate that the bioavailability of curcumin is not only increased by combining it with fenugreek, but is also dose dependent.

Summary:

The observed physiologic effects of curcumin and its use as a therapeutic agent have been limited due to its poor systematic bioavailability upon oral administration which is related to its inadequate intestinal absorption, rapid metabolism, and short half-life. Krishnakumar et. al. (2012) investigated the bioavailability of curcumin combined with the herb fenugreek in animals and humans. In the human subjects who received the formulated curcumin supplement, absorption was 15.8 times higher in oral dosages of 1500 mg (equivalent to 600 mg curcumin), and 12.9 times higher at 250 mg (equivalent to 100 mg curcumin) as compared to 1000 mg of unformulated curcumin. The results of this study indicate that the bioavailability of curcumin is dose dependent and increased by combining it with fenugreek.

2.4 Development of the Physical Working Capacity at the Fatigue Threshold Model deVries et al. (1982)

This study examined the relationship between two fatigue thresholds (critical power [CP] and fatigue threshold [FT]), derived from unique methodologies. The CP was determined from four, exhaustive, constant power output cycle ergometer workbouts. The CP was defined as the slope of the relationship between the total work performed and the time to exhaustion for the four workbouts. In addition, the anaerobic threshold (AT) was

determined from gas exchange and ventilatory parameters measured during an incremental test to exhaustion. The FT was then estimated by measuring integrated electromyographic amplitude (IEMG) from the quadriceps femoris muscle during four, constant power output cycle ergometer workouts. This study utilized a unipolar lead system, with surface electrodes placed on the lateral portion of the dominant quadriceps femoris muscle and reference electrode over the anterior superior iliac spine (ASIS). The IEMG data was plotted over 10 second periods as a function of time during three or four workouts of various power outputs on the cycle ergometer. The slope coefficients from each power output were determined and then power output was plotted against the slope coefficients for each of three or four workouts (fatigue slope coefficient on the x axis and power output in W on the y). The FT was identified as the point of zero slope, estimated from the Y intercept value and expressed in W. The FT was defined as the highest constant power output that could be maintained during cycle ergometry without an increase in IEMG activity. This study demonstrated a linear relationship between time and increased IEMG activity ($r^2 = 0.88-0.99$). The FT ranged from 124-284W, with a mean value of 190.5 ± 14.0 W. In addition, the CP and FT as well as the AT and FT were compared. They found that the relationship between AT and FT ($r = 0.903$, $p < 0.001$) was stronger than that between FT and CP ($r = 0.860$, $p < 0.001$) and there was no significance between AT and FT, CP was less than FT. The authors concluded that due to the correlation found among the three variables, CP, AT and FT there may be a single phenomenon that underlies them all, and IEMG estimation of FT may provide an alternative to the measurement of AT.

deVries et al. (1987)

The purpose of this study was to develop a test utilizing electromyographic (EMG) responses from submaximal discontinuous work bouts on the bicycle ergometer to determine the physical working capacity at the fatigue threshold (PWC_{FT}). The subjects for this study included 32 males, 18-29 years old, with fitness levels ranging from highly trained to untrained and sedentary. The same IEMG equipment and procedures were used in this study as in the previous study conducted by deVries et al. (1982). The onset of blood lactate accumulation (OBLA), percent heart rate range (%HRR) at the PWC_{FT} , heart rate- workload (HR-WL) relation, critical power (CP) and ratings of perceived exertion (RPE) at the PWC_{FT} were measured for each subject. These measurements were used to examine the validity of the PWC_{FT} model through correlational and regression analyses. The procedures for measuring the PWC_{FT} included multiple discontinuous work bouts on the bicycle ergometer at increasing intensities, with the measurement of IEMG amplitude. The subject's heart rate returned to within $10 \text{ b} \cdot \text{min}^{-1}$ of the resting value before the next work bout was initiated. The first work bout was performed at $420 \text{ kpm} \cdot \text{min}^{-1}$ and increased by $420 \text{ kpm} \cdot \text{min}^{-1}$ for each subsequent work bout (3 total). The PWC_{FT} was determined as the average of the highest power output with no change in IEMG amplitude over time and the lowest power output that resulted in an increase in IEMG amplitude. Seventeen of the subjects were retested on a different day to determine the reliability of the PWC_{FT} test. The authors found that PWC_{FT} was highly reproducible, with a test-retest correlation of $r = 0.947$ and a SEE of $145.6 \text{ kpm} \cdot \text{min}^{-1}$ (9.7% of the mean PWC_{FT}). The results also showed the OBLA, % HRR, and

HR-WL relationship accounted for 70% of the total variance in the PWC_{FT} . In conclusion, the PWC_{FT} model was shown to be valid and highly reproducible, providing an attractive alternative to supramaximal tests for untrained or sedentary individuals.

deVries et al. (1990)

The purpose of this study was to examine alternative methodological procedures for the determination of the physical working capacity at the fatigue threshold (PWC_{FT}) to improve the practical application and extend the utility of the test. This study examined the use of a continuous test protocol rather than a discontinuous protocol. In addition, the PWC_{FT} estimates were compared for treadmill and bicycle ergometer exercise, from a bipolar electromyographic (EMG) lead system and a unipolar lead system, as well as the potential for residual fatigue from tests repeated 24 hours apart. Twenty subjects participated in this study, with a wide age (19-70 years) range and different fitness levels. Each subject participated in one or more of the experiments conducted. For each experiment EMG data and fatigue curves were recorded as previously described by deVries et al. (1987). The initial power output was set based on the subject's estimate of their fitness level and abilities and was then incrementally increased by 12-25 W at each stage for the bicycle ergometer tests and by one to two percent grade increases for the treadmill test. The first experiment was a comparison continuous versus discontinuous protocol on the bicycle ergometer. For the discontinuous test, the subjects rested until their HR was within $10 \text{ b} \cdot \text{min}^{-1}$ of their resting value between each power output or grade increase. The continuous test followed the same procedures as the discontinuous test except for there was no rest interval between each increase in intensity. The PWC_{FT} was determined as the average of the highest power output with no change in IEMG

amplitude over time and the lowest power output that resulted in an increase in IEMG amplitude. There was no mean difference between the PWC_{FT} estimates from the discontinuous versus continuous test on the bicycle ergometer and they were highly correlated ($r=0.856$). The PWC_{FT} estimates from the treadmill versus bicycle ergometer involved 6 of the 20 subjects, for both the continuous and discontinuous protocols. Since there is no direct measurement of work or power for exercise on a treadmill, to determine PWC_{FT} the highest heart rates (HR) achieved during the two-minute period in which the fatigue threshold was reached (i.e., increase in EMG amplitude) were compared from the bicycle ergometer test and treadmill test. The EMG data were collected from the quadriceps and gastrocnemius muscles for the treadmill test. The results showed that there was no significant difference between the mean HR at the PWC_{FT} between the two different muscle groups, however, the HR at the PWC_{FT} was $20-35 \text{ b} \cdot \text{min}^{-1}$ lower on the bicycle ergometer compared to the treadmill. The unipolar versus bipolar lead systems test showed that the mean voltages from the unipolar measurements were significantly greater than for the bipolar, but they were well correlated ($r=0.841$). The PWC_{FT} estimates from unipolar and bipolar were not significantly different ($t=1.09 \text{ } p>0.05$). Furthermore, the effect of 24-hour repeated tests showed that there was no residual fatigue effect for discontinuous PWC_{FT} tests repeated at a 24 hour interval.

Summary

The Physical Working Capacity at the Fatigue Threshold Model (PWC_{FT}) was developed by deVries et al. (1982) to examine the onset of neuromuscular fatigue. This model utilized surface electromyography (EMG) from the quadriceps muscle. The amplitude (AMP) of the EMG signal was plotted versus time for a series of constant

work rate rides. The PWC_{FT} was defined as the highest constant power output that could be maintained during cycle ergometry without an increase in EMG AMP. deVries et al. expanded upon the initial study, and in 1990 an experiment to determine factors that may affect the estimation of the PWC_{FT} . Overall, it was concluded that the model could be extended to treadmill running, conducted using bipolar or unipolar leads, but most importantly, that a continuous exercise protocol could be implemented instead of the previously utilized, discontinuous protocol.

2.5 Utilization of PWC_{FT} to Examine the Efficacy of Nutritional Supplements to Improve Performance

Camic et al (2010a)

The purpose of this study was to examine the effects of 4 weeks of arginine-based supplementation on the physical working capacity on the fatigue threshold ($PWCFT$). Arginine serves as a precursor in the production of nitric oxide (NO) and plays an important role in endothelium-dependent vasodilation. Due to these physiological effects, arginine supplementation may help to increase blood flow to the myocardium and skeletal muscle. This was a double-blind, placebo-controlled, parallel design which included 50 college aged men who were randomly placed into one of three groups: 1) placebo, 2) 1.5 g arginine or 3) 3.0 g arginine. All of the subjects performed a pre-supplementation (PRE) incremental test to exhaustion on a cycle ergometer to determine the $PWCFT$ and performed the exact same protocol after 4 weeks of arginine supplementation. The subjects were instructed to take one dose (4 pills) of their assigned supplement on an empty stomach immediately after they woke up every morning with 16

oz. of water for 28 days. The subjects were instructed not to change their dietary or exercise habits during the supplementation period. At their post supplementation (POST) visit subjects took one dose 60 minutes prior to the test. The incremental test to exhaustion protocol has previously been described by deVries et. al. (1987) Surface EMG signals were recorded from the vastus lateralis using a bipolar electrode arrangement during the incremental tests for the determination of the PRE and POST supplementation PWCFT values. The results of the study indicated significant increases in the PWCFT values for the 1.5 g (22.4%) and 3.0 g (18.8%) supplement groups, but no change for the placebo group (-1.6%). These findings were likely due to the physiologic actions of arginine combined with grape seed extract, which help reduce the concentrations of metabolic by-products produced during exercise, improved blood flow associated with increased NO synthesis and decreased endothelin production. In conclusion, arginine based supplements can be used as an ergogenic aid for untrained individuals to increase time to fatigue and potentially improve exercise performance.

Summary

Arginine has been shown to serve as a precursor in the production of nitric oxide (NO) and plays an important role in endothelium-dependent vasodilation (Camic et al. 2010a). Due to these physiological effects, arginine supplementation may help to increase blood flow to the myocardium and skeletal muscle and may have an affect on the PWC_{FT}. Camic et al. (2010a) conducted a study in which examined the effects 28 days of supplementation with a placebo, 1.5g arginine, and 3.0g arginine on the PWC_{FT}. The results of the study (Camic et al. 2010a) indicated significant increases in the PWC_{FT} for the 1.5 g (22.4%) and 3.0 g (18.8%) supplement groups, but no change for the placebo

group (-1.6%). These findings were likely due to the physiologic actions of arginine combined with grape seed extract, which help reduce the concentrations of metabolic by-products produced during exercise, improved blood flow associated with increased NO synthesis, and decreased endothelin production. In conclusion, supplementation with a compound (arginine) that improves endothelial function and blood flow can be used as an ergogenic aid for untrained individuals to increase time to fatigue and potentially improve exercise performance.

CHAPTER 3. METHODS

3.1 Experimental Design and Supplementation

The present study used a randomized, double-blind, placebo- controlled, parallel design. For this study, there were three groups, two experimental groups and one control. Due to the low bioavailability of curcumin, it was taken in combination with galactomannan soluble dietary fiber from fenugreek seeds in a supplement called CurQfen®, which has been shown to significantly increase plasma concentrations of curcumin (Krishnakumar et al. 2012). The first visit to the laboratory was to determine each subject's PWC_{FT} -PRE, $\dot{V}O_2$ peak-PRE, and T_{lim} -PRE from graded exercise test to exhaustion (GXT) on an electronically braked ergometer. Following the PRE-test, 47 subjects were randomly assigned to either the control group (placebo: PLA, n=15), CurQfen® (CUR, n=18), or fenugreek soluble fiber (FEN) (n=14) and were given instructions regarding the supplementation process. The supplements were taken daily for 28 days, in the morning before eating, with 16 oz. of water. The CurQfen® dosage was 500 mg with 100 g of CurQfen® powder that contained 37 to 38 g of natural curcuminoids (including curcumin (31g), demethoxy curcumin (6g) and bisdemethoxy curcumin (1.1g)) along with 60 g of de-bitterised fenugreek dietary fiber rich in galactomannans, and a 2 to 4% moisture content. The fenugreek only group was included to account for any extraneous effects of fenugreek soluble fiber and contained 300mg. During the supplementation period, the subjects were instructed not to change their dietary habits or increase their activity level. Food logs were kept for three days prior to the PRE- and POST-test to determine the total grams consumed for each macronutrient as well as total kilocalories. In total, 41 of the 47 subjects completed and returned the food logs for PRE- and POST-test and were included

in the subsequent analyses of total kilocalories and grams of macronutrients consumed. The subjects checked in every week to ensure adherence to the protocol and compliance to the supplementation regimen. To be included in the final analyses, subjects had to demonstrate > 80% compliance (compliance = [# of capsules consumed / # of capsules provided] x 100). After 28 days of supplementation, the subjects returned to the laboratory for their POST-test to determine PWC_{FT} -POST, $\dot{V}O_2$ peak-POST, and T_{lim} -POST.

3.2 Subjects

The subjects recruited for the present investigation were college-aged, aerobically untrained individuals. The subjects were not highly trained, specifically in bicycling, and participated in no more than 3 days a week of regular activity (<4 hours per week). In total, 67 subjects were recruited to participate in this study, and 47 were included in the final analyses. Of the initial 67 subjects, three subjects withdrew from the study due to scheduling conflicts, one withdrew due to illness, and five were excluded due to equipment malfunctions. From the remaining 58 subjects, two were excluded due to an inability to complete minimal stage requirements for the GXT, and nine subjects did not have a detectable PWC_{FT} at either the PRE- or POST-test. All of the 47 subjects included in the final analyses were > 80% compliant (mean $\pm$ SD: 27.88 ± 0.51). All of the subjects completed a health history questionnaire and met the following criteria: (a) no history of medical or surgical events that could significantly affect experimental results or increase the subjects risk of injury, these included cardiovascular disease, metabolic, renal, hepatic, or musculoskeletal disorders; (b) were not taking any medication that

could significantly affect experimental results; (c) not currently using any nutritional supplements that could significantly affect experimental results; and (d) not presently participating in another clinical trial or ingestion of another investigational product. The study was approved by the University's Institutional Review Board for Human Subjects, and all subjects were given a health screening and signed a written informed consent document before testing.

3.3 Determination of $\dot{V}O_2$ peak

Each subject performed a GXT to exhaustion on a calibrated electronically-braked cycle ergometer (Lode Corival, Groningen, The Netherlands) to determine the PWC_{FT} , $\dot{V}O_2$ peak, and T_{lim} at PRE- and POST-test. The ergometer seat height was adjusted so that the subject's legs reach near full extension at the bottom of the pedal revolution. Toe clips were used to maintain pedal contact throughout the test and all subjects were equipped with a nose clip and a 2-way valve mouth piece to collect all expired air. Expired gas samples were collected and analyzed using a calibrated metabolic cart (TrueMax 2400, ParvoMedics, Sandy, UT). The gas analyzers were calibrated with room air and gases of known concentration prior to all testing sessions. In addition, subjects wore a Polar Heart Rate Monitor (Polar Electro Inc., Lake Success, NY), synchronized with the metabolic cart to obtain heart rate measures throughout the ride. The test began at 50 W at a cadence of $70 \text{ rev} \cdot \text{min}^{-1}$ and the power output increased by 30 W every 2 min until voluntary exhaustion or the subject's pedal rate dropped below $70 \text{ rev} \cdot \text{min}^{-1}$ for more than 10 seconds, despite strong verbal encouragement. The $\dot{V}O_2$ peak was defined as the highest $\dot{V}O_2$ value in the last 30 seconds of the test that met two of the following three

criteria: 1) 90% of age-predicted heart rate; 2) respiratory exchange rate > 1.1 ; and 3) a plateau of oxygen uptake (less than $150 \text{ mL} \cdot \text{min}^{-1}$ in $\dot{V}\text{O}_2$ over the last 30 seconds of the test).

3.4 Electromyographic Measurements

The EMG signals were measured from the vastus lateralis (VL) on the dominant leg during the incremental test. Prior to electrode placement, the skin at each site was shaved, carefully abraded, and cleaned with alcohol. A bipolar surface electrode (circular 24 mm, Kendall disposable EMG electrodes, Covidien LTD; Gosport Hampshire, UK) arrangement (2.0 cm center-to-center) was placed based on the recommendations from the SENIAM Project for EMG electrodes placement (Hermans et al. 1999). Specifically, a reference line was drawn over the VL, one-third of the distance between the lateral superior border of the patella and the anterior superior iliac spine. In addition, the electrode-placement site was located 5 cm lateral to the reference line so that the electrodes were over the VL muscle (Malek et al. 2006). A goniometer (Smith & Nephew Rolyan, Inc., Menomonee Falls, WI) was used to orient the EMG electrodes at a 20° angle to the reference line to approximate the pennation angle of the muscle fibers for the VL (Abe et al. 2000). The EMG signal was amplified (gain: $\times 1,000$) using differential amplifiers (EMG 150 BIOPAC Systems, Inc., Santa Barbra, CA, bandwidth = 10–500 Hz).

3.4.1 Signal Processing

The raw EMG signals were digitized at 1,000 Hz, stored in a personal computer (MacBook Pro OSX, version 10.6.8, Apple Inc., Cuperino, CA) for subsequent analysis

and processed with a custom program written with LabVIEW programming software (version 7.1, National Instruments, Austin, TX). The EMG signals will be bandpass-filtered (zero phase shift, fourth-order Butterworth) at 10-500 Hz. Continuous 10 s epochs for the EMG AMP (microvolts root mean square, μV_{rms}) and EMG MPF (MPF in Hz) were calculated. For the MPF analyses, each data segment was processed with a Hamming window and a discrete Fourier transform (DFT) algorithm in accordance with the recommendations of Hermens et al. (1999). The MPF were selected to represent the power spectrum on the basis of the recommendations of Hermans et al. (1999) and was calculated as described by Kwatny et al. (1970).

3.5 Determination of the Physical Working Capacity at the Fatigue Threshold (PWC_{FT})

The protocol used to determine the PWC_{FT} has previously been described in detail by deVries et al. (1987) and was conducted simultaneously with the GXT. Briefly, the test began at a power output of 50 W, and was increased by 30 W every 2 min until voluntary exhaustion or the subject could no longer maintain a pedal cadence of 70 rpm. During each 2-minute power output, 12, 10-second EMG samples were recorded from the VL muscle. The EMG amplitude values for each of the 12, 10-second periods were plotted across time for each power output of the test. The PWC_{FT} was defined as the average of the highest power output that results in a non-significant slope coefficient and the lowest power output that results in a significant positive slope coefficient.

3.6 Statistical Analyses

Separate, one-way ANOVAs were used to determine if there were any significant differences among the PLA, FEN, and CUR groups for age, height, weight, PWC_{FT} , $\dot{V}O_2$ peak, and T_{lim} , at PRE-test. The test-retest reliability (PRE- and POST-test values) for each dependent variable was determined using the $ICC_{2,1}$ model (Weir, 2005) for the 15 subjects in the PLA group. The standard error of the measurement ($SEM = SD\sqrt{1 - ICC}$) and minimal difference need to be considered real (MD) ($MD = SEM \times 1.96 \times \sqrt{2}$) were also calculated for the PLA group for each dependent variable. In addition, three separate paired samples t-test were used to determine if there were any significant changes in the PWC_{FT} , $\dot{V}O_2$ peak, or T_{lim} from PRE- to POST-test for the PLA. Three separate, one-way ANCOVAs were used to examine differences among group for each dependent variable, PWC_{FT} , $\dot{V}O_2$, and T_{lim} . The respective PRE-test value was used as the covariate. The total caloric (kilocalories) and macronutrient (grams of carbohydrate, fat, and protein) intake was analyzed with separate, 3 (Group: CUR, FEN, and PLA) x 2 (Time: PRE and POST) mixed-factorial ANOVAs. A significant main effect was followed up with one-way ANOVAs and independent samples t-test. An alpha of $p \leq 0.05$ was considered statistically significant for all comparisons.

CHAPTER 4. RESULTS

The results of the one- way ANOVAs comparing pre-test values indicated that there were no significant mean group differences for the PWC_{FT} ($F=0.705$, $p=0.500$), $\dot{V}O_2$ ($F=1.367$, $p=0.266$), or T_{lim} ($F=0.988$, $p=0.381$) determined from the GXT or for age ($F=1.469$ $p=0.241$), height ($F=0.050$ $p=0.951$), or weight ($F=1.62$ $p=0.209$) values.

Table 1. Demographic information and PRE test values for time to exhaustion (T_{lim}), $\dot{V}O_2$ peak, and the physical working capacity at the fatigue threshold (PWC_{FT}).

Variable	Placebo (n= 15)	Fenugreek (n=14)	Curcumin (n= 18)
Age (years)	20.27 $\pm$ 1.44	20.86 $\pm$ 1.56	21.78 $\pm$ 3.66
Height (cm)	174.81 $\pm$ 6.77	175.07 $\pm$ 7.58	175.62 $\pm$ 8.09
Weight (kg)	73.24 $\pm$ 12.83	79.91 $\pm$ 12.14	71.90 $\pm$ 13.89
Time to exhaustion (min)	13.87 $\pm$ 2.69	15.13 $\pm$ 2.53	15.25 $\pm$ 3.60
$\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	39.20 $\pm$ 8.95	37.82 $\pm$ 6.35	42.32 $\pm$ 8.22
PWC_{FT} (W)	153.00 $\pm$ 54.93	176.43 $\pm$ 43.12	170.00 $\pm$ 63.64

4.1 Reliability Analysis

The paired samples t-tests indicated that there were no significant mean differences between PRE- and POST-test for the PLA group for the PWC_{FT} ($t = -0.764$ $p = 0.458$), $\dot{V}O_2$ peak ($t = 0.452$ $p = 0.658$), and T_{lim} ($t = -0.137$ $p = 0.893$). The $ICC_{2,1}$ values for the PWC_{FT} , $\dot{V}O_2$ peak, and T_{lim} were 0.834, 0.944, and 0.953, respectively. The SEM and MD values for the PWC_{FT} , $\dot{V}O_2$ peak, and T_{lim} are presented in Table 2.

Table 2. Results of the reliability analyses for the placebo group using PRE- and POST- test values for the physical working capacity at the fatigue threshold (PWC_{FT}), $\dot{V}O_2$ peak, and time to exhaustion (T_{lim}).

Subject	PRE- PWC_{FT} (W)	POST- PWC_{FT} (W)	PRE- $\dot{V}O_2$ peak ($L \cdot min^{-1}$)	POST- $\dot{V}O_2$ peak ($L \cdot min^{-1}$)	PRE- T_{lim} (min)	POST- T_{lim} (min)
1	95	155*	2.41	2.28	12.52	11.02
2	275	275	4.07	4.20	19.01	18.76
3	155	185	3.54	3.24	15.00	15.51
4	185	125**	3.43	2.72	14.39	13.01
5	185	155	3.42	3.51	15.50	15.52
6	155	125	2.47	2.29	12.49	11.52
7	155	155	2.15	2.08	11.01	11.40
8	125	125	2.2	2.14	11.50	11.51
9	215	245	3.61	3.72	17.51	18.40
10	65	95	2.89	3.13	14.01	15.01
11	65	95	1.58	1.40	8.51	8.01
12	125	155	3.01	3.27	15.00	16.50
13	155	155	2.56	2.61	14.00	13.32
14	155	155	2.06	2.22	11.51	12.46
15	185	185	3.31	3.46	16.00	16.51
Mean	153 $\pm$	159 $\pm$	2.85 $\pm$	2.82 $\pm$	13.87 $\pm$	13.90 $\pm$
$\pm$ SD	54.93	49.25	0.708	0.760	2.69	3.02
ICC	0.834		0.944		0.953	
SEM	21		0.171		0.61	
MD	58		0.474		1.69	

*Indicates that the subject had an increase in the PWC_{FT} that exceeded the minimal difference to be considered a real change (MD). ** Indicates that the subject had a decrease in the PWC_{FT} that exceeded the MD.

4.2 Mean Group Comparisons

The one-way ANCOVA for the PWC_{FT} values indicated there were no significant differences among groups ($F = 3.141$, $p = 0.053$), however pairwise comparisons indicated a significant difference between the CUR and PLA groups ($p = 0.016$), but no differences between FEN and CUR ($p = 0.321$) or FEN and PLA ($p = 0.171$). Therefore, separate one-way ANCOVAs were used to examine the adjusted PWC_{FT} means for the PLA vs. CUR ($F = 4.906$, $p = 0.035$) and the PLA vs. FEN ($F = 2.969$, $p = 0.097$). The adjusted PWC_{FT} mean ($\pm SD$) for the CUR ($192.88 \pm 58.41W$; 95% CI 175.98W-209.77W) was significantly greater ($p = 0.035$) than the PLA ($165.55 \pm 49.25W$; 95% CI 147.02W-184.07W). The adjusted POST PWC_{FT} means ($\pm SD$) for the PLA ($166.66 \pm 49.25W$; 95% CI 153.14W-180.18W) and FEN ($183.22 \pm 31.53W$ (95% CI 169.21W-197.23W) were not significantly different ($p = 0.097$) (Figure 1 and 2). The one-way ANCOVA for $\dot{V}O_2$ peak ($F = 0.612$, $p = 0.612$) and T_{lim} ($F = 0.688$, $p = 0.508$) indicated there were no significant differences among groups (Figure 3 and 4).

The 3 x 2 mixed factorial ANOVAs resulted in no significant group x time interactions, main effects for group, or main effects for time for total kilocalories, or carbohydrates (g) and protein (g). However, there was a significant main effect for group for fat (g) ($F = 4.327$, $p = 0.020$). A follow up one-way ANOVA ($F = 4.327$, $p = 0.020$) and between subject t-test indicated that the fat intake (collapsed across time) was greater for FEN compared to PLA ($p = 0.015$) and CUR ($p = 0.014$), but there was no difference between CUR and PLA ($p = 0.954$). Supplement compliance was recorded with supplementation logs and demonstrated a mean ($\pm SD$) compliance rate of $99.5\% \pm 0.51\%$.

Figure 1. Adjusted POST-test physical working capacity at the fatigue threshold (PWC_{FT}) (mean $\pm$ SEM) values (covaried for PRE PWC_{FT} scores) for the feugreek soluble fiber (FEN) and placebo (PLA) groups.

Figure 2. Adjusted POST-test physical working capacity at the fatigue threshold (PWC_{FT}) (mean $\pm$ SEM) values (covaried for PRE PWC_{FT} scores) for the curcumin+feugreek soluble fiber (CUR) and placebo (PLA) groups.

*Significantly (p < 0.05) greater than placebo.

Figure 3. Adjusted POST-test peak oxygen consumption ($\dot{V}O_2$ peak) (mean $\pm$ SEM) values (covaried for PRE $\dot{V}O_2$ scores) for the curcumin+fénugreek soluble fiber (CUR), fénugreek soluble fiber (FEN), and placebo (PLA) groups.

Figure 4. Adjusted POST-test time to exhaustion (T_{lim}) (mean $\pm$ SEM) values (covaried for PRE T_{lim} scores) for curcumin+fénugreek soluble fiber (CUR), fénugreek soluble fiber (FEN), and placebo (PLA) groups.

4.3 Individual Responses

Four of the 18 subjects in the CUR group (Figure 5), one of the 14 subjects in the FEN group (Figure 6), and one of the 15 subjects in the PLA group (Figure 7) had a value greater than the minimal difference for the PWC_{FT} , but none of the subjects in the PLA, FEN, or CUR groups exceeded the MD for $\dot{V}O_2$ peak or T_{lim} (Figures 8-13).

Figure 5. Individual responses for the physical working capacity at the fatigue threshold (PWC_{FT}) from PRE- to POST-test for the curcumin+fenureek soluble fiber (CUR) supplement group. Solid line indicates the subject had an increase greater than the minimal difference (MD=60W). Dashed line (---)= no change, dashed and dotted line (- .) = decrease, and dotted line (...) = increase, not greater than the MD. Note there were 4 subjects who had increases greater than MD, two solid subject lines overlap at 155-245W.

Figure 6. Individual responses for the physical working capacity at the fatigue threshold (PWC_{FT}) from PRE- to POST-test for the fenugreek soluble fiber (FEN) supplement group. Solid line indicates the subject had an increase greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . -) = decrease, and dotted line (...) = increase, not greater than the MD.

Figure 7. Individual responses for the physical working capacity at the fatigue threshold (PWC_{FT}) from PRE- to POST-test for the placebo (PLA) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . -) = decrease, and dotted line (...) = increase, not greater than the MD.

Figure 8. Individual responses for $\dot{V}O_2$ peak from PRE- to POST-test for the curcumin+fenugreek soluble fiber (CUR) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . .) = decrease, and dotted line (...) = increase.

Figure 9. Individual responses for $\dot{V}O_2$ peak from PRE- to POST-test for the fenugreek soluble fiber (FEN) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . .) = decrease, and dotted line (...) = increase.

Figure 10. Individual responses for $\dot{V}O_2$ peak from PRE- to POST-test for the placebo (PLA) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . -) = decrease, and dotted line (...) = increase.

Figure 11. Individual responses for time to exhaustion from PRE- to POST-test for the curcumin+fenugreek soluble fiber (CUR) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . -) = decrease, and dotted line (...) = increase.

Figure 12. Individual responses for time to exhaustion from PRE- to POST-test for the fenugreek soluble fiber (FEN) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . .) = decrease, and dotted line (...) = increase.

Figure 13. Individual responses for time to exhaustion from PRE- to POST-test for the placebo (PLA) supplement group. Solid line indicates the subject had an increase greater than the minimal difference, dashed, double dot, dashed (- . . -) line indicates the subject had a decrease greater than the minimal difference. Dashed line (---) = no change, dashed and dotted line (- . .) = decrease, and dotted line (...) = increase.

CHAPTER 5. DISCUSSION

The primary findings of the current study were that 28 days of curcumin+fenugreek soluble fiber (CUR) and fenugreek soluble fiber (FEN) supplementation did not affect $\dot{V}O_{2peak}$ or T_{lim} , but the PWC_{FT} was greater at POST-test for the CUR, compared to the PLA group. In addition, there was no difference in the PWC_{FT} at POST-test between CUR and FEN. The PWC_{FT} increased PRE- to POST-test by 16.7% in the CUR group, 8.5% in the FEN group, and only by 3.9% in the PLA group. The increase in the PWC_{FT} for the CUR group in this study were consistent with the 22.4% and 18.8% increases in the PWC_{FT} from PRE- to POST-test previously reported (Camic et al. 2010) after 4 weeks of daily oral administration of arginine-based supplements (Camic et al. 2010). Like arginine, one of the potential physiological actions of curcumin is its role as a precursor to nitric oxide (NO) production, which plays an important role in endothelium-dependent vasodilation (Boonla et al. 2014). Thus, the changes in the PWC_{FT} in the CUR supplementation group in the present study were consistent with the changes previously reported following 28 days of a nutritional supplement intervention without exercise training, and may be related to increased blood flow and metabolic byproduct clearance, triggered by increased NO bioavailability, a potent vasodilator (Camic et al 2010).

The changes observed for the PWC_{FT} for the CUR group in this study were likely related to the factors that underlie neuromuscular fatigue and the detection of the threshold. The PWC_{FT} is an electromyographic (EMG) technique that theoretically estimates the highest power output that can be maintained for an extended period of time without evidence of neuromuscular fatigue. Previous studies have examined the potential

mechanisms underlying neuromuscular fatigue, which are reflected by increases in the EMG signal and include additional motor unit recruitment and/or increases in firing rate of the initially recruited motor units to maintain exercise at a constant power output (Camic et al 2010, Zuniga et al). For example, it has been suggested (Camic et al 2010, Zuniga et al 2010, Bergstrom et al 2013) that the fatigue-induced changes in EMG amplitude may be related to the accumulation of various metabolic byproducts and/or ions (i.e., inorganic phosphate, hydrogen ions, ammonia and potassium). Further studies have shown that the accumulation of these metabolites and ions result in fatigue-induced decreases in membrane excitability (Clausen 2013, Enoka and Stuart 1992), excitation-contraction coupling involving Ca^{++} release and uptake from the sarcoplasmic reticulum, myofibrillar Ca^{++} sensitivity for binding with troponin, actin and myosin binding, and ATP production and breakdown (Gladden 2004, MacLaren et al 1989, Robergs et al 2004, Westerblad et al 2002). Other studies attribute the recruitment of additional motor units to maintain force or power output during a fatiguing task to the decrease in intracellular pH as a result of non-mitochondrial ATP hydrolysis, which contributes to the decline in muscle fiber conduction velocity (Lindstrom et al 1970, Moritani et al 1982). It has been hypothesized (Camic et al 2010) that, during incremental cycle ergometry, intracellular $[\text{H}^+]$ may rise to a critical level, decreasing intracellular pH, and interfering with excitation-contraction coupling. These effects may result in fatigue changes in induced motor unit activation and an increase in EMG amplitude observed at the PWC_{FT} .

The physiological mechanisms responsible for the ergogenic effects of curcumin have not been fully identified, but some reported effects include increased metabolic

byproduct clearance (Sahin et al 2016, Huang et al 2015), potentially linked to increased NO bioavailability and the subsequent vasodilatory response (Boonla et al 2014), as well as increased blood antioxidant capacity, and reduced oxidative stress during exercise from decreased levels of reactive oxygen species (ROS) (Takahashi et al 2013).

Curcumin is an active ingredient of polyphenolic curcuminoids, which can be found in the spice turmeric and has been well studied in clinical investigations over the last few decades (Boonla et al 2014). It is possible the purported (Boonla et al 2014, Sahin et al 2016, Takahashi et al 2013) effects of curcumin supplementation to increase clearance of metabolic byproducts through improved endothelial function, increased neuromuscular time to fatigue and the PWC_{FT} in this study. For example, curcumin has been shown to decrease the accumulation of metabolic byproducts such as lactate and ammonia (Sahin et al 2016), metabolites that have previously been linked to neuromuscular fatigue as they accumulate during repeated muscle contractions (Camic et al 2010, Zuniga et al 2010, Bergstrom et al 2013). Thus, it is possible curcumin improves clearance of metabolic byproducts in the muscle cells through NO mediated vasodilation may increase the time to neuromuscular fatigue, and allow the working muscle to perform at a higher power output without the changes in muscle activation, thus delaying the PWC_{FT}.

In the present study, fenugreek soluble fiber was added to increase the bioavailability of curcumin (Krishnakumar et al 2012), but it may also have physiological effects. Fenugreek is a naturally growing plant, originating in India and Northern Africa (Srichamroen et al 2008). The seeds and leaves have been used for hundreds of years in Ayurvedic and Chinese medicine, however recently, preliminary animal and human trials suggest that oral fenugreek seed powder may have possible hypoglycemic and

antihyperlipidemic properties (Basch et al 2003). In the present study, the galactomannan component of fenugreek was used in the CUR supplement to increase absorption from the small intestine (Krishnakumar et al 2012). Galactomannan is a soluble fiber guar gum, and a potential active component of fenugreek seeds (Srichamroen et al 2008). Specifically, galactomannans from fenugreek have been shown to have effects on glycemic and lipidemic status in rats (Srichamroen et al 2008). Specifically, galactomannan-fed rats demonstrated significantly less adipose tissue compared to the control, suggestive of a high metabolic rate in the tissue that were also associated with increased release of FFA into the circulation and decreased plasma insulin levels (Srichamroen et al 2008). The authors suggested that the reduction of plasma insulin level in galactomannan-fed rats may increase lipolysis in adipose tissue, and consequently increase plasma FFA levels and reduce adipocyte size (Srichamroen et al 2008). Thus, it is possible the fenugreek component in the CUR and FEN groups increased FFA release and decreased insulin levels. These factors may have contributed to the increased neuromuscular time to fatigue for the CUR group by delaying the reliance on anaerobic energy production and accumulation of metabolic byproducts from glucose metabolism. This may also explain, in part, the non-significant difference between the CUR and FEN group at POST-test.

In this study, it appears the purported effects of curcumin to increase NO production and aid in metabolic byproduct clearance were effective to increase the submaximal neuromuscular fatigue threshold (PWC_{FT}), but did not affect indices of maximal performance such as T_{lim} and $\dot{V}O_{2peak}$. It is possible the intensity specific effects were related to the factors affecting $\dot{V}O_{2peak}$ and the mode of exercise to

determine T_{lim} . Previous investigators (Sahin et al. 2016) have reported decreased levels of lactate, ammonia, and muscle malondialdehyde in conjunction with increased run times to exhaustion in rodents supplemented with curcumin compared to a placebo. Consistent with the findings by Sahin et al. (2016), Huang et al. (2015) reported that curcumin supplementation dose-dependently increased grip strength and endurance performance and increased muscular glycogen content and reduced levels of physical fatigue-associated biomarkers serum lactate, ammonia, and blood urea nitrogen compared to the vehicle treatment (Huang et al 2015). Each of these previous endurance tests in animal models used submaximal protocols to examine changes in T_{lim} (Sahin et al 2016, Huang et al 2015). In the present study, however, a maximal GXT on a bicycle ergometer was utilized, which may not have been sensitive to the physiological changes induced by curcumin, due to the observed non-significant increases in time PRE to POST. Specifically, although improved metabolic byproduct clearance may have delayed neuromuscular fatigue and the PWC_{FT} , an increase in T_{lim} for the GXT would likely be dependent factors that increased the cellular and blood buffering capacities as well as improved cellular respiration at maximal intensities. Thus, the results of the present study indicated curcumin and fenugreek soluble fiber were effective to delay neuromuscular fatigue but did not increase T_{lim} during a GXT. Future studies should explore the effects of curcumin on the sustainability of submaximal exercise performed at or below the PWC_{FT} to determine if the factors that improved the threshold might also increase T_{lim} .

Similar to T_{lim} , there were no effects of supplementation on $\dot{V}O_2$ peak in the present study. Maximal oxygen uptake ($\dot{V}O_2$ peak) is primarily limited by the ability of the cardiorespiratory system to deliver oxygen to the exercising muscles (Basset et al

2000), but is also dependent on peripheral factors related to skeletal muscle O_2 extraction such as capillary and mitochondrial density (Basset et al 2000), which play a critical role in improving submaximal endurance performance. Of the many factors which may increase mitochondrial biogenesis, effects of endurance training have been well studied and shown to cause an increase in mitochondrial enzyme activities, which improves performance by enhancing fat oxidation and decreasing lactic acid accumulation at a given $\dot{V}O_2$ (Zamora et al 1995). A study by Zamora et al (1995) found that following 10 weeks of endurance training in men there was a significant increase in the number of mitochondria per area and in the relative surface occupied by the total mitochondria profile area. In the present study, it appears 28 days of curcumin supplementation was not effective to alter physiological mechanisms which would lead to increases in $\dot{V}O_2$ peak, however previous research has demonstrated cellular effects of curcumin supplementation in mice that were amplified with exercise (Hamidie et al 2015). Specifically, the combination of curcumin supplementation and endurance training has the potential to accelerate mitochondrial biogenesis in skeletal muscle by increasing cAMP levels (Hamidie et al 2015). In the present study, subjects were only supplemented with CUR for 28 days, during which time they were instructed not to change dietary or exercise habits. Without an increase in endurance training during supplementation time, it is possible that CUR supplementation alone did not have potent enough effects to increase cardiac output, which, theoretically, would result in an increase of $\dot{V}O_2$ peak. Additionally, according to Basset et al (2000), peripheral cellular changes have been shown to improve submaximal performance. Therefore, any potential increases in capillary and mitochondrial density as a result from curcumin supplementation may have

had an effect on the PWC_{FT} , a submaximal work rate, but not $\dot{V}O_2$ peak, which represents maximal exercise. Future studies should aim to investigate whether a longer supplementation period, or higher dosages of curcumin may lead to increases in $\dot{V}O_2$ peak. Furthermore, curcumin supplementation with endurance training should be investigated, to determine if the combination of the two interventions may lead to an increase in $\dot{V}O_2$ peak.

In the present study, a reliability analysis was used to observe individual responses and establish the MD, to determine the magnitude of change in PRE- to POST-test values necessary to be considered real (Weir 2005). The MD speaks to the sensitivity of the test in distinguishing a “real” change from variation or error in measurement (Weir 2005). The MD for the change to be real for the PWC_{FT} was 60 W in this study, which reflected a delay in neuromuscular fatigue by two stages from PRE to POST-test. In the PLA group, one of the 15 subjects had a change greater than the MD, and one subject had a change that was less than the MD (~7% of the subjects). In the FEN group, one of the 14 subjects exceeded the MD (7% of the subjects), and four out of the 18 exceeded the MD (22% of the subjects) in the CUR group. Thus, in general the individual subject responses in the CUR group were not necessarily reflective of the mean response for the CUR group. Therefore, the mean difference at POST-test for the CUR was likely driven by a few high responders (exceeded the MD) and more moderate changes for the remaining subjects (Figures 5-13). Extended to a larger population, 4-weeks of supplementation with curcumin may only be effective for ~20% of individuals. It is possible that the subjects who showed improvement in this study had more potential for change, which predisposed them to be more sensitive to actions of curcumin supplementation. Factors

that may impact an individual's ability to respond to supplementation include prior training status, health history, and potential genetic factors (Roberts et al 2018). Of these genetic factors, Bamman et al. (2007) reported that different skeletal muscle biomarkers exist between high responders, who showed a marked increase in muscle fiber cross-sectional area (fCSA), relative to low responders following 16 weeks of resistance training. A proposed mechanism for this difference in high versus low responders is the difference in levels of skeletal muscle insulin-like growth factor-1 (IGF-1) mRNA variants as well as an mRNA indicative of satellite cell differentiation (myogenin). These markers were expressed higher in the high responders relative to low responders following training (Bamman et al 2007). To date, there is limited evidence whether there are high responders versus low responders to nutritional supplements, and the underlying mechanisms. Future studies should aim to investigate factors affecting a subject's response to nutritional supplementation. Although the subjects in this study reported training frequencies and volume consistent with moderately trained, college aged individuals, there was a wide range of cardiorespiratory fitness levels (very poor to superior) based on $\dot{V}O_{2peak}$ values measured from the GXT (ACSM's Guidelines for Exercise Testing and Prescription). In addition, subjects were eliminated from participation if they had a history of cardiovascular or respiratory illnesses, however, no additional measures of health status were taken beyond resting heart rate and blood pressure. Previous investigators have observed the effects of curcumin on arterial stiffness in mice due to aging and concluded that curcumin supplementation improves large elastic arterial stiffening as a result of aging, NO-mediated vascular endothelial dysfunction, decreases oxidative stress and increases collagen and advanced glycation

end products (AGEs) in mice (Fleenor et al 2013). It is possible that the subjects who demonstrated significant improvements after supplementation were more sensitive to change due to factors like arterial stiffness. Future studies should examine cardiovascular health measures and prior training status to determine if these factors are related to the potential for an individual to respond to curcumin supplementation.

Potential limitations of this study may be the result of the inability of to consistently monitor subject's activities and habits over the duration of the study. To ensure compliance, subjects were given only 7 days of the supplement at a time, along with a dosing log and were required to check in with investigators weekly to report if they had missed any doses before receiving their next supply. Subjects were only included in the study if they were 80% compliant with their supplementation (compliance = (# of doses consumed / total # of doses provided) x 100), however, there was no measure included to determine levels of curcumin in the circulation. Furthermore, to rule out the potential effects of exercise or nutrition on the results, the subjects kept an exercise log over the 28 days as well as a food log for the three days leading up to the PRE-and POST tests and were instructed not to change dietary or exercise habits. Lastly, other factors that may affect performance, such as sleep, and study habits were not measured and quantified, which could have an effect if changed or disrupted over the course of the study.

In conclusion, CurQfen® supplementation increased the PWC_{FT} compared to a placebo, but not compared to fenugreek alone. However, there were no effects of CurQfen® on $\dot{V}O_2$ peak or T_{lim} . The mechanisms responsible for delaying time to neuromuscular fatigue may include increased NO production, and increased blood flow to remove metabolic byproducts. Considering individual responses, CurQfen®

supplementation resulted in a real change in the PWC_{FT} for a small portion of the subjects (~20%). These findings suggested that CurQfen® supplementation without exercise training may help to improve time to neuromuscular fatigue in certain individuals, but the group mean analyses were not necessarily reflective of the responses for a majority of the subjects.

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