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EFFECTS OF CURCUMIN AND FENUGREEK SOLUBLE FIBER SUPPLEMENTS ON SUBMAXIMAL AND MAXIMAL AEROBIC PERFORMANCE INDICES IN UNTRAINED COLLEGE-AGED SUBJECTS

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THESIS

A thesis submitted in partial fulfillment of the
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By

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2019

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EFFECTS OF CURCUMIN AND FENUGREEK SOLUBLE FIBER SUPPLEMENTS ON SUBMAXIMAL AND MAXIMAL AEROBIC PERFORMANCE INDICES IN UNTRAINED COLLEGE STUDENTS

Submaximal exercise performance is, in part, limited by the accumulation of metabolic byproducts and energy system capacities. Curcumin and the combination of curcumin and fenugreek soluble fiber (CurQfen®) have been shown to increase endogenous antioxidants and metabolic byproduct clearance as well as reduce inflammation and lipid peroxidation, and therefore, may enhance submaximal aerobic thresholds. In addition, there is evidence that the galactomannan component of fenugreek, used to enhance bioavailability of curcumin, may also have potential physiological effects related to the up regulation of free fatty acid oxidation Therefore, the purpose of this study was to examine the effects of curcumin and fenugreek soluble fiber supplementation on the ventilatory threshold (VT), respiratory compensation point (RCP), maximal oxygen consumption ($\dot{V}O_2$ peak), and time to exhaustion (T_{lim}) derived from a graded exercise test (GXT). Forty-five untrained, college-aged, male (n = 24) and female (n = 21) subjects (mean age ± SD: 21.2 ± 2.5 yr) were randomly assigned to one of three supplementation groups; placebo (PLA, n=13), 500 mg·day^{-1} CurQfen® (CUR, n=14), or 300 mg·day^{-1} fenugreek soluble fiber (FEN, n=18). All of the subjects completed a maximal GXT on a cycle ergometer to determine the VT, RCP, $\dot{V}O_2$ peak, and T_{lim} before (PRE) and after (POST) 28 days of daily supplementation. The VT and RCP were determined from the V-slope method for the ventilation ($\dot{V}_E$) vs. $\dot{V}O_2$ and $\dot{V}_E$ vs. $\dot{V}CO_2$, respectively. Separate, one-way ANCOVAs were used to examine the between group differences for adjusted POST VT, RCP, $\dot{V}O_2$ peak, and T_{lim} values, with the respective PRE test value as the covariate. The adjusted POST VT-$\dot{V}O_2$ for the CUR (mean ± SD= 1.593 ± 0.157 L·min^{-1}) and FEN (1.597 ± 0.157L·min^{-1}) groups were greater than (p= 0.04 and p= 0.03, respectively) the PLA (1.465 ± 0.155L·min^{-1}) group, but the FEN and CUR groups were not different (p = 0.94). The one-way ANCOVAs for RCP (F = 3.177, p = 0.052), $\dot{V}O_2$ peak (F = 0.613, p = 0.547), and T_{lim} (F = 0.654, p = 0.525) indicated there were no significant differences among groups. These findings suggested that CurQfen® and/or fenugreek soluble fiber may improve submaximal, but not maximal, aerobic performance indices in untrained subjects.

KEYWORDS: Curcumin, Galactomannan, Ventilatory Threshold, Nutritional Intervention, Performance

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Curcumin is a polyphenol that targets multiple signaling pathways and has been shown to positively influence health at the cellular level (Gupta, 2013). It is an active ingredient of a rhizomatous herbaceous perennial plant of the ginger family called turmeric and has been widely used as a spice and medicine in various cultures throughout history (Boonla, 2014; Gupta 2013). These uses range from colorants, cosmetics, teas, and taste enhancers to anti-inflammatory agents and supplements. In populations where curcumin (100–200 $mg \cdot day^{-1}$) is consumed, epidemiological data have indicated the incidences of some chronic diseases (e.g., large bowel cancer) are lower, compared with populations of non-consumption (Mohandas, 1999; Sinha, 2003). Curcumin has been shown to have strong antioxidant, anti-hypertensive, anti-inflammatory, anti-diabetic effects, potential body composition benefits, as well as the positive mediation of various cardio-health risk markers (Sahin, 2016; Gupta, 2013; Anand, 2007, Santos-Parker, 2017). Curcumin supplementation has also been shown to reduce the vascular restructuring and endothelial dysfunction prevalent in diabetes, metabolic syndrome, and hypertension (Boonla, 2014; Santos-Parker, 2017). In addition, curcumin supplementation has been shown to result in an up-regulation of the endogenous nitric oxide (NO) production (Boonla, 2014; Gupta S.C., 2013), which mediates endothelial-dependent vasodilation. Therefore, it is possible curcumin may enhance blood flow to the working cardiac and skeletal muscles (Santos-Parker Jessica R., 2017; Boonla, 2014; Gupta S.C., 2013).

One of the primary limitations to curcumin supplementation is its poor bioavailability. Curcumin has a poor absorption, rapid metabolism, and rapid systemic elimination (Krishnakumar, 2012; 2014). These characteristics result in the inability for

curcumin supplementation alone to be effective at increasing plasma and tissue concentrations of curcumin to physiologically relevant values of 0.1 micromolar in vitro (Sharma, 2004). It has been documented (Anand, 2007) that even at high doses ($12g \cdot day^{-1}$), the plasma and tissue concentrations are still lower than the necessary threshold for physiological effects. Therefore, approaches to slow digestion of curcumin, increase its absorption, and slow its systemic elimination have been examined (Krishnakumar, 2014; Lao, 2006; Tu, 2014). For example, curcumin has been combined with piperine, which interferes with glucuronidation (Tu, 2014) as well as fenugreek, where the combination slows release and protects curcumin from acidic gastrointestinal conditions (Krishnakumar, 2012). A liposomal curcumin has also been developed that has been shown to slow systemic elimination (Li, 2007). Curcumin, combined with fenugreek soluble fiber (galactomannans) has been shown to positively affect the bioavailability of curcumin, by increasing the absorption and saturation to up to 20 times compared to curcumin alone (Krishnakumar, 2014).

The main role of fenugreek soluble fiber in combination with curcumin is to increase plasma and tissue concentrations of curcumin by slowing down its digestion and elimination (Neelakantan, 2014; Srichamroen, 2008; Krishnakumar, 2012). However, it is important to note that galactomannan supplementation alone has potential physiological effects (Mathern, 2009; Poole, 2010). Previous investigators have shown significantly slower gastric emptying, increased plasma sensitivity, decreased plasma insulin levels, decreased hepatic cholesterol concentration, and increased plasma free fatty acid (FFA) levels in circulation after 28 days of supplementation of galactomannans from fenugreek (Srichamroen, 2008; Mathern, 2009). These effects, particularly decreased plasma insulin

levels and increased FFA levels, have been linked to increased rates of the FFA oxidation (Neelakantan, 2014; Romijn J. A., 1985; Srichamroen, 2008). Thus, in addition to increasing the absorption of curcumin from the small intestine, fenugreek may also have the potential to improve metabolic parameters.

A number of previous investigators have examined the effects of curcumin on indices of vascular function and other markers of cardiovascular health (Gupta, 2013Boonla, 2014; Cheng, 2001). The purported effects of curcumin on inflammatory pathways, nitric oxide production, and anti-oxidative capabilities (Boonla, 2014; Huang, 2015, Sahin, 2016), have recently lead to the examination of curcumin as an ergogenic aid to delay fatigue and enhance recovery from exercise (Huang, 2015; Davis, 2007; McFarlin, 2016). In general, these studies have demonstrated improvements in measured thresholds at submaximal levels (ventilatory threshold and gas exchange threshold). Curcumin has been shown to significantly decrease cytokine production in inflammatory pathways as well as reduce various markers of exercise-induced muscular damage (EIMD) from repeated eccentric muscle actions (Davis, 2007; McFarlin, 2016). These effects have resulted in lower decrements in grip strength after fatiguing eccentric exercise (Huang, 2015) compared with placebo. There is also evidence curcumin may increase glycogen stores following 28 consecutive days of supplementation (Huang, 2015) as well as decrease the accumulation of metabolic byproducts (i.e., hydrogen ions, ammonia, etc.) (Sahin, 2016), which may increase the time to fatigue and enhance recovery from longer duration (>60 min) exercise (Huang, 2015; Davis, 2007; McFarlin, 2016). For example, Huang et al. (2015) showed curcumin supplementation significantly increased swim time to exhaustion in mice, dose-dependently, while decreasing injury markers by approximately

fifty percent, when compared to the placebo. Thus, currently there is evidence curcumin may enhance endurance performance and increase time to exhaustion as well as improve recovery from EIMD (Huang, 2015; Davis, 2007; McFarlin, 2016).

Fatigue thresholds such as the ventilatory threshold (VT) and respiratory compensation point (RCP) provide a non-invasive assessment of metabolic responses during incremental exercise (Beaver, 1986; Gaskill, 2001). Theoretically, the VT demarcates the moderate from heavy exercise intensity domain (Burnley & Jones, 2007; Gaesser & Poole 1996), and provides information about the exercise intensity above which aerobic adenosine triphosphate (ATP) production is supplemented with anaerobic energy metabolism. Exercise performed above the VT (within the heavy domain) results in increased blood lactate concentration and hydrogen ion (H^+) production (Gaesser & Poole, 1996). The VT reflects the increased ventilation ($\dot{V}_E$), relative to oxygen consumption ($\dot{V}O_2$) in response to excess carbon dioxide (CO_2) generated from the bicarbonate buffering of the H^+ (Beaver, 1986). The VT has been used to assess physical fitness in both clinical (Thin, 2002) and athletic populations (Malek, 2007) and has been shown to be sensitive to training and nutritional interventions (Jones, 2000)

The RCP, theoretically, demarcates the heavy from severe exercise intensity domains and defines the threshold where minute ventilation ($\dot{V}_E$) increases at a greater rate than the volume of CO_2 ($\dot{V}CO2$) produced (Beaver, 1986; Bergstrom, 2013). Exercise performed above the RCP (within the severe domain) relies on increased rates of anaerobic energy metabolism to supplement aerobic ATP production compared with exercise in the heavy domain (below the RCP) (Gaesser & Poole, 1996). The RCP reflects the hyperventilation that occurs when the bicarbonate buffering system is overwhelmed by the

H^+ production (Beaver, 1986) from anaerobic energy metabolism. Exercise performed above the RCP is typically not sustainable for more than 20 minutes, while exercise below this threshold can be maintained for at least 30 minutes (Bergstrom, 2013). Thus, the RCP provides a non-invasive assessment of the highest sustainable exercise intensity (Bergstrom, 2013; Gaesser & Poole, 1996)

Previous studies have indicated the potential for curcumin supplementation to increase NO production, decrease metabolic byproduct accumulation (Sahin, 2016), and increase time to exhaustion (T_{lim}) (Huang, 2015; Davis, 2007; McFarlin, 2016). It is possible these effects may also improve submaximal fatigue thresholds (VT and RCP) as well as increase the $\dot{V}O_2$ peak and T_{lim} on a graded exercise test (GXT). In addition, there is evidence that galactomannan component of fenugreek, used to enhance bioavailability of curcumin, may also have potential effects on up regulating FFA oxidation (Srichamroen, 2008; Neelakantan, 2014). This may delay the reliance on anaerobic energy production and the increase the VT. No previous studies, however, have examined the effects of curcumin on submaximal and maximal endurance performance markers such as the VT, RCP, $\dot{V}O_2$ peak, and T_{lim}. Therefore, the purpose of this study was to examine the effects of curcumin and fenugreek soluble fiber supplementation on the VT, RCP, $\dot{V}O_2$ peak, and T_{lim} derived from a GXT. We hypothesized that 28 days of curcumin and fenugreek soluble fiber supplementation would result in increases in the VT, RCP, $\dot{V}O_2$ peak, and T_{lim} compared to a placebo.

CHAPTER 2. LITERATURE REVIEW

2.1 Curcumin: Mechanisms of Action

Boonla et al. 2014

Curcumin (diferuloymethane) has been suggested to have anti-inflammatory, anti-hypertensive, anti-diabetic and antioxidant properties and has been shown to inhibit migration of vascular smooth muscle cells and reduce oxidative stress in hypertensive rats. The purpose of this study was to determine whether curcumin can reverse hypertension, endothelial dysfunction, and vascular structural remodeling in hypertensive rats. Hypertension was induced in male Sprague-dawley rats by clipping the left renal artery with a silver clip (0.2mm. The rats were divided into five groups of 16: 1) sham vehicle only; 2) sham with 100 mg curcumin (Cur100); 3) Hypertension induced with vehicle; 4) Hypertension induced with 50 mg curcumin (Cur50); and 5) Hypertension induced with Cur100. A dose of Cur100 in rats corresponded to a 950mg dose for a 60kg human. Dosing was maintained for six weeks after a five-day post-surgery recovery. The rats were assessed for angiotensin converting enzyme, vascular reactivity testing and enzymes affecting nitric oxide production and endothelial dysfunction. The aortas of the rats were embedded and stained for morphometric analysis. The results of this study showed that curcumin attenuated systolic blood pressure and hemodynamic disturbances in a dose-dependent manner, but there were no hypotensive effects for the sham animals. Assessment of the isolated aortic rings showed significant impairment in response of acetylcholine (Ach) in the hypertension rats compared to the sham; treatment with curcumin significantly enhanced ACh response ($p<0.05$) and had increased nitrite/nitrate levels. In addition, curcumin supplementation prevented morphological changes in the hypertension-induced

rats. Curcumin treated rats had attenuated hypertension-induced increases in enzymes that remodel the endothelial wall. Finally, angiotensin converting enzyme (ACE) levels superoxide production, and protein carbonyl levels that were increased due to hypertension treatment were significantly attenuated by curcumin treatment. It was suggested that curcumin directly scavenged free radicals and decreased ROS activity. The main findings of this study suggested that curcumin treatment ameliorated blood pressure increases, improved endothelial function, and prevented vascular remodeling. The key finding was that oral supplementation of curcumin (400mg·day^{-1} significantly blunted serum creatine kinase and inflammatory cytokines concentrations (IL-8 and TNF-α) during recovery post EIMD.

Sahin et al. 2016

The present study was undertaken in an animal model to investigate the effects of the water-soluble curcumin formulation (CurcuWIN) on oxidative stress markers, exercise (Ex) time of exhaustion, and the antioxidant status in muscles. A total of 28 Wistar rats were divided into four arms: control (no exercise or CurcuWIN), No Ex + CurcuWIN, Ex + no CurcuWIN, and Ex + CurcuWIN. CurcuWIN was administered at 100 mg·kg^{-1}, providing 20 mg of CurcuWIN daily for 6 weeks. The CurcuWIN dose at 100 mg·kg^{-1} was chosen based on previously reported value for effective antioxidant activity in rodents (Ma, 2013; Anand, 2008). The exercise protocols were performed on a motor driven rodent treadmill over a 5-day period. The Ex protocol was as follows: day 1 – 10 m·min^{-1} for 10 mins, day 2 – 20 m·min^{-1} for 10 mins, day 3 – 25 m·min^{-1} for 10 mins, day 4 – 25 m·min^{-1} for 20mins, day 5 – 25 m·min^{-1} for 30 mins. Animals were euthanized after the last exercise within the same hour and the blood, muscle and tissue samples were stored for

injury and oxidative stress markers. The sample size was based on a power of 85% to obtain a P-value of 0.05 and seven animals per treatment were examined. ANOVA and Tukey tests for post hoc analyses were conducted on each dependent variable between treatments and within treatments. The results showed that time to exhaustion was lower for the control (no exercise rats, no CurcuWIN) and no exercise rats with CurcuWIN (average of 72 mins) versus the exercise rat groups (average of 174 mins), ($P<0.01$). Within the exercise rats, CurcuWIN supplementation significantly affected the time to exhaustion (Ex: 173.45 mins, Ex w/ Cur: 185.14mins) ($P<0.05$). Chronically exercised rats had less cardio-metabolic health markers than controls ($P<0.001$) and the cardio-metabolic health markers in Ex + CurcuWIN groups were decreased significantly compared to the other groups. Serum low-density lipoprotein cholesterol (LDL-C) levels were reduced in the Ex + CW treatment groups (7.00 ± 1.55 mg·dL^{-1}) compared to those in the untreated rats (avg: 10.7 mg·dL^{-1}) ($P<0.0001$). The serum lactate levels in the Ex + CW group was decreased compared to all other groups ($P<0.0001$). Curcumin supplementation and exercise significantly ($P<0.001$) decreased muscle oxidative stress metabolites (control: 74.29 ± 7.48 nmol·mg protein, Ex w/ Cur: 42.00 ± 2.65 nmol·mg protein) and increased antioxidant enzymes compared to all other groups. This study shows that CW supplementation enhances the antioxidant activity by upregulating antioxidant enzymes production, downregulating a transcription factor that affects multiple inflammatory pathways, and was shown to increase time to exhaustion during exercise.

Summary:

Curcumin is an extremely versatile chemical noted for its ability to mediate mechanisms and cytokines affecting inflammatory pathways, reversing blood pressure, and even

physiological restructuring such as vascular remodeling and endothelial dysfunction. It increases response to neurotransmitter acetylcholine, enzymes affecting nitric oxide production, and attenuates hypertensive response enzymes (Boonla, 2014). Curcumin also has been shown to increase antioxidant capacity by upregulating antioxidant enzymes production, positively affect numerous cardio-health markers, decrease low-density lipoprotein cholesterol levels, and ameliorate effects of exercise-induced muscle damage (Sahin, 2016). Curcumin supplementation also decreases the weight of the epididymal fat pad significantly in a murine model (Huang, 2015). While there is a suggested serum concentration level for effectiveness, it can be noted that the effects of curcumin supplementation are dose-dependent (Boonla, 2014; Huang, 2015; Lao, 2006)

2.2 Physiological and Performance Effects of Curcumin Supplementation

Huang et al. 2015

This study aimed to evaluate the potential benefits of curcumin (CCM) supplementation in a mouse model of physical performance test and exhaustive swimming. It was hypothesized that CCM supplementation may decrease exercise-induced metabolites, energy distribution, and improve physical performance. The dosage of CCM given to the rats was based on the daily-recommended dose of CCM at 60 $mL \cdot serving^{-1} \cdot day^{-1}$ for humans (mouse CCM dose @12.3 $mL \cdot kg^{-1}$). The mouse CCM dose (12.3 $mL \cdot kg^{-1}$) we used was converted from a human equivalent dose (HED) based on body surface area by the following formula from the US Food and Drug Administration: assuming a human weight of 60 kg, the HED for 60 (mL) $\cdot 60\ (kg)^{-1} = 1 \times 12.3$ = a mouse dose of 12.3 $mL \cdot kg^{-1}$; the conversion coefficient 12.3 was used to account for differences

in body surface area between mice and humans (Chen, 2014). The mice were split into four groups: vehicle, 1 x recommended dosage, 2 x dosage, and 5 x dosage (10 mice per group). The dependent variables tested were forelimb grip strength, swim exercise performance test, blood biochemical variables related to fatigue and injury, glycogen, and a biochemical profile. The differences were analyzed by a one-way ANOVA and the Cochran–Armitage test for dose-effect trend analysis. The results showed that grip strength was higher by 1.2 and 1.34 times in the CCM1x and 5X group respectively compared to the vehicle, with a dose-dependent increase on the trend analysis ($P<0.0001$). The exhaustive swimming times in the CCM1x, CCM2x, and CCM5x dosage swim times were longer by 1.98, 2.17, and 2.22-fold, respectively, to the vehicle. The exercise fatigue biochemical and induced injury indicators all showed significant dose-dependent effects in which all were decreased by levels averaging approximately 40%, up to 60% (creatine kinase). The muscle glycogen levels were significantly increased by 1.39-1.49- fold with CCM supplementation. Finally, CCM supplementation also decreased the weight of the epididymal fat pad significantly. This study found that CCM supplementation improved exercise performance including grip strength and endurance by increasing muscle glycogen content and had significant benefits for physiological indicators after exercise.

Davis et al. 2007

The purpose of this study was to evaluate the potential benefits of curcumin supplementation using an eccentrically biased downhill treadmill running murine model. The pilot study from the same research team proved that voluntary and involuntary running were significantly reduced for up to 4 days after a bout of downhill running in mice. The mice were randomly assigned to four groups of uphill/placebo, downhill/placebo,

uphill/curcumin, and downhill/curcumin with placebo and curcumin given 3 days prior to each exercise session. The first experiment was a treadmill run to fatigue at an 8% incline or decline. The second experiment was an assessment of voluntary activity on the exercise wheel following the exercise bout session. The third arm of the study was analysis of muscle cytokine and plasma creatine kinase inflammatory and damage markers. These data were analyzed using a 2-way ANOVA. The results showed that downhill running significantly reduced treadmill run to fatigue time as compared to the uphill run; curcumin blocked over 100% reduction in treadmill times for the downhill, and no supplementation effect was shown for uphill running. Curcumin also completely blocked reduction in voluntary running times post exercise session. Plasma creatine kinase was significantly elevated following downhill run compared to the uphill run. Curcumin blunted the increase for the downhill run ($P<0.05$), with no effect shown for the uphill run. Finally, curcumin feedings blunted the increases in all muscle cytokine levels (IL-β, IL-6, TNFα: 24hr, and 48hr). The primary results of this study suggested that curcumin may speed recovery of voluntary and involuntary running performance following exercise induced muscle damage. The study also concluded that since anti-inflammatory properties of curcumin directly influence inflammatory regulators and mimic similar activity of non-steroidal anti-inflammatory drugs, but without many of the same side effects, curcumin could possibly replace repeated usage of NSAIDs in addressing inflammatory issues.

McFarlin et al. 2016

Curcumin modifies the signaling pathway of inflammatory cytokines and reduces its production. This known ability is similar to the effect of non-steroidal inflammatory drugs, making curcumin an ideal supplement for the treatment of exercise-induced

muscular damage (EIMD) and delayed onset muscle soreness (DOMS). The purpose of this study was to determine the effects of an optimal dosage of bioavailable oral curcumin supplementation (400mg·day^{-1}) on subjective quadriceps muscle soreness, serum creatine kinase (CK), and serum inflammatory cytokines following 60 repetitions of eccentric-only dual leg press exercise at 100% of the 1RM. A pilot study was done to determine the optimal dosage of oral curcumin with 200, 400, and 1000mg. A curcumin dose of 400mg·day^{-1} (effect size=0.42 compared to placebo) resulted in at least 19% blunting of inflammatory cytokines compared to 6% in a 200mg·day^{-1} dose (effect size=0.20 compared to placebo) at one to two days. There was no significant increase in effect size of 1000mg·day^{-1} dosage (effect size = 0.44 compared to placebo) compared to the 400 mg·day^{-1} dosage (effect size=0.42 compared to placebo). Forty subjects were randomized into placebo and supplement (curcumin at 400 mg·day^{-1}) groups. Ten days before completing a muscle damaging session, the subjects were required to complete a muscle strength test for a 1RM and familiarization for the eccentric session. The subjects were given either the supplement or placebo 2 days prior to EIMD and 3 days after. For the eccentric session, the subjects completed 6 sets of 10 repetitions with 5 second eccentric contractions at a beginning load of 110% of their 1RM. The resistance was reduced by 2.2kg if subject was unable to maintain the 5 second contraction and continued for subsequent sets. The subjects were given 5 mins of passive seated rest between sets. The variables for assessment were subjective quadricep muscle soreness, activities of daily living soreness, serum creatine kinase, and serum inflammatory cytokines. The results showed no significant difference in supplemental effects of muscle soreness for both subjective quadriceps and activities of daily living soreness. For serum creatine kinase,

curcumin supplementation resulted in a significant blunted CK response after EIMD session day: Day 1 (-44%), Day 2 (49%), Day 3 (57%), Day 4 (69%) compared to the placebo. The curcumin group had a significantly blunted increase in CK after EIMD that returned to baseline at day 2 post damage. Curcumin supplementation significantly decreased 3 out of the 4 inflammatory cytokines tested with an average of 20% decrease. The key finding of this study was that oral supplementation of curcumin (400 $mg \cdot day^{-1}$) significantly blunted serum creatine kinase and inflammatory cytokines concentrations (IL-8 and TNF-α) during recovery post EIMD for up to 4 days but had no effect on the perceived soreness.

Summary:

Curcumin's anti-inflammatory, anti-oxidative, blood flow facilitative, and a host of other benefits has a profound impact on not only the mental and medical applications, but also in the realm of performance capability and physiological enhancement during exercise (Davis, 2007; Huang, 2015; McFarlin, 2016). In a murine model study, Huang et al. (2015) showed dose dependent effects of curcumin supplementation on increased grip strength, glycogen stores, and exhaustive swim times as well as decreased markers of fatigue (e.g., lactate, ammonia, blood urea nitrogen, and creatine kinase) compared to a placebo. Another murine model study showed that during fatiguing exercise, curcumin supplementation compared with a placebo, negated the reduction in time to fatigue, significantly speed up recovery time, and significantly blunted increases in injury and inflammatory pathways in manners mirroring use of non-steroidal anti-inflammatory drugs post-activity (Davis, 2007). In addition, McFarlin et al. (2016) reported curcumin supplementation significantly blunted injury markers and inflammatory cytokine levels

post eccentric workout session in humans when compared to a placebo but had no effect on perceived soreness.

2.3 Physiological and Performance Effects of Fenugreek Supplementation

Poole et al. 2010

The purpose of this study was to determine the effects of a commercially available supplement containing *Trigonella foe- num-graecum* (fenugreek) on strength, body composition, power output, and hormonal profiles in resistance-trained males over the course of a structured resistance-training program. This study included 49 resistance-trained males at an average age of 20 years old. This was a double-blind, placebo-controlled design with parallel groups matched by weight. The two groups were randomly assigned to the placebo (N = 23) or the supplement (N = 26) condition. The independent variables were the condition (500mg placebo or 500mg fenugreek supplement) and the time points (week 1, week 4, and week 8) and the dependent variables were the estimated dietary energy intake, body composition, upper and lower body 1-RM strength, muscle endurance (80% of 1RM), anaerobic sprint power, fasting clinical blood profiles, anabolic/catabolic hormones, and metabolic hormones (insulin and leptin). The performance measures, fasting clinical blood profiles, and hormone levels were assessed at week 1 (prior to training), week 4 (mid-training cycle), and week 8 (post-training). All food and fluid intakes were recorded four days prior to each testing day. The capsules were ingested once per day in the morning on non-training days and before the workout on training days. The training protocol consisted of a periodized 4-day per week resistance-training program, split into two upper and two lower extremity workouts per week, for a total of 8-weeks.

The results showed a significant increase in lean body mass at week 4 and 8 ($P<0.001$) compared to baseline for the fenugreek group, with no changes observed in the placebo group ($P<0.005$). There were significant decreases in body fat percentages ($P<0.001$) at weeks 4 and 8 in the fenugreek group compared to baseline, but there were no changes for the placebo group ($P<0.005$). A significant group × time interaction ($p = 0.008$) and main effect for time ($p < 0.001$) was observed between fenugreek and placebo groups for bench press 1-RM, however pairwise comparisons revealed no significant differences between fenugreek ($P<0.001$) and placebo ($P<0.008$) bench press 1-RM's at any time point. Pairwise comparisons indicate significant difference in the fenugreek (334 ± 74 - 419 ± 87 kg) and placebo groups (316 ± 63 - 364 ± 68kg) for leg press at week 8 compared to baseline ($P<0.019$). A significant main effect for time ($p = 0.002$) was observed for Wingate peak power FEN (1141 ± 222 - 1183 ± 200) to PLA (1091 ± 215 - 1132 ± 237), and further pair-wise comparison showed a significant increase in peak power for FEN at week 8 ($p = 0.008$) compared to the placebo. There were no significant interactions or effects for clinical blood profiles. No significant between or within group changes occurred for any other serum hormone variables ($p > 0.05$) but free testosterone with significant differences between groups at week 4 ($p = 0.018$) FEN (40 ± 33 - 33 ± 22) and week 8 ($p = 0.027$) FEN (40 ± 33 - 36 ± 22). The findings of this study suggested that ingesting 500 mg of a commercially available botanical extract once per day for eight weeks in conjunction with a structured resistance training program can significantly impact body composition and strength in resistance trained males when compared to a placebo.

Summary:

Fenugreek (*Trigonella foenum-graecum)* is an herb widely used and consumed in India. Fenugreek is also a commercially used as a supplement with purported benefits on increased strength and improved body composition (Poole, 2010). Fenugreek supplementation has been shown to increase free testosterone, strength in resistance training (e.g., bench press, leg press), and peak power output, and significantly decrease body fat percentages, to a greater degree than a placebo, when used in conjunction with exercise programs (Poole, 2010).

2.4 Dosage and Bioavailability of Curcumin Supplementation

Lao et al. 2006

Few systematic studies have been done on the toxicology of curcumin in humans; though up to 8000mg dosing shows minimal toxicity and peak plasma concentration have been identified at 1-2 hours after singular dose of 4000mg or higher. (Cheng et al, 2001). This study aimed to determine the maximum tolerated dose, safety profile, and resultant serum concentration of a single dose of standardized curcumin powder extract obtained from Alleppey finger turmeric (C3 Complex™, Sabinsa Corporation). This dosage contained a minimum 95% concentration of three curcuminoids: curcumin, bisdemethoxycurcumin, and demethoxycurcumin. The sample size consisted of 24 participants (13 male, 11 female) with a mean age of 34 years and who had not consumed any curcumin rich food within the past 14 days. The study had five dosage levels (1000mg, 4000mg, 8000mg, 10000mg, and 12000mg) with 3 subjects in each level. Safety was assessed for 72 hours after dosing where blood specimens were obtained prior to, one hour, two hours, and four hours after dosing. The results showed 7 adverse results all at grade 1,

spread evenly among the dosing where grading was based on the *National Cancer Institute, Common Toxicity Criteria version 2.0 [10].* No toxicity appeared to be dose-related. Curcumin was detected only in the serum of two subjects taking the 10000mg and 12000mg dose levels (10000mg 1hr: 30.4ng·ml^{-1}, 2hr: 39.5ng·ml^{-1}, 4hr: 50.5ng·ml^{-1}; 12000mg 1hr: 29.7ng·ml^{-1}, 2hr: 57.6ng·ml^{-1}, 4hr: 51.2ng·ml^{-1}). The authors of this study concluded there was minimal toxicity of curcumin supplementation up to a 12000mg dosage level from a standardized powder extract obtained from Alleppey finger turmeric, and low levels of curcumin were only found in doses higher than 8000mg.

Krishnakumar et al. 2014

The purpose of this study was to examine an original formulation for enhancing the bioavailability of curcuminoids through use of an extensive gel-forming non-digestible soluble dietary fiber galactomannan, containing proteins from fenugreek. Curcuminoids are impregnated in the soluble fiber matrix to produce microencapsulates that has unique binding, enhanced solubility, and degradation protectivity of curcuminoids from the upper gastrointestinal tract environment, and facilitation of slow release for better absorption. The fenugreek polysaccharide is non-digestible and swells extensively in the upper GI tract; the curcumin that is bound to this gel matrix is protected from the enzymes responsible for rapid degradation, is very stable, and leaches out very slowly. The advantage of this system of delivery over prior art is the negating of the limitation of poor bioavailability via oral delivery (20 times greater than that of 95% unformulated curcumin absorption), the saturation rate is at a physiologically relevant level (minimum concentration of 0.1uM in plasma in vitro), for a considerable duration (5-hour peak compared to the 3 hour peak of unformulated curcumin). In addition, 24 hours past dosing,

the curcumin concentration was 0.21ug·g^{-1}, compared to 0.0008ug·g^{-1} of the unformulated curcumin. Therefore, these findings suggested the combination of fenugreek and curcumin, enhanced the bioavailability of curcumin.

Summary:

Although there are many purported benefits to linked to the consumption of curcumin (Boonla, 2014; Sahin, 2016; Gupta, 2013; Huang, 2015), the supplemental form of curcumin alone has been shown to have poor bioavailability (Lao, 2006; Krishnakumar, 2014; Li, 2007; McFarlin, 2016). Curcumin has a poor absorption, rapid metabolism, and rapid systemic elimination (Krishnakumar, 2012; 2014; Lao, 2006; Li, 2007) These properties result in an inability for efficient consumption without a vehicle to increase its bioavailability. It has been documented (Lao, 2006; Anand, 2007) that even at high doses (12g·day^{-1}), the plasma and tissue concentrations are still at physiologically irrelevant levels; a study by Garcea et al. (2004) states that most curcumin activity required a minimum of approximately 0.1 micromolar levels in vitro, the Lao et al. (2006) study showed curcumin was only detected in the serum of subjects taking greater than 10000mg dosages, and the concentration was still too low (10000mg 1hr: 30.4ng·ml^{-1} ;12000mg 1hr: 29.7ng·ml^{-1}) to be considered to have physiological effects. The study also showed that curcumin supplementation of dosing up to 12g has minimal toxicity risk. Therefore, other ingredients have been added to curcumin in an attempt to improve its absorption (Krishnakumar, 2014; Lao, 2006; Tu, 2014). For example, some manufactures have combined curcumin with piperine which interferes with glucuronidation (Tu, 2014), while liposomal curcumin or the addition of fenugreek have been used to slow digestion, increase absorption, and slow systemic elimination (Anand, 2007). In addition, a gel-forming non-

digestible soluble dietary fiber galactomannan (Fenugreek) vehicle that encapsulates the curcumin has been used and shown to prevent rapid elimination, slow the release to counteract high metabolism, and increased peak saturation for an extended duration (Krishnakumar, 2012; 2014). The formulated fenugreek curcumin complex exhibited 20 times greater absorption and saturation than that of 95% unformulated curcumin (Krishnakumar, 2014). These studies indicate a minimum serum concentration 0.1um of curcumin (Garcea, 2004) is necessary for curcumin to have physiological effects. Although curcumin alone has been shown to have poor bioavailability, serum concentrations have been shown to be increased through synergist supplementation combination with fenugreek.

2.5 Anaerobic Thresholds/Gas Exchange Thresholds

Beaver et al. 1986

The purpose of this study was to measure the changes in respiratory gas exchange during an incremental exercise session and derive an objective mathematical method that can reliably locate the anaerobic threshold (AT) based on the buffering of lactic acid, and that is independent of the sensitivity of ventilatory control mechanisms. Ten male subjects ages 19-39 performed a cycle ergometer incremental exercise to the limit of tolerance in which work rate was increase by $15W \cdot min^{-1}$ increments. Gas exchange measurements of minute ventilation ($\dot{V}_E$), alveolar $\dot{V}O_2$ and $\dot{V}CO_2$ were collected and arterial blood analyses for lactate and bicarbonate were taken via an indwelling brachial artery catheter. The gas exchange analysis was done by the V-slope method and independent experts. The v-slope method involves analyzing the behavior of $\dot{V}CO_2$ as a function of $\dot{V}O_2$ as the lactate

threshold is exceeded in which the buffering of lactic acid via HCO_3^- leads to a consequential increase of carbon dioxide production. The gas exchange data were also analyzed by six experienced reviewers using a visual identification technique; each judge independently reviewed ten subjects' plots of $\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$, end-tidal CO_2 pressure, end-tidal O_2 pressure, and R vs time. The determination of the (AT) was performed by dividing the $\dot{V}CO_2$ versus $\dot{V}O_2$ relationship into two linear segments and fitting them with linear regression. The tentative AT is the intersection between the two linear regression segments and the point is moved until the two lines best fit the data by maximizing the ratio of the greatest distance of the intersection point from the single regression line of the data to the mean square error of regression. The estimated 95% confidence intervals of 10 studies ranged from 0.05L·min^{-1} to 0.1 L·min^{-1}, averaging 0.07 L·min^{-1} or ± 3.8% of the $\dot{V}O_2$ at the intersection point. The panel vs the calculated AT differed only by 0.02 L·min^{-1} (not significant). Only 5 out of 10 ATs were detected by all the panelists while the V-slope method yielded ATs for all subject data. Respiratory compensation point (RCP) is defined in this study as the intersection between the two linear segments of the data slopes in the plot of $\dot{V}_E/\dot{V}CO_2$ if the change in slope between them is greater than a preselected amount (15% of the initial slope). The mean $\dot{V}O_2$ at the respiratory compensation point (RCP) was 75% of the $\dot{V}O_2$ max and the mean $\dot{V}$O2 for AT was 73% of the mean $\dot{V}O_2$ value of the RCP. The AT found by the V-slope method occurred at a mean lactate increase of 0.50 meq·L^{-1} above the lactate value at LT. The most significant finding for the bicarbonate study was that the mean $\dot{V}O_2$ at AT (1.83 ± 0.30 L·min^{-1}) was not significantly different from the estimated $\dot{V}O_2$ at the mean HCO_3^- threshold (1.78 ± 0.24 L·min^{-1}). Overall this study validates the V-slope method and its reliability, further validates previous studies

that RCP demarcates heavy to severe domains, AT demarcates moderate to heavy domains, and indicates that the lactate threshold and AT are not significantly different.

Bergstrom et al. 2013

This study examined the relationships between physical working capacity at the fatigue threshold (PWC_{FT}), gas exchange threshold (GET), respiratory compensation point (RCP), and critical power (CP) to identify and compare possible physiological mechanisms underlying the onset of muscular fatigue. The authors hypothesized that the differences between these thresholds would reflect the parameters used to estimate them, and there would be possible differences in the physiological mechanisms that underlie them. The participants consisted of six men and four women (mean ± SD age: 20 ± 1 year; body weight 69.9 ± 12.6 kg; height 171.6 ± 9.0 cm). Each participant performed an incremental test to exhaustion on a calibrated Lode electronically-braked cycle ergometer at a pedal cadence of 70 rev min^{-1} where testing began at 50W and increased by 30W per minute until voluntary exhaustion or the participant's pedal rate fell below 70 rev min^{-1} for more than 10 s, despite strong verbal encouragement. The GET was determined using the V-slope method and the RCP was determined using the $\dot{V}_E$ versus $\dot{V}CO_2$ relationship. The PWC_{FT} values were determined from 10 s epocs of electromyographic amplitude (EMG AMP) signals recorded from the vastus lateralis muscle during each 2 min stage of the test. 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 AMP versus time relationship, and the lowest power output that resulted in a significant ($p > 0.05$) positive slope coefficient. The CP was the average power output over the final 30 s of the 3-min all-out test. The mean differences in fatigue thresholds were analyzed using a one-way

repeated measures ANOVA with least significant difference post-hoc comparisons, and the relationships among the PWC_{FT}, GET, RCP and CP were described using Pearson-product moment correlations and a zero-order correlation matrix. The PWCFT (197 ± 55 W), RCP (212 ± 50 W) and CP (208 ± 63 W) were significantly greater than the GET (168 ± 40 W), but there were no significant differences among the PWC_{FT}, RCP and CP. The thresholds PWC_{FT}, GET, RCP and CP represented 75 ± 11%, 65 ± 5%, 82 ± 3% and 79 ± 9% of peak power respectively; and were shown to be significantly inter-correlated (r = 0.794–0.958). The results of the current study indicated: 1) the PWC_{FT} was 17% greater than, but highly correlated (r = 0.847) with the GET; 2) the PWC_{FT} was not significantly different from, and highly correlated (r = 0.835) with, the RCP and that a similar physiological mechanism may underlie the determination of these fatigue thresholds; and 3) the PWC_{FT} and CP were not significantly different and moderately correlated.

Summary:

The gas exchange method (GET) was developed as an objective mathematical method to reliably locate the anaerobic threshold (AT) based on the buffering of metabolic acidosis by measuring the changes in respiratory gas exchange during an incremental exercise session (Beaver, 1986). This method involved quantifying and analyzing the oxygen consumption ($\dot{V}O_2$) and the carbon dioxide production $\dot{V}CO_2$ through the V-slope method. The V-slope method plots $\dot{V}CO_2$ as a function of $\dot{V}O_2$, and separates the data into two linear segments, then fits these segments with linear regression. The tentative AT (or GET) is the intersection between the two linear regression segments. The respiratory compensation point (RCP) was defined as the intersection between the two linear segments of the $\dot{V}_E \, versus \, \dot{V}CO_2$ relationship (Beaver, 1986). It has been suggested the GET

demarcates the moderate from heavy exercise intensity domains, while the RCP is typically greater than 75% of $\dot{V}O_2$ max and demarcates the heavy from severe exercise intensity domains. The GET, VT and RCP can be determined from the measurement of respiratory gas exchange during incremental exercise (Beaver, 1986; Bergstrom, 2013).

CHAPTER 3. METHODS

3.1 Experimental Approach

This study used a randomized, double-blind, placebo-controlled, parallel design with two experimental groups and one placebo group. Sixty subjects were randomly assigned to the placebo group (PLA, n=13), curcumin+ fenugreek supplement, CurQfen® (CUR, n=14), or fenugreek soluble fiber supplement (FEN, n=18). The subjects visited the testing center located in University of Kentucky Kinesiology and Health Promotion education facility a total of six times; the second and sixth sessions lasted approximately two hours, and there were weekly check-in visits (4 total= visits three through six) during the 28-day supplementation period. During the first visit, each subject completed a health history questionnaire and signed an informed consent. During the second visit, the subjects completed a pre-test graded exercise test (GXT_{pre} prior to 28 days of supplementation), followed by a 28-day supplementation protocol. The subjects were asked to ingest one dose (PL, CUR, or FEN) every day for 28 days and one dose 60 minutes prior to the post-test (GXT_{post} after 28 days of supplementation). The GXT was used to derive the pre-test VT, RCP, $\dot{V}O_2$peak, and T_{lim}. Following 28-days of supplementation, each subject completed a post-test GXT to derive the post-test (VT), (RCP), $\dot{V}O_2$peak, and T_{lim}. Dietary intakes three days prior to test days were recorded with food logs. In addition, supplement compliance was recorded with supplementation logs.

3.2 Subjects

In total, 67 subjects were screened and enrolled in the study. Three of the subjects withdrew due to scheduling conflicts, two of subjects were excluded due equipment malfunctions, and one subject was excluded due to inability to complete PRE/POST-test as a result of illness. Four of the subjects were excluded as they did not exhibit landmarks for threshold calculation, and two were excluded due to inability to complete minimal stage requirements needed for this test. The subjects were untrained in aerobic exercise and engaged in no more than 4 hours of recreational activity per week. To account for variations in low- and high- fitness levels, subjects were excluded if they fell below (very poor) or above (superior) the 10^{th} percentile of cardiorespiratory fitness based on age and sex, according to the American College of Sports Medicine (Thompson, 2014). Five of the subjects had a $\dot{V}O_2$peak that was below, and five of the subjects had a $\dot{V}O_2$peak that was above the 10^{th} percentile for cardiorespiratory fitness and were excluded from the analyses. Thus, there were 45 men (n =) and women (n =) (age: 21.2 ± 2.4 yrs; height: 174.4 ± 8.2cm; weight: 73.1 ± 13.4kg) who completed this study (PLA = 13, FEN = 18, CUR = 14). 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 include cardiovascular disease, metabolic, renal, hepatic, or musculoskeletal disorders; (b) were not taking any medication that could significantly affect experimental results (such as vasodilators/vasoconstrictors); (c) were not currently using any nutritional supplements that could significantly affect experimental results; and (d) were not presently participating in another clinical trial or ingestion of another investigational product. The subjects were instructed to not consume any caffeine on the testing day and avoid alcohol consumption for 24 hours prior to testing. The study

was approved by the University's Institutional Review Board for Human Subjects, and all subjects signed a written informed consent document before testing.

3.3 Supplementation

A limitation to curcumin supplementation is its low bioavailability. The supplement CurQfen® combines curcumin extract and fenugreek to significantly increase plasma concentrations of curcumin (Krishnakumar, 2012). Galactomannan soluble dietary fiber from fenugreek seeds slows the digestion and rapid elimination of curcumin to allow better absorption into the bloodstream, improving absorption by 15.8 times of the curcumin standalone (Krishnakumar, 2012). The 500mg CurQfen® capsule contained 190 mg of total curcuminoids (curcumin- 81%, demethoxycurcumin - 15.7% and bisdemethoxycurcumin - 2.6%) and 300mg FenuMAT (de-bitterised fenugreek dietary fiber containing 5 to 80% galactomannans with 2-4% moisture). The fenugreek soluble fiber only group was included to account for any extraneous effects of fenugreek soluble fiber and contained 300 mg. The subjects consumed one dose daily and received the capsules on a weekly basis according to their randomly assigned group of either the PLA, CUR, or FEN. The pills were ingested with 16 oz. of water every morning before eating for 28 days. The subjects completed a dosing log and checked in weekly with their pill bottles to ensure adherence to proper dosing procedures and to receive the following weeks supplement. The dosing log was used to check compliance (compliance = (# of doses taken / total # of doses provided] x 100). A compliance rate of > 80% was required for inclusion in the data analyses. In addition, during supplementation, the subjects were instructed to keep a three-day food and activity log prior to each testing session and were asked not to change their diet and activity level during the study. The three-day food logs prior to each

testing day were further analyzed to ensure consistency in diet. A total of 42 of the 45 subjects (PLA = , FEN = , CUR =) completed and returned food logs that were used for subsequent analyses.

3.4 Graded Exercise Tests

Each subject performed an incremental cycle test to exhaustion on an electronically braked cycle ergometer (Lode Corival, Groningen, Netherlands) to determine the VT, RCP, $\dot{V}O_2$peak, and T_{lim}. The subjects were familiarized with the equipment before proceeding with the GXT. 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 mouthpiece to collect all expired air. A calibrated metabolic cart (TrueMax 2400, ParvoMedics, Sandy, UT) was used to collect and analyze the expired gas samples. The gas analyzers were calibrated with room air and gases of known concentration prior to all testing sessions. The O_2, CO_2, and ventilatory parameters were expressed as 30 s averages. In addition, the heart rate was recorded with a Polar Heart Rate Monitor (Polar Electro Inc., Lake Success, NY) that was synchronized with the metabolic cart. A Borg Rating 6-20 of Perceived Exertion (RPE) scale was used to quantify the subjective effort of the participant at the end of each minute during the test (Borg, 1982). Following a one-minute warm up at 0 W, the resistance was increased to 50W and increased by 30W every 2 min until the subjects were unable to maintain 70 rev·min^{-1}, or until volitional fatigue. This protocol was consistent with the study protocol previously used to assess $\dot{V}O_2$ peak, gas exchange and ventilatory thresholds, as well as the electromyographic fatigue threshold in college aged males (Bergstrom, 2013). 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 mL·min^{-1} in $\dot{V}O_2$ over the last 30 seconds of the test).

3.5 Determination of Fatigue thresholds: VT, and RCP

The VT was determined from the $\dot{V}_E$ versus $\dot{V}O_2$ relationship and defined as the $\dot{V}O_2$ value that corresponds with the point of non-linear increase in $\dot{V}_E$ relative to $\dot{V}O_2$ (Beaver, 1986). The RCP was determined using the $\dot{V}_E$ versus $\dot{V}CO_2$ relationship and defined as the $\dot{V}O_2$ value that corresponds with the point of non-linear increase in $\dot{V}_E$ relative to $\dot{V}CO_2$ (Beaver, 1986).

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, VT$\dot{V}O_2$, RCP$\dot{V}O_2$, $\dot{V}O_2$peak, and T_{lim} among the groups prior to supplementation. The PLA group (n = 13) PRE- and POST-test values were used for the calculation of reliability, which consisted of the intraclass correlation coefficient model 2,1 ($ICC_{2,1}$), the standard error of the measurement (SEM), and the minimal difference needed to be considered real (MD) for each dependent variable (VT$\dot{V}O_2$, RCP$\dot{V}O_2$, $\dot{V}O_2$ peak, and T_{lim}) (Weir, 2005). The SEM was calculated as the SD x $\sqrt{1 - ICC}$; and the MD was calculated as the SEM x 1.96 x $\sqrt{2}$ (Weir, 2005). In addition, three separate paired samples t-test were used to determine if there were any significant changes in the dependent variable for the PLA group from PRE- to POST-test. Our testing model consists of two independent variables (group and

time). Four separate, one-way ANCOVAs (one for each dependent variable, VT, RCP, $\dot{V}O_2$peak, and T_{lim}) were used to determine if there were any differences between adjusted POST-test values (VT$\dot{V}O_2$, RCP$\dot{V}O_2$, $\dot{V}O_2$ peak, and T_{lim}) and the respective PRE-test values were used as the covariate. Follow-up analyses consisted of independent samples t-tests. Separate 2 (Time: PRE and POST) x 3 (Group: PL, CUR, FEN) mixed factorial ANOVAs were performed for the total kilocalories and grams for each macronutrient (carbohydrates, fats, and proteins). The analyses were conducted using Statistical Package for the Social Sciences software (v. 24.0 IMB SPSS Inc., Chicago, IL, USA). An alpha level of $p \leq 0.05$ was considered statistically significant for all analyses.

CHAPTER 4. RESULTS

4.1 PRE-test ANOVAs

The results of the one- way ANOVAs comparing PRE-test values indicated that there were no significant mean group differences for the VT$\dot{V}O_2$ (F= 0.039 p= 0.200), RCP$\dot{V}O_2$ (F= 0.148 p= 0.863), $\dot{V}O_2$peak (F= 0.068 p= 0.934), or T_{lim} (F= 0.181 p= 0.835) determined from the GXT; or for age (F= 1.753 p= 0.186), height (F= 0.241 p= 0.787), or weight (F= 1.001 p= 0.376) values (Table 4.2).

4.2 Reliability Analyses

A paired samples t-test of the PLA group indicated that there were no significant mean differences between PRE- and POST-test for the VT$\dot{V}O_2$ (t= 1.224 p= 0.244), RCP$\dot{V}O_2$ (F= -0.492 p= 0.631), $\dot{V}O_2$peak (t= -0.293 p= 0.775), and T_{lim} (t= -0.054 p= 0.958). The ICC values for the VT$\dot{V}O_2$, RCP$\dot{V}O_2$, $\dot{V}O_2$ peak, and T_{lim} were 0.959, 0.917, 0.971, and 0.957, respectively. The SEM and MD values for the VT$\dot{V}O_2$, RCP$\dot{V}O_2$, $\dot{V}O_2$peak, and T_{lim} are presented in Table 4.2.

Table 4.1 Demographic information (Mean ± SD) and PRE-test values for the ventilatory threshold (VT), respiratory component point (RCP), $\dot{V}O_2$ peak, and time to exhaustion (T_{lim}) from the graded exercise test.

	PLA	**FEN**	**CUR**
Age (yrs)	20.5 ± 1.5	20.9 ± 1.4	22.1 ± 3.8
Height (cm)	175.3 ± 7.6	173.5 ± 7.9	173.7 ± 9.1
Weight (kg)	71.5 ± 11	76.7 ± 12.9	70.5 ± 16.2
$\dot{V}O_2$peak (L·min^{-1})	2.8 ± 0.7	2.9 ± 0.7	2.8 ± 0.7
VT (L·min^{-1})	1.507±0.325	1.480±0.328	1.514±0.440
RCP (L·min^{-1})	2.343±0.586	2.435±0.534	2.341±0.568
T_{lim} (min)	13.956±2.698	14.48±2.68	14.61±3.58

PLA (n = 13)= Placebo**, FEN** (n = 18) = Fenugreek**, CUR** (n = 14) = CurQfen®

Table 4.1 Results of the reliability analyses for the placebo group using PRE-test and POST-test values for the ventilatory threshold ($VT\dot{V}O_2$), respiratory compensation point ($RCP\dot{V}O_2$), $\dot{V}O_2$ peak, and time to exhaustion (T_{lim}).

Subject	Pre$\dot{V}O_2$Peak	Post$\dot{V}O_2$Peak	Pre$VT\dot{V}O_2$	Post$VT\dot{V}O_2$	Pre$RCP\dot{V}O_2$	Post$RCP\dot{V}O_2$	PreT_{lim}	PostT_{lim}
4	3.782	3.826	1.500	1.520	3.168	3.230	18.02	17.49
7	2.876	3.164	2.118	2.287	2.623	3.010	15.34	14.51
10	2.413	2.282	1.310	1.220	2.193	2.078	12.52	11.02
22	3.537	3.245	1.860	1.730	2.716	2.688	15.00	15.51
27	2.065	1.921	1.170	0.980*	1.694	1.571	11.01	11.50
32	3.423	3.509	1.971	1.890	2.533	2.685	15.50	15.52
36	2.472	2.289	1.400	1.300	1.986	1.911	12.49	11.52
47	3.614	3.715	1.430	1.484	3.309	3.100	17.51	18.40
51	2.890	3.127	1.390	1.500	2.180	2.847*	14.01	15.01
57	1.578	1.403	0.990	0.925	1.330	1.225	8.51	8.01
62	2.565	2.609	1.626	1.592	2.331	2.078	14.00	13.33
66	2.057	2.219	1.224	1.162	1.656	1.810	11.51	12.46
67	3.308	3.464	1.599	1.561	2.739	2.680	16.01	17.00
Mean ± SD	**2.814 ±0.691**	**2.829 ±0.758**	**1.507 ±0.325**	**1.473 ±0.372**	**2.343 ±0.586**	**2.829 ±0.636**	**13.96 ±2.70**	**13.94 ±2.98**
ICC	**0.971**		**0.959**		**0.917**		**0.957**	
SEM	**0.119**		**0.066**		**0.170**		**0.56**	
MD	**0.330**		**0.183**		**0.471**		**1.55**	

ICC = intraclass correlation coefficient; SEM = standard error of the measurement; MD = minimal difference to be considered a real change. (*) denotes an increase or decrease from PRE-test to POST-test that exceeded the MD.

4.3 Fatigue Thresholds and Maximal Testing Parameters

4.3.1 Analysis of Covariance

The one-way ANCOVA for the VT$\dot{V}O_2$ values indicated there were significant differences among the groups (F = 3.224, p= 0.05) (Figure 4.1). The pairwise comparisons indicated a significant difference between the CUR and PLA groups (p= 0.039) and between the FEN and PLA (p= 0.025), but no differences between FEN and CUR (p= 0.943). The adjusted VT$\dot{V}O_2$ mean (±SD) for the Placebo, FEN, and CUR were 1.465 ± 0.155 L·min^{-1} (95% CI= 1.378-1.552 L·min^{-1}), 1.597 ± 0.157 L·min^{-1} (95% CI= 1.522-1.671 L·min^{-1}), and 1.593 ± 0.157 L·min^{-1} (95% CI= 1.509-1.677 L·min^{-1}) (Figure 1). The one-way ANCOVAs for $\dot{V}O_2$ peak (F = 0.613, p = 0.547), RCP$\dot{V}O_2$ (F = 3.177, p = 0.052), and T_{lim} (F = 0.654, p = 0.525) indicated there were no significant differences among groups (Figures 4.2, 4.3, 4.4 respectively).

The 3 x 2 mixed factorial ANOVAs resulted in no significant group x time interactions (p = 0.430 -0.802), main effects for group (p = 0.222 -0.652), or main effects for time (p = 0.335-0.870) for the total kilocalories or macronutrients consumed. Supplement compliance was recorded with supplementation logs and demonstrated a mean (± SD) compliance rate of 98.6% ± 2.6%.

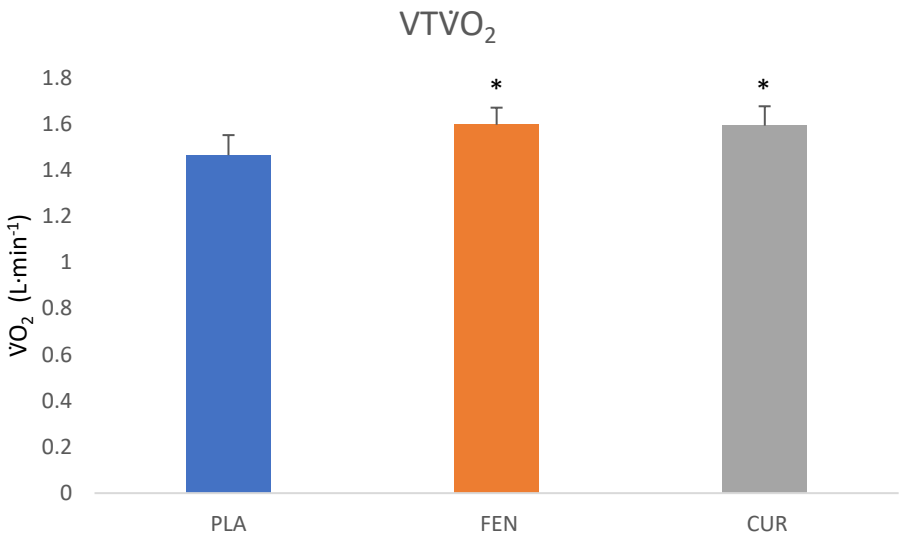

Figure 4.1 Adjusted POST-test ventilatory threshold (VT) $\dot{V}O_2$ (mean ± SEM) values (covaried for PRE-test VT$\dot{V}O_2$ scores) for placebo (PLA), fenugreek (FEN), and the CurQfen® (CUR) groups. *Significantly ($p < 0.05$) greater than placebo.

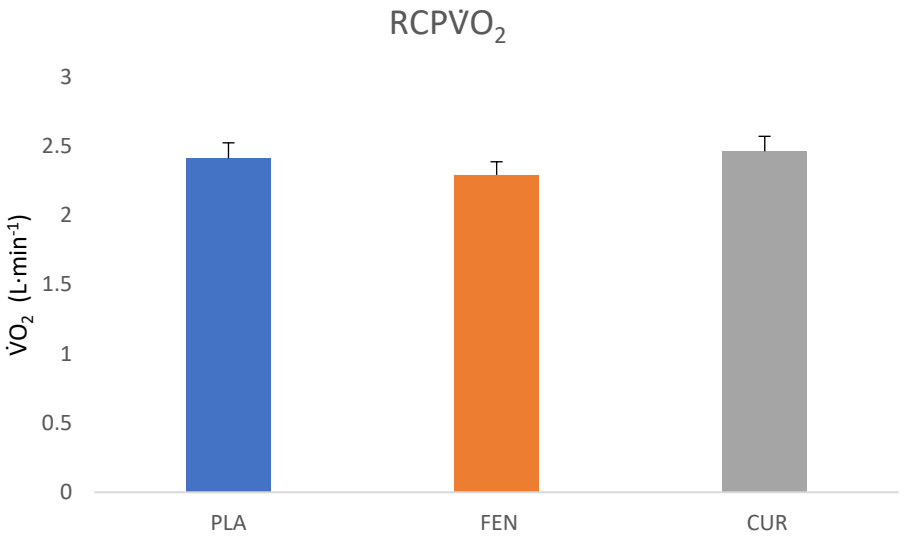

Figure 4.2 Adjusted POST-test respiratory compensation point (RCP) $\dot{V}O_2$ (mean ± SEM) values (covaried for PRE-test RCP$\dot{V}O_2$ scores) for placebo (PLA), fenugreek (FEN), and the CurQfen® (CUR) groups.

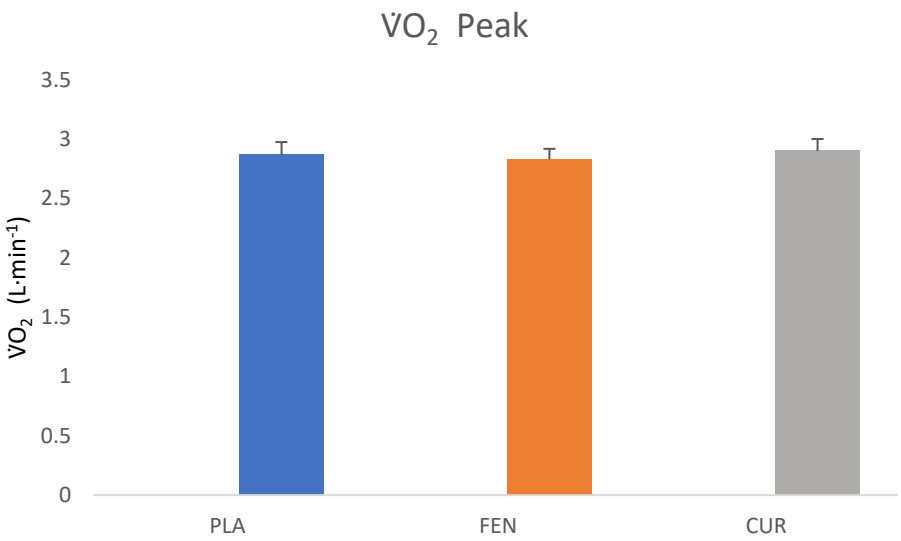

Figure 4.3 Adjusted POST-test $\dot{V}O_2$ peak (mean ± SEM) values (covaried for PRE-test $\dot{V}O_2$ Peak scores) for placebo (PLA), fenugreek (FEN), and the CurQfen® (CUR) groups.

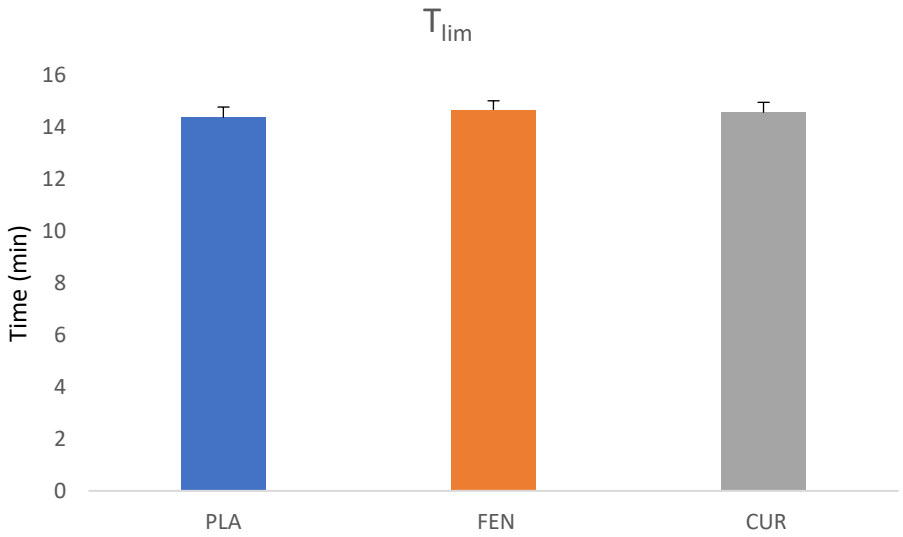

Figure 4.4 Adjusted POST-test time to exhaustion (T_{lim}) (mean ± SEM) values (covaried for PRE-test T_{lim} scores) for placebo (PLA), fenugreek (FEN), and the CurQfen® (CUR) groups.

4.3.2 Individual Responses for Ventilatory Threshold ($VT\dot{V}O_2$)

One subject of the 13 subjects in the PLA group showed a decrease greater than MD (Figure 4.5). Four of the 18 subjects in the FEN group (Figure 4.6) and two of the 14 subjects from the CUR group (Figure 4.7) showed an increase greater than MD.

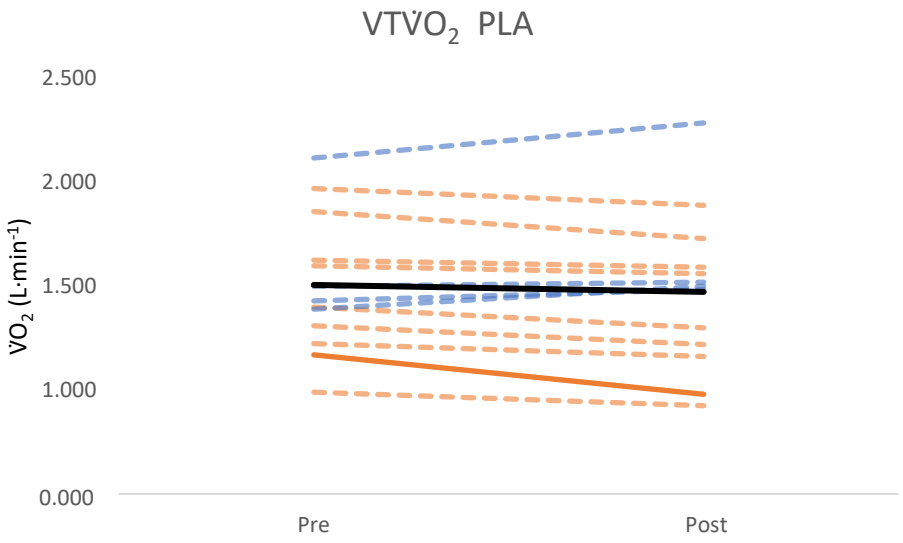

Figure 4.5 Individual responses for the ventilatory threshold $\dot{V}O_2$ from PRE- to POST-test for the placebo (PLA) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

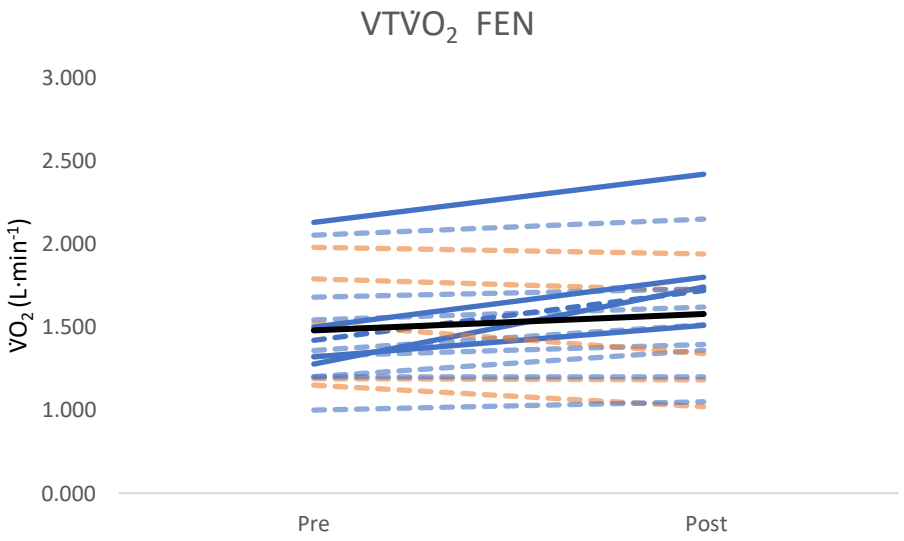

Figure 4.6 Individual responses for the ventilatory threshold $\dot{V}O_2$ from PRE- to POST-test for the fenugreek (FEN) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

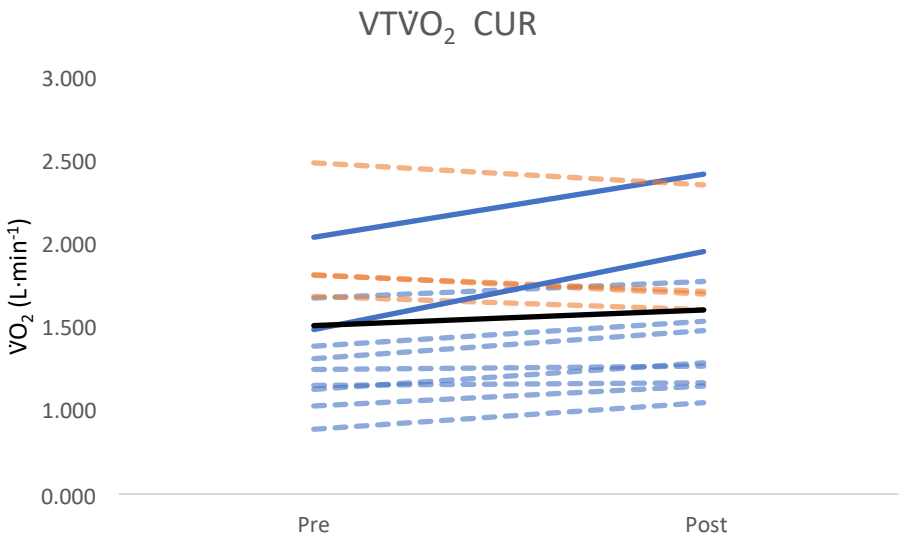

Figure 4.7 Individual responses for the ventilatory threshold $\dot{V}O_2$ from PRE- to POST-test for the CurQfen® (CUR) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

4.3.3 Individual Responses for the Respiratory Compensation Point ($RCP\dot{V}O_2$)

One of the 13 subjects in the PLA group (Figure 8) showed an increase greater than MD while there were no subjects who exceed the MD in the FEN (Figure 9) or CUR (Figure 10) groups.

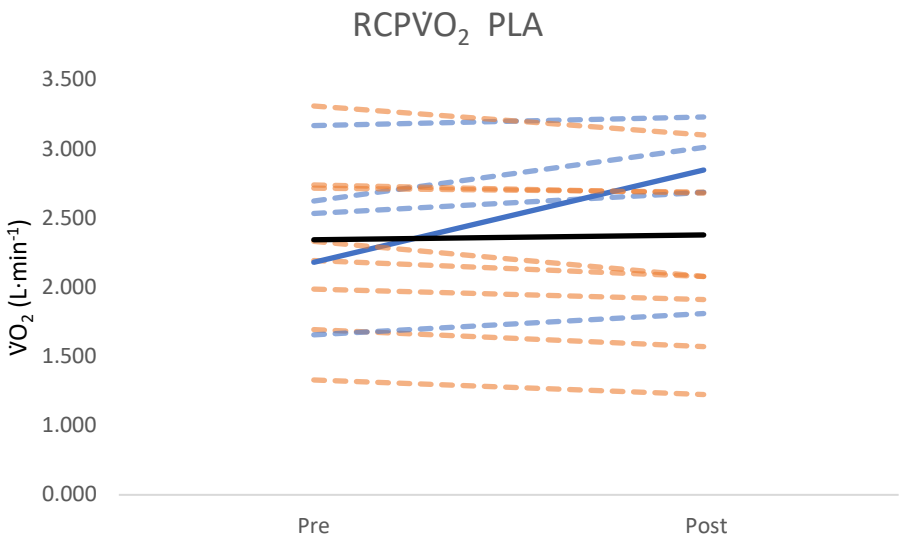

Figure 4.8 Individual responses for the respiratory compensation point $\dot{V}O_2$ from PRE- to POST-test for the placebo (PLA) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

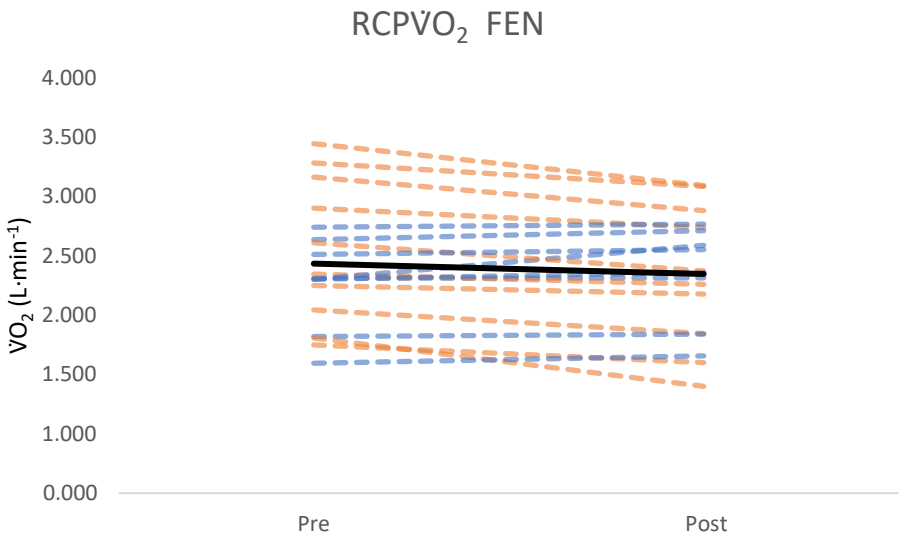

Figure 4.9 Individual responses for the respiratory compensation point $\dot{V}O_2$ from PRE- to POST-test for the fenugreek (FEN) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

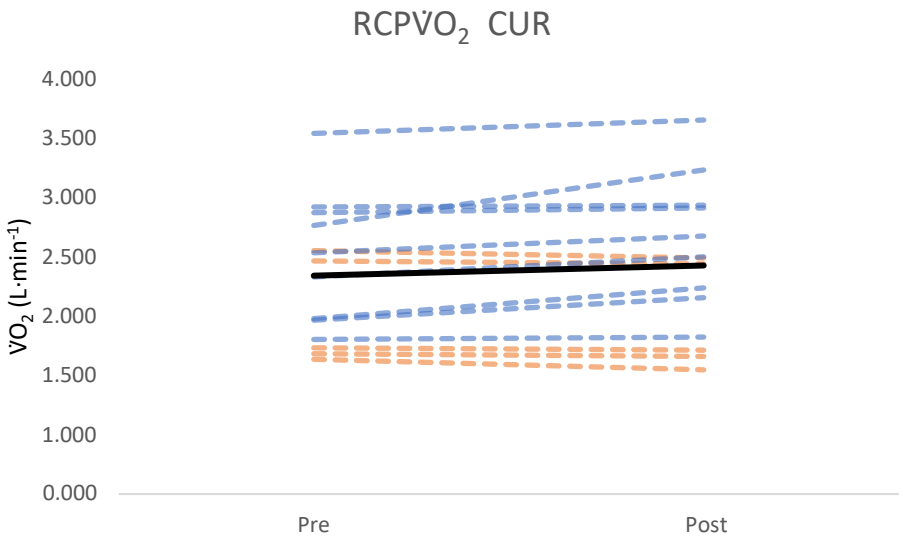

Figure 4.10 Individual responses for the respiratory compensation point $\dot{V}O_2$ from PRE- to POST-test for the CurQfen® (CUR) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

4.3.4 Individual Responses for $\dot{V}O_2$ Peak

None of the 13 subjects in the PLA group showed a change in $\dot{V}O_2$peak greater than the MD (Figure 11). Two of the 18 subjects in the FEN group (Figure 12) showed a decrease greater than the MD and one of the 14 subjects in the CUR Figure 13) showed an increase greater than the MD.

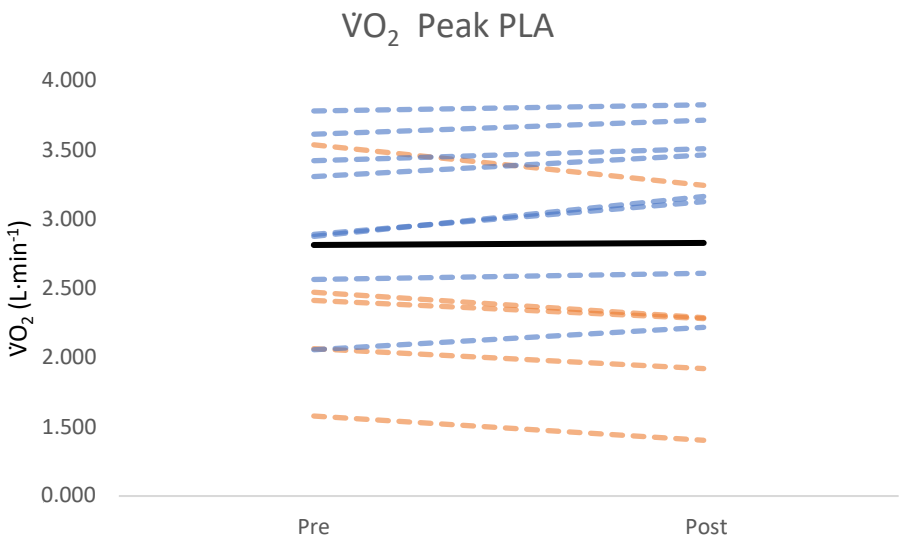

Figure 4.11 Individual responses for the $\dot{V}O_2$ Peak from PRE- to POST-test for the placebo (PLA) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

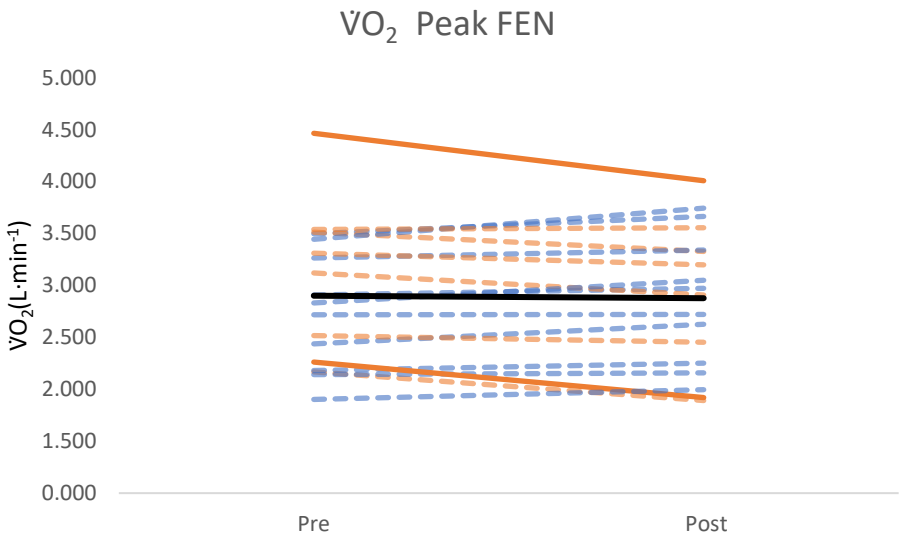

Figure 4.12 Individual responses for the $\dot{V}O_2$ Peak from PRE- to POST-test for the fenugreek (FEN) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

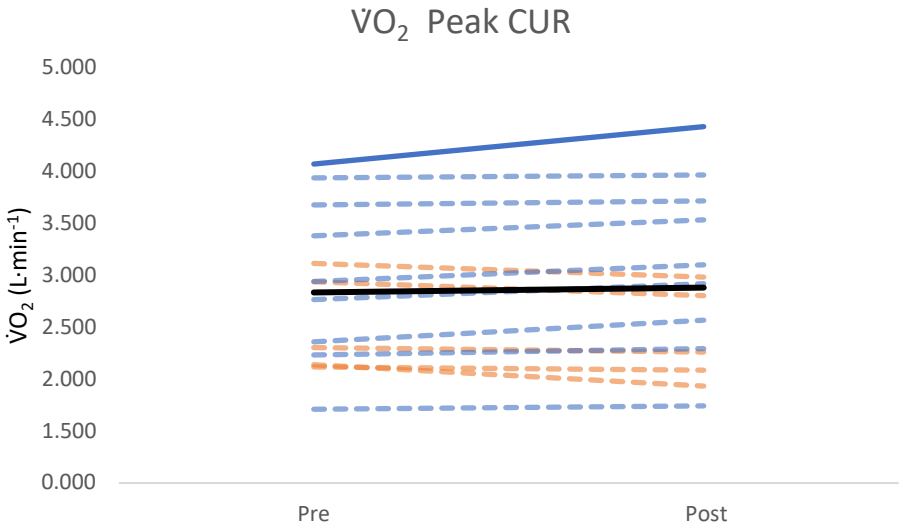

Figure 4.13 Individual responses for the $\dot{V}O_2$ Peak from PRE- to POST-test for the CurQfen® (CUR) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

4.3.5 Individual Responses for Time to Exhaustion

There were no subjects in any of the groups that showed and increase or decrease greater than MD (Figures 14, 15, 16) for the T_{lim}.

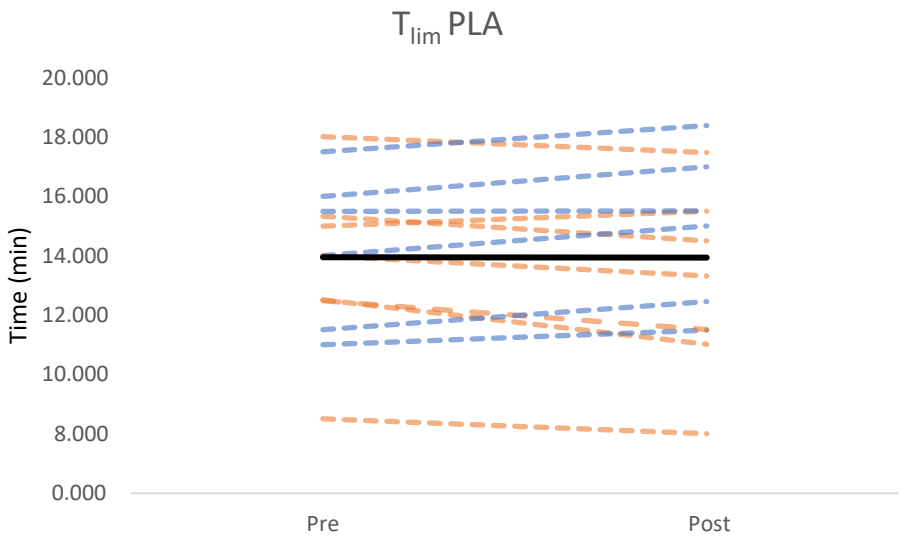

Figure 4. 14 Individual responses for the time to exhaustion (T_{lim}) from PRE- to POST-test for the placebo (PLA) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. The black line indicates the mean response.

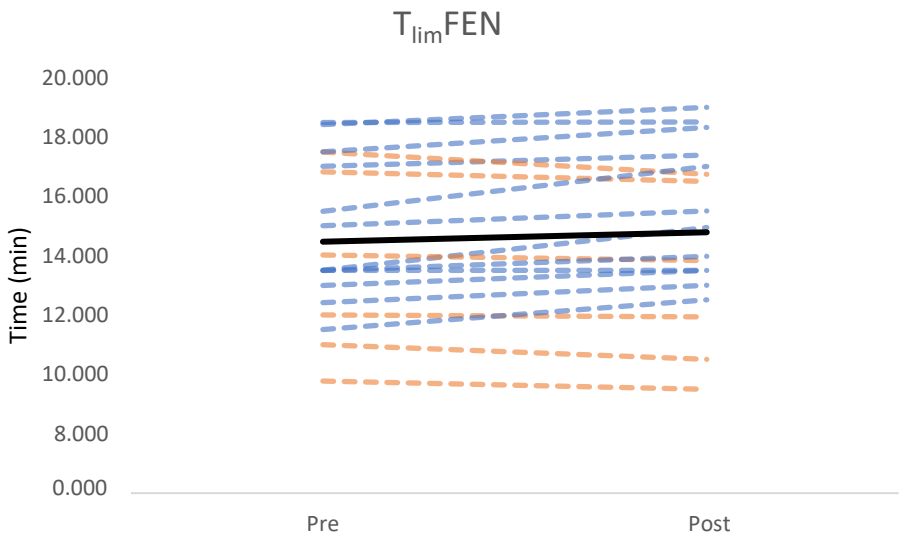

Figure 4.15 Individual responses for the time to exhaustion (T_{lim}) from PRE- to POST-test for the fenugreek (FEN) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. The black line indicates the mean response.

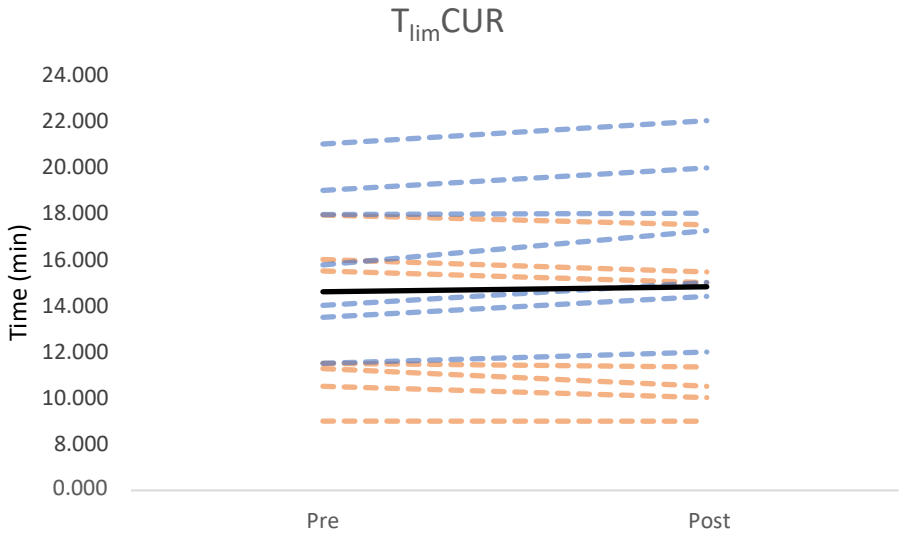

Figure 4.16 Individual responses for the time to exhaustion (T_{lim}) from PRE- to POST-test for the CurQfen® (CUR) supplement group. Dashed blue lines indicate an increase and the dashed orange lines a decrease from PRE- to POST-test. Solid colored lines indicate an increase/decrease greater than the minimal difference. The black line indicates the mean response.

CHAPTER 5. DISCUSSION

The purpose of this study was to examine the effects of a 28 day dosing period of curcumin and fenugreek soluble fiber on submaximal and maximal endurance performance indices measured during a GXT. The primary findings were that the VT was greater for the CUR and FEN compared to the PLA at post-test, but there were no differences in the RCP, $\dot{V}O_2$peak, or T_{lim} values. In this study, the VT increased 6.2% (increase = 0.094 $L \cdot min^{-1}$) and 6.7% (increase = 0.099 $L \cdot min^{-1}$) from PRE- to POST-test for the CUR and FEN, respectively, but was not improved for the PLA - 2.2% (decrease = 0.034 $L \cdot min^{-1}$). To our knowledge, no previous studies have examined the effects of curcumin and fenugreek on submaximal fatigue thresholds, however, the relative changes (6.2 – 6.7%) in the VT in this study were consistent with the 4.1 to 5.4% increases previously reported for the gas exchange threshold (GET) after 28 days of arginine supplementation (Camic, 2010). Interestingly, these increases in the GET were also not accompanied by changes in $\dot{V}O_2$peak. Thus, the results of the present study showed increases in a submaximal fatigue threshold (VT) for both CUR and FEN, without changes in maximal endurance performance ($\dot{V}O_2$peak, T_{lim}, and RCP), that were consistent with previously reported (Camic, 2010) changes of a similar threshold after a nutritional intervention.

5.1 Supplementation Effects on a Submaximal Endurance Performance Threshold

The sensitivity of the VT to curcumin and fenugreek supplementation is likely related to the mechanisms underlying the fatigue threshold. Although there is conflicting evidence regarding the true underlying mechanism(s) for the breakpoints in the $\dot{V}_E$ versus $\dot{V}O_2$ and

$\dot{V}CO_2$ versus $\dot{V}O_2$ relationships that define the VT and GET, respectively, these thresholds have been demonstrated across multiple studies ((Beaver, 1986; Wasserman, 1973; Gaesser, 1996) and are likely related to the accumulation of metabolic byproducts (i.e., H^+, inorganic phosphate, ammonia, and potassium) of muscular contractions. Thus, the VT and GET demarcate the moderate from the heavy exercise intensity domains and reflect the point of increased reliance on anaerobic ATP production, as the aerobic system can no longer fully support the energy demands of the exercise intensity (Powers, 2015). One of curcumin's purported physiological benefits is the up regulation of enzymes involved in nitric oxide (NO) production and enhanced acetylcholine-induced vasodilation (Boonla, 2014; Santos-Parker, 2017). Nitric oxide bolsters tissue respiration and endothelium-dependent vasodilation by relaxing smooth muscle cells in the vasculature (Maiorana, 2003; Chen, 2008). In addition, curcumin supplementation has been shown to reduce the accumulation of metabolic byproducts (lactate and ammonia) of muscular contraction (Davis, 2007; Huang, 2015) in rodents. It is possible the reduction of these metabolites after curcumin supplementation were a result of increased NO production and enhanced endothelium-dependent vasodilation (Boonla, 2014). Similar to these responses, the increases in the GET previously reported after arginine supplementation were hypothesized to be related to the amino acids essential role in the synthesis of NO production and the subsequent vasodilatory response to enhance metabolic byproduct clearance (Camic, 2010; Wasserman, 1973). Thus, it is possible the increases observed in the VT for CUR group were related to increased metabolic byproduct clearance from NO induced vasodilation. Future studies should focus on the measurement of metabolic byproducts and their

relationship with the VT in humans supplemented with varying levels of curcumin, to observe any dose-dependent responses.

Fenugreek soluble fiber, also known as galactomannan, was added to curcumin (CurQfen®) to increase the bioavailability of the supplement (Krishnakumar, 2012). Theoretically, galactomannans slow digestion, especially in the small intestine, resulting in a greater absorption of curcumin and greater plasma curcumin concentrations (Krishnakumar, 2014). However, galactomannans from fenugreek have also been shown to have physiological effects after 28 days of supplementation. Two of the purported benefits of chronic galactomannan supplementation are an increased plasma free fatty acid (FFA) concentration in circulation and decreased plasma insulin levels (Neelakantan, 2014; Srichamroen, 2008). It has been shown (Romijn, 1985) that FFA oxidation rates were increased by greater concentrations of FFA in circulation. During exercise in the moderate domain (i.e., below the VT), FFA's are the primary energy substrate for aerobic ATP production. Thus, greater plasma FFA in circulation may increase the rate of FFA utilization, potentially delaying the reliance on anaerobic glycolytic metabolism and attenuating metabolic byproduct accumulation. Furthermore, supplementation of galactomannans from fenugreek has been shown to increase plasma insulin sensitivity, decrease plasma insulin levels, and decrease blood glucose levels in mice models, and has been replicated in human models for both fasting and post oral glucose tests (Neelakantan, 2014). Insulin suppresses lipolysis by directly inhibiting transcription of lipase via the mTOR pathway (Meijssen, 2001; Chakrabarti, 2013). Increased insulin sensitivity and subsequent decreases in insulin levels would, theoretically, increase lipolysis and favor fat mobilization. Previous investigators have reported a significant, positive relationship

between insulin sensitivity and oxidative capacity (Bruce, 2003; Srichamroen, 2008). Thus, it is possible the VT was improved in the FEN group from increased FFA oxidation that delayed reliance on anaerobic glycolysis and attenuated the accumulation of metabolic byproducts. Future studies should further examine the effects of fenugreek, in particular, the galactomannan component, on FFA concentrations and insulin sensitivity to determine its relationship with submaximal exercise performance indices.

5.2 Synergistic Effects of Curcumin and Galactomannan Soluble Fiber

It is also possible the purported effects of fenugreek were responsible, at least in part, for the increases in VT for the CUR group (CurQfen® = curcumin + fenugreek: 300mg). Due to the poor bioavailability of curcumin, it is difficult to achieve plasma curcumin levels of physiological effect without a bioavailability booster such as fenugreek or piperine (Krishnakumar, 2012; 2014). Therefore, we could not isolate the individual effects of curcumin in this study. Based on the purported effects of curcumin and fenugreek, it would seem logical that the combination of both would exhibit synergistic effects to improve performance. Unexpectedly, both the CUR and FEN group demonstrated a greater VT at post-test compared to the placebo, but the VT was not different between the CUR and FEN. In this study, the 500 mg dose of of CurQfen® contained 190mg of curcuminoids and 300mg of fenugreek soluble fiber (75-80% galactomannans). It is possible at this relative dosage that any differences between the supplementation groups (CUR and FEN) were too small to detect. Future studies should examine the effects of supplementation with various doses of curcuminoids, without additional fenugreek fiber, to determine if there are any differences between curcumin and fenugreek supplementation on the VT. In addition, future studies should examine the

effects of supplementation fenugreek fiber alone and curcumin in combination with other ingredients (to increase absorption), such as piperine, to determine if there are similar changes in submaximal endurance performance indices.

5.3 Supplementation Effects on the Respiratory Compensation Point and Maximal Endurance Indices

Curcumin and fenugreek soluble fiber supplementation had no effects on the RCP, $\dot{V}O_2$peak, or T_{lim} in this study. The RCP is defined as the breakpoint in the $\dot{V}CO_2$ versus $\dot{V}E$ relationship and has been suggested to reflect the involuntarily hyperventilation associated with metabolic acidosis due to the failure of regional buffering systems (e.g., carnosine and sodium bicarbonate) (Powers, 2015). Theoretically, the RCP demarcates the heavy from severe exercise intensity domains and is typically identified at intensities greater than 75% of $\dot{V}O_2$ max in healthy, active adults (Beaver, 1986). By this definition, the VT and GET may be more sensitive to changes affecting aerobic adaptations or interventions such as oxygen supply and substrate availability, while the RCP may be more sensitive to changes affecting anaerobic metabolic system buffering capacities (Takano, 2000; Beaver, 1986; Gaesser & Poole, 1996). Thus, it is possible that NO mediated vasodilation and increased FFA concentrations as a result of curcumin and fenugreek supplementation, respectively, were effective to improve aerobic metabolic system efficiency and the VT, but did not alter the cellular and blood buffering capacities (e.g., carnosine and sodium bicarbonate, respectively) that would increase the RCP. Furthermore, the lack of change in $\dot{V}O_2$peak and T_{lim} after curcumin or fenugreek supplementation may also be related to the mechanisms of action of the supplements and the mode of testing. Specifically, previous literature has demonstrated that increased local

vasodilation did not equate to a higher local and systemic $\dot{V}O_2$peak during maximal incremental studies (Calbet, 2006). Thus, the potential NO mediated vasodilation and increased metabolic byproduct clearance as a result of curcumin supplementation would likely not alter $\dot{V}O_2$peak. In addition, curcumin supplementation has been reported to increase glycogen stores by 1.39-1.49- fold in mice (Huang, 2015). Because our incremental test was designed to encourage failure and $\dot{V}O_2$Peak within 15 min, it is unlikely that the muscle or liver glycogen stores were depleted and, therefore, would not limit these parameters ($\dot{V}O_2$peak and T_{lim}). The primary action of galactomannans to slow digestion, increase insulin sensitivity, and decrease blood glucose to promote FFA oxidation appeared to be ineffective at altering measures of maximal performance after 28 days of supplementation in this study. These findings are supported by previous literature that reported no effects on $\dot{V}O_2$max after eight weeks of FEN supplementation (Gholaman, 2018) Thus, in the healthy, untrained subjects, it seems that chronic non-stimulant, spice related nutritional supplementation affects submaximal thresholds that demarcate the moderate from heavy domains but are not effective for higher thresholds or maximal performance indices ($\dot{V}O_2$peak and T_{lim}). Future studies should examine the effects of curcumin and/or fenugreek on T_{lim} at a submaximal intensity, such as the VT, to examine potential effects to improve the sustainability of aerobic exercise.

5.4 Individual Responses

Typically, overall conclusions regarding the effectiveness of an intervention are drawn from mean responses, however, the MD analyses in this study indicated there were a small percentage of subjects that respond strongly to CUR and FEN supplementation. In this study, although there were significant effects of supplementation on the mean VT

responses at post-test for the FEN and CUR groups (Figures 5 and 6, p = 0.025, p= 0.039 respectively, compared to PLA) and the mean responses of the groups were similar (CUR = 6.2%, FEN = 6.7), only 22% of subjects exceeded minimal difference (MD) to be considered a real increase in the FEN group and 14% subjects exceeded MD in the CUR group. Conversely, no subjects in the PLA demonstrated an increase in the VT that exceeded the MD, while 7.7% exceeded the MD to be considered a real decrease. In this study, the MD for the VT was 0.183 $L \cdot min^{-1}$ and was calculated from the placebo group pre- to post-test reliability analyses. The MD defines "the difference needed between separate measures on a subject for the difference in the measures to be considered real" (Weir, 2005, P. 238) and speaks to the sensitivity of the test in distinguishing a "real" change from variation or error in measurement. Although there were a greater number of subjects in the FEN group who exceeded the MD compared to the CUR group, 71.4% of the subjects in the CUR supplemented group demonstrated a small increase from pre- to post-test, compared to 66.6% of the subjects in the FEN group, while only 30.7% demonstrate a small increase for the PLA group. The inherent limitation of simplifying results to the mean response is the assumption that all individuals have the same metabolic structure and capacities, where biological variability and biological noise such as circadian rhythm, nutritional intake, and motivation were not accounted for (Lampe, 2013; Swinton, 2018) Thus, the current findings demonstrated a small percentage (14-22%) of strong responders (exceeded the MD) and a larger percentage (67-72%) of subjects who demonstrated small increases that did not exceed the MD drove the mean responses for the CUR and FEN groups.

The further understanding of the underlying mechanisms related to the high, low, and non-responders would likely require the measurement of additional biomarkers. We did not measure any physiological markers outside of resting blood pressure, heart rate, and patient self-reported medical history to confirm that the subjects were healthy and asymptomatic of any metabolic, cardiovascular, renal, or pulmonary diseases. However, baseline measurement of other markers such as arterial stiffness, lipid profiles, total cholesterol, fasting glucose level, and plasma insulin may have better informed the likelihood to demonstrate responses to an intervention. Based on previous evidence (Boonla, 2014; Srichamroen, 2008; Sahin, 2016; Neelakantan, 2014), it appears subjects with above average arterial stiffness, hypertension, endothelial dysfunction, and insulin resistance may be more sensitive to the effects of curcumin and fenugreek soluble fiber interventions. It is possible that the subjects who exceed the MD in this study might have had biological differences affecting sensitivity to the nutritional interventions. In addition, the responsiveness to an intervention is also likely related to an individuals' genotype. For example, genetic predisposition has been shown to influence differences in low and high responders regarding hypertrophic responses specific to resistance exercise (Roberts, 2018). Subjects that were homozygous for a specific genotype or alleles expression were observed to experience greater or lower degrees of hypertrophy (Roberts, 2018). These observations were centered on hypertrophy responsiveness; however, it is possible that genetic variances may make an individual more receptive to the effects of nutritional interventions and/or aerobic exercise interventions. Additionally, it is possible that the diet of responders during the 28 days of supplementation might have further enhance the effectiveness of supplementation. Based on the current findings, we recommend that

interventions are examined not only by the mean response, but also on an individual-by-individual basis to provide further information on the sensitivity of the interventions (e.g., CurQfen® and/or galactomannans supplementation) to affect performance outcomes. Furthermore, baseline measurement of arterial stiffness, lipid profiles, total cholesterol, fasting glucose level, and plasma insulin in addition to individual responses and a full 28 day log of caloric consumption should be considered to further explain the proportion of population that may demonstrate a meaningful or real change.

Factors related to study design might also help explain the individual variability in response to CurQfen® and/or galactomannans supplementation. Specifically, the relatively low percentage of responders (i.e., exceeded the MD) in this study may be related to the duration of the supplementation period, the relative dosage of supplementation, and/or the exclusion of an exercise intervention. It is possible a longer supplementation period and/or a higher relative dosage are necessary for the effects of curcumin and/or galactomannan's to fully manifest as previous investigators have indicated a dose-dependency (Mathern, 2009; Boonla, 2014; Huang, 2015; Lao, 2006). Furthermore, this study did include an exercise intervention or examine the benefits of curcumin on recovery or inflammation. Previous studies that examined have curcumin supplementation in conjunction with exercise have demonstrated a greater magnitude of change compared to a PLA when the two intervention are combined (Davis, 2007; Huang, 2015; Sahin, 2016). These effects have been attributed to curcumin's anti-inflammatory effects and enhanced recovery (Boonla, 2014; Davis, 2007; Huang, 2015)). Thus, future studies should examine longer supplementation periods (>6 weeks) of curcumin and galactomannan at higher relative doses (>500mg·day^{-1}) in conjunction with an exercise training protocol to determine if the

effects on the VT in this study for a few subjects (14 – 22%) are extended to a larger portion of the sample.

5.5 Limitations

One of the primary limitations of the current study was the dependence on subject compliance. The subjects were not confined to the laboratory throughout the supplementation and testing periods, therefore, sleep and the dietary intake outside of the three days prior to pre- and post-testing were not accounted for. However, there were no differences in the macronutrient and total caloric intakes from the self-reported three-day food logs at pre- and post-test. Having a measurement of physical activity and diet of the months prior to testing would have provided a baseline to determine if these habits changed during the intervention period. In addition, many of our subjects were college aged and it is possible that academic calendar and social stressors might influence their pre- to post-test responses. Furthermore, the laboratory availability for testing was limited and the time of day for pre- to post-test was kept consistent as much as possible but not always identical. To control for these limitations as much as possible, we accounted for any prior supplementation through the health history review as well as encouraged subjects not to change exercise or dietary habits during enrollment.

5.6 Conclusion

The current findings indicated 28 days of CUR and FEN supplementation improved a submaximal threshold (VT), but did not alter the RCP, T_{lim}, or $\dot{V}O_2$Peak. The changes in the VT are supported by previous literature and are most likely related to the vasodilatory properties of curcumin and the increased FFA availability from fenugreek soluble fiber

(Boonla, 2014; Davis, 2007; Huang, 2015; Neelakantan, 2014; Mathern, 2009; Romijn, 1985; Srichamroen, 2008) Previous investigators have indicated that curcumin had a small effect on $\dot{V}O_2$peak in mice, and these effects may be amplified with the addition of an exercise intervention (Davis, 2007; Huang, 2015). Thus, the lack of change in the RCP, $\dot{V}O_2$peak, and T_{lim} in this study may be related to the inclusion of only a supplementation intervention without exercise. Potentially examining these same markers with an exercise intervention group might yield significant results that were not demonstrated from supplementation alone. The primary implications of the current study are that CurQfen® and fenugreek soluble fiber demonstrated equal effects on a submaximal exercise intensity. These findings demonstrate the potential for these nutritional interventions to delay fatigue and improve aerobic performance in healthy, asymptomatic individuals.

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