Effect of curcumin supplementation on exercise-induced oxidative stress, inflammation, and muscle damage

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Oxidative stress (OS) and inflammation can be detrimental to exercise performance. Curcumin has shown to reduce OS, inflammation, muscle damage, and soreness. The purpose of this study was to examine the effect of curcumin on biomarker markers of OS (MDA, TAC), inflammation (TNF-α), muscle damage (CK) and soreness. Participants performed a muscle damaging protocol, before and after supplementation. Subjects were randomly assigned to curcumin (1.5 g/day) or placebo for 28 days. Blood was sampled pre and immediately post exercise (additional post: 60 min, 24, and 48 h). No significant differences were observed for OS or inflammation. There was a treatment X condition interaction for CK, where CK were significantly lower post supplementation in the curcumin group ($p < 0.0001$). Curcumin resulted in significantly lower muscle soreness compared to the placebo ($p = 0.0120$). In conclusion, curcumin may reduce muscle damage, and soreness without affecting OS and inflammatory after exercise.

DEDICATION

This project is dedicated to my family and their unconditional support throughout this entire process. Although this project has been very rewarding, it has been very challenging. Without unwavering love and motivation given from my family, this project would not have been successful.

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CHAPTER I

INTRODUCTION

Background

Oxidative stress (OS) and inflammation may be detrimental to exercise performance (Cesari et al., 2004) and health (Huang et al., 2015). OS is the result of an imbalance between reactive oxygen species (ROS), reactive nitrogen species (RNS) and antioxidants (Sies, 1997). Antioxidants serve as a protectant mechanism from the damaging effects of ROS (Kunwar & Priyadarsini, 2011). Excessive ROS production has been shown to impair metabolic function (Costa, Amorim, Quintanilha, & Moradas-Ferreira, 2002; Grant, Quinn, & Dawes, 1999), which may cause further risk for cardiometabolic and cardiovascular diseases (Montezano & Touyz, 2012). Although exercise is a protectant against many diseases (Kullo, Khaleghi, & Hensrud, 2007), it elicits acute OS and inflammation (Park & Kwak, 2016; Tiidus, 1998), with single bouts of exercise shown to increase OS (Koska et al., 2000; Maughan et al., 1989). In addition to aerobic exercise, unaccustomed resistance training (RT) (eccentric muscle actions) also elicits an OS response (Proske & Morgan, 2001), along with muscle damage (Newham, McPhail, Mills, & Edwards, 1983) and muscle soreness (Maughan et al., 1989). Following the onset of muscle damage, an initial inflammatory response initiates for muscle regeneration (Philippou, Maridaki, Theos, & Koutsilieris, 2012). Under normal conditions, the inflammatory process is regulated shortly after by anti-

inflammatory processes (Philippou et al., 2012). However, if chronic inflammation persists, it may hinder exercise performance (Cesari et al., 2004).

Although exercise may reduce OS (Hollander et al., 1999), it may not be sufficient; thus, antioxidant supplementation may aid in reducing OS and inflammation (Kodama et al., 2009). Curcumin, the active ingredient in the spice turmeric, supplementation reduces OS (Kim et al., 2007; Ruby, Kuttan, Dinesh Babu, Rajasekharan, & Kuttan, 1995) and inflammation (Gupta et al., 2011; Kim et al., 2007; Kondamudi, Kovelamudi, Nayak, Rao, & Shenoy, 2015), while enhancing antioxidant capacity (Hamidie, Yamada, Ishizawa, Saito, & Masuda, 2015; Sarker et al., 2015; Takahashi et al., 2014). Curcumin may act on several different cytokine/ROS mediated inflammatory pathways. Curcumin has shown to specifically reduce expression of nuclear factor kappa-light-chain-enhancer of activated β cells (NF-kB)(Kang, Lee, Price, & Kim, 2009)/cyclooxygenase-2 (COX-2) (Kang et al., 2004), while increasing antioxidants via activation of nuclear factor-like 2 (NrF2) (Shehzad & Lee, 2013). While there is a consensus on the potent anti-inflammatory (Davis et al., 2007; McFarlin et al., 2016) and antioxidant (Kawanishi et al., 2013; Takahashi et al., 2014) effects of curcumin, there are inconsistent reports for reduced muscle damage and soreness (Drobnic et al., 2014; Jäger et al., 2017; McFarlin et al., 2016). Most recent studies have demonstrated reduced creatine kinase concentration (i.e. biomarker of muscle damage) (Jäger et al., 2017; McFarlin et al., 2016) following exercise-induced muscle damage. However, both reported confounding results for inflammation and muscle soreness. McFarlin et al. (2016) reported a reduction in tumor necrosis factor alpha (TNF-α) up to 25%, whereas, Jäger et al. (2017) demonstrated no changes. In contrast, Jäger et al.

(2017) reported a significant reduction in perceived muscle soreness, while McFarlin et al. (2016) did not. Differences may lie in the assessment technique for subjective measurements, subject training status, and exercise protocol. In an animal model, Davis et al. (2007) utilized a similar protocol of downhill running (Jäger et al., 2017), and reported a reduction in both inflammation and muscle damage using more invasive techniques (plasma vs. tissue sample), thus, suggesting differences may also lie in species/tissue sampling.

Further, rather than acute supplementation (e.g. hours-days) used in previous studies (Drobnic et al., 2014; McFarlin et al., 2016; Nicol, Rowlands, Fazakerly, & Kellett, 2015), this study consisted of 28 days of curcumin supplementation. The primary aim of this study was to examine the relationship between a commercially available curcumin (CurcuFresh^{TM}) on markers of inflammation, total antioxidants, muscle damage, and muscle soreness. We hypothesized curcumin supplementation would reduce inflammation and increase antioxidant status at rest. Additionally, we hypothesized curcumin supplementation would reduce inflammation, muscle damage, and muscle soreness following exercise-induced muscle damage (EIMD).

CHAPTER II

LITERATURE REVIEW

Introduction

Oxidative Stress (OS) may negatively affect exercise performance and health, thus, controlling OS may be critical for optimizing both. This review will define OS and the different mechanisms that can perpetuate OS. Moreover, this review will specifically examine exercise-induced OS, inflammation, and muscle damage. Aerobic exercise and eccentric actions have been shown to induce OS (Fisher-Wellman & Bloomer, 2009; Powers & Jackson, 2008; Proske & Morgan, 2001). Further, endogenous antioxidant status and exogenous supplementation with regard to exercise will be discussed. Lastly, this review will focus on the effects of curcumin on inflammation and antioxidants regarding exercise-induced responses.

Defining Oxidative Stress, Reactive Oxygen Species

Although the mitochondria are the energy producing organelle in the cell, they are the source of potentially harmful reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) (Balaban, Nemoto, & Finkel, 2005). Aerobic metabolism is responsible for the greatest production of ROS (Balaban, Newmoto, & Finkel, 2005). The electron transport chain may lead to an incomplete reduction in oxygen which gives rise to the

superoxide anion (O_2^-) (Jastroch, Divakaruni, Mookerjee, Treberg, & Brand, 2010). Superoxide is relatively unreactive; however, in the presence of other radicals, such as nitric oxide, may react very quickly to form peroxynitrate, which is a relatively strong oxidizing agent (Halliwell & Gutteridge, 2015). Within the cell membrane of the mitochondria, "Complex I" and "Complex III" is reported to generate the majority of superoxide under resting conditions (Chen, Vazquez, Moghaddas, Hoppel, & Lesnefsky, 2003; Liu, Fiskum, & Schubert, 2002). Further, superoxide may be reduced to a less active molecule, hydrogen peroxide (H_2O_2) (Loschen, Azzi, Richter, & Flohe, 1974), where it can readily cross cell-membranes with a relatively longer half-life compared to superoxide. Although hydrogen peroxide is relatively stable, in the presence of iron, it may be converted to a more highly reactive radical, hydroxyl radical ($\cdot OH$), in the Fenton reaction (Powers & Jackson, 2008). Due to the dependency of aerobic metabolism, the generation of ROS may be inevitable. However, the production of ROS may be a trigger for endogenous antioxidants (Brieger, Schiavone, Miller, & Krause, 2012) and improved mitochondrial redox environment (Hollander et al., 1999; Lander, 1997). The imbalance among antioxidants and the generation of oxidants or ROS has been termed OS (Sies, 1985, 1986, 1991). OS signifies a disruption/challenged redox environment most notably observed through molecular changes to redox sensitive molecules and increased production of ROS (Powers & Jackson, 2008). There is an unwavering consensus that OS is the underlying pathological cause for impaired metabolic function (Costa et al., 2002; Grant et al., 1999), thus, leading to increased risk for cardiovascular disease (Huang et al., 2015).

Exercise-Induced Oxidative Stress

Most research has confirmed as mitochondria the main site for ROS production (Davies, Maguire, Brooks, Dallman, & Packer, 1982), however, some researchers suggest that it is only at rest when the mitochondria is the main culprit, and there is less ROS generation in the mitochondria during exercise in contrast to previous theories (Kozlov et al., 2005). Other, more prominent sites of ROS production during exercise, may be to blame, which include the sarcoplasmic reticulum, transverse tubules, plasma membranes and cytoplasmic spaces within the muscle cell (Powers & Jackson, 2008). In these areas, other ROS generating enzymes such as NADPH oxidases (Ushio-Fukai, 2006) and xanthine oxidases (McNally et al., 2003) have been identified as viable sources of ROS production. ROS production from these enzymes have been shown to alter the function of calcium pumps, and contractile structures in the muscle fiber, thus, possibility leading to ROS-induced muscle fatigue (Powers & Jackson, 2008). Nonetheless, exercise can increase ROS generation resulting in an OS response (Park & Kwak, 2016); however, several exercise training elements dictate the extent of ROS production and damage.

The extent of oxidative damage is related to both the duration (Bloomer, Davis, Consitt, & Wideman, 2007) and the intensity (Goto et al., 2003) of exercise. Most literature investigating acute aerobic exercise consisting of longer durations have examined elite runners following marathons and triathlons (McAnulty et al., 2005; Nieman et al., 2004; Radák et al., 2003), as well as other modalities such as cycling (McAnulty et al., 2007). OS is prevalent via increased lipid peroxidation measurements such as thiobarbituric acid reactive substances (TBARS) which is typically measured as malondialdehyde (MDA) equivalents (Fisher-Wellman & Bloomer, 2009). Other modes

of exercise, such as short-term anaerobic exercise has also shown to increase ROS/RONS resulting is OS (Groussard et al., 2003). Additionally, other anaerobic exercise modalities such as RT elicits similar responses of increased ROS/OS as seen from aerobic exercise (Fisher-Wellman & Bloomer, 2009). Studies that have examined similar outcomes in increased biomarkers of lipid peroxidation have used full-body RT paradigms with multiple sets (McBride, Kraemer, Triplett-McBride, & Sebastianelli, 1998; Ramel, Wagner, & Elmadfa, 2004; Vina et al., 2000; Vincent, Morgan, & Vincent, 2004), while some have demonstrated elevated markers of OS only utilizing one exercise (Bloomer, Falvo, Schilling, & Smith, 2007; Subudhi, Davis, Kipp, & Askew, 2001; Volek et al., 2002). In contrast, Bloomer et al. (2005) investigated differences in blood markers of OS in ten cross-trained men during 30-minutes of cycling at 70% $\dot{V}O_{2max}$ and dumbbell squats at 70% of 1-RM (repetition maximum). When compared to cycling, blood markers of OS were significantly higher in the RT protocol post exercise (Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005). These results suggest there may be differences between aerobic and anaerobic exercises (i.e. resistance exercise) and their respective roles for perpetuating OS. A later study by the same authors demonstrated training status may modulate the OS response to exercise (Bloomer et al., 2006). In this study (Bloomer et al., 2006), 12 anaerobically trained males performed six, 10-s sprints, on a separate occasion participants performed barbell squats matching the same volume (total work [kJ]). There were no significant changes in MDA following either exercise. The authors note, repeated exposure to OS may potentiate protective mechanisms, resulting in greater resistance to OS. Given the association between OS and RT, exact mechanisms responsible for excessive ROS during RT should be identified.

Eccentric Muscle Contraction

During dynamic exercise, muscles undergo concentric and eccentric actions (D. B. Hollander et al., 2015). Morgan and Allen (1999), and Warren, Ingalls, Lowe, and Armstrong (2001) identified two major components that contribute to muscle damage, damaged sarcomeres and an impaired "excitation-contraction coupling" system. Ultimately, eccentric muscle actions elicit the most significant muscle damage (Newham et al., 1983; Proske & Morgan, 2001) and are linked to muscle soreness (Maughan et al., 1989; Radak, Pucsok, Mecseki, Csont, & Ferdinandy, 1999). A universally accepted method to promote muscle damage is through downhill running, allowing for controlled lengthening, while stimulating damage (Davis et al., 2007; Drobnic et al., 2014; Powers & Jackson, 2008). Maughan et al. (1989) showed increases in MDA after downhill-running, while also reporting evidence indicative of muscle damage via increased creatine kinase (CK) levels. Another study observed increased CK levels of approximately 150 fold, compared to resting levels after a one-legged eccentric exercise protocol (Hellsten, Frandsen, Orthenblad, Sjødin, & Richter, 1997). Other studies have used other methods including timed eccentric contractions during dynamic resistance exercises resulting in similar muscle damage (Nicol et al., 2015). However, other studies reported no change in MDA after eccentric muscle contractions (Saxton, Donnelly, & Roper, 1994). Following exercise-induced muscle damage, an inflammatory response results lead to more ROS production (Azlina, Kamisah, Chua, Ibrahim, & Qodriyah, 2015).

Inflammation/Cytokine Production and Muscle Damage/Recovery

Muscle damage involves an inflammatory response which is initiated to modulate the repair process or degradation of muscle tissue before the final repair stages (Philippou et al., 2012). ROS generation may be responsible for the initial inflammatory response in damaged muscle tissue (Tiidus, 1998). Specific cells such as neutrophils, macrophages, and lymphocytes engorge the damaged tissue (Pedersen & Hoffman-Goetz, 2000; Smith, Kruger, Smith, & Myburgh, 2008), which secrete cytokines that modulate repair of damaged muscle tissue (Pedersen, Ostrowski, Rohde, & Bruunsgaard, 1998a; Tidball & Villalta, 2010). The main cytokine/ROS mediated inflammatory cascades consist of the COX-2 and nuclear factor kappa-light-chain-enhancer of activated β cells (Nf-kB) (Peterson, Bakkar, & Guttridge, 2011). The COX-2 pathway is responsible for the production of prostaglandins, which are responsible for the regeneration of damage muscle tissue and muscle soreness (Bondesen, Mills, Kegley, & Pavlath, 2004). Stimulation of cytokines are found within the damaged muscle tissue, in addition to other tissues and circulation, and can promote localized inflammation (Pedersen & Hoffman-Goetz, 2000; Pedersen, Ostrowski, Rohde, & Bruunsgaard, 1998b). The responses to damaged muscle tissue consists of break-down, repair, and regeneration of muscle tissues (Charge & Rudnicki, 2004; Pedersen et al., 1998a; Smith et al., 2008), whereas muscle repair after damage can be explained declination, inflammation, and growth (Mourkioti & Rosenthal, 2005; Prisk & Huard, 2003; Tidball, 2005; Tidball & Villalta, 2010). However, if inflammatory response is prolonged, additional damage will occur, disrupting muscle growth (Philippou et al., 2012).

One major cytokine involved in the initial inflammatory response is tumor necrosis factor alpha (TNF-α) (Figarella-Branger, Civatte, Bartoli, & Pellissier, 2003; Peake, Nosaka, & Suzuki, 2005). Increased secretion of this pro-inflammatory cytokine perpetuates muscle damage (Philippou et al., 2012; Tidball & Villalta, 2010) which, may inhibit performance (Cassatella, 1995). Hardin et al. (2008) reported decreased contractile force in mice diaphragms after TNF-α administrations, which supports the relationship between cytokines and performance. Further, TNF-α activates the transcriptional factor Nf-kB which initiates the inflammatory cascade, ultimately increasing adhesion molecules in the endothelial lining of vessels; thus TNF-α is strongly associated with the progression and initiation of atherogenesis/atherosclerosis (Lastra, Manrique, & Hayden, 2006). Unlike the pro-inflammatory cytokine TNF-α, interleukin 6 (IL-6), also known as a myokine due to direct secretion from muscle fibers (Pedersen, Akerstrom, Nielsen, & Fischer, 2007), is an anti-inflammatory cytokine (Philippou et al., 2012). IL-6 plays pivotal roles in mobilizing energy stores during exercise (Febbraio & Pedersen, 2002) while also regulating several neurotransmitters and other signaling cascades (Gleeson, 2000). The reported increased production of IL-6 during exercise increases the synthesis of other anti-inflammatory cytokines, which can inhibit TNF-α production (Petersen & Pedersen, 2005). Although IL-6 is classified as anti-inflammatory (Tilg, Dinarello, & Mier, 1997), if chronically elevated, it can act as a pro-inflammatory cytokine (Pedersen et al., 2007; Pedersen & Hoffman-Goetz, 2000). IL-6 is related to exercised-induced muscle damage (Bruunsgaard et al., 1997; Hellsten et al., 1997; Pedersen et al., 1998a; Steensberg et al., 2000). Hellsten et al. (1997) and Bruunsgaard et al. (1997) both reported a substantial increase in IL-6, 90 min and 120

min after eccentric protocols, respectively. Many studies have also demonstrated a relationship between increased IL-6 and strenuous aerobic exercise (Castell et al., 1996; Nieman et al., 2003; Ostrowski et al., 1998; Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999; Sprenger et al., 1992; Ullum et al., 1994). Pedersen and Hoffman-Goetz (2000) and Pedersen et al. (2007) further discuss the possible relationship between increased IL-6 secretion and the type of exercise modality, intensity, and duration. Ultimately, increased levels of inflammatory cytokines are associated with deferments in performance (Cesari et al., 2004; Taaffe, Harris, Ferrucci, Rowe, & Seeman, 2000; Visser et al., 2002). If inflammation persist, it may inhibit muscle regeneration and possibly reduce exercise performance.

Antioxidants

Endogenous

Improved endogenous antioxidant status may be a result from exercise training (Wiecek et al., 2016). Although some studies have demonstrated increased endogenous antioxidants with RT (Çakir-Atabek, Demir, PinarbaSili, & Gündüz, 2010; Ramel et al., 2004), research is scarce, and the relationship between RT and endogenous antioxidants remains unclear. Although excessive ROS production can be detrimental to health (Ji, Gomez-Cabrera, & Vina, 2006) and performance (Donges, Duffield, & Drinkwater, 2010), acute ROS production may improve the mitochondrial redox environment (Dada et al., 2003; Hollander et al., 1999). Cellular processes depend heavily on the mitochondria aerobic capacity (Jastroch et al., 2010). In response to ROS production, endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and

glutathione peroxidase (GPX) are up-regulated (Brieger et al., 2012). These antioxidants help scavenge ROS and reduce oxidative strain. First discovered in 1969, SOD is the first line of protection against the anion superoxide (McCord & Fridovich, 1969), which catalyzes the reaction to create hydrogen peroxide.

However, chronic aerobic training is shown to increase endogenous antioxidants (Brites et al., 1999; Mena et al., 1991; Ortenblad, Madsen, & Djurhuus, 1997). Mena et al. (1991) reported an increase in SOD in professional cyclist after a 2800-kilometer ride in 20 days. Brites et al. (1999) and Ortenblad et al. (1997) both observed increased SOD activity in soccer and volleyball players, respectively. Similarly, Miyazaki et al. (2001) reported increased SOD levels in 9 untrained males after a 12-week endurance program consisting of running on a treadmill at 80% maximal heart rate. Increased antioxidants such as SOD are strongly related to decreased OS (Fielding & Meydani, 1997). Increased fitness levels appear to positively influence OS during exercise (Kocabas et al., 2016). This is due to the activation of peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1-α), a major metabolic adaptation to exercise, which increases endogenous antioxidants through binding to its' downstream targets (St-Pierre et al., 2006). Other possible mechanisms such as direct activation of Nrf2 improves the mitochondria redox environment (Cantó & Auwerx, 2009; Kobayashi & Yamamoto, 2005). Although these studies reported increased antioxidants with trained individuals, literature is inconsistent with these findings. One study reported no changes in SOD regarding untrained individuals after an 8 week cycling program (Tiidus, Pushkarenko, & Houston, 1996). Likewise, Tauler, Gimeno, Aguilo, Guix, and Pons (1999) reported no increases in SOD, but did find increased CAT activity in aerobically trained athletes. It is

pertinent to mention that observable changes in SOD concentrations differ among exercise intensity and duration (Powers et al., 1994), which may give rise to possible explanations for confounding results.

Once superoxide is catalyzed to hydrogen peroxide, secondary enzymes, GPX and CAT, may reduce the radical to water. Similar to SOD, many studies have identified increased concentrations of GPX contingent upon both exercise intensity and duration (Powers et al., 1994). In one study Powers et al. (1994) examined significant increases GPX activity and expression following high-intensity/long-duration exercises when compared to low-intensity/short-duration exercises. Further, it was reported that elevated concentration/activity of GPX was increased in Type I/IIa muscle fibers. In the same study, there were no observable differences found for CAT concentration/activity, suggesting CAT activity and expression is independent of changes seen in GPX and SOD. Along with exercise, dietary interventions consisting of increased fruit and vegetable consumption are attributed to increase endogenous antioxidant status (Teixeira, Mill, Pereira, & Molina Mdel, 2016). Exercise as well as dietary interventions are well established factors that can enhance endogenous antioxidants.

Exogenous

In conjunction with exercise, supplemental or exogenous antioxidants may be used to decrease OS and/or improve antioxidant status. Some supplemental antioxidants that have demonstrated reduced OS include (but are not limited to): quercetin (Duarte et al., 2002; Duarte et al., 2001; Garcia-Saura et al., 2005), resveratrol (Ates et al., 2007; Rege et al., 2013; Venturini et al., 2010), and coenzyme Q10 (CoQ10) (Pala et al., 2016; Zhang et al., 2013). Pala et al., (2016) reported anti-inflammatory potential through

supplementation with CoQ10. Other dietary supplements such as fish oil or flax seed oil are shown to lower cytokines (IL-6) and upregulate endogenous antioxidants such as SOD, GSH, and CAT (Jangale, Devarshi, Bansode, Kulkarni, & Harsulkar, 2016). However, these studies reported decreased markers of OS and inflammatory cytokines in animals. One study examined quercetin supplementation in humans and found no evidence of decreased OS, but demonstrated decreased blood pressure, which may reduce the risk of cardiovascular disease (Edwards et al., 2007). Edwards et al., (2007) hypothesized possible metabolic differences between species, which may result in contraindicating findings in human models. Further, Edwards et al., (2007) suggested that quercetin may have a localized antioxidant effect that was undetectable.

In addition, two of the most popular antioxidants are vitamin C and E, where they work collectively to provide protection against lipid peroxidation (Knez, Coombes, & Jenkins, 2006). Multiple studies have reported decreased biomarkers of exercise-induced OS following vitamin C (Alessio, Goldfarb, & Cao, 1997; Goldfarb, Patrick, Bryer, & You, 2005; Sastre et al., 1992) and vitamin E supplementation (Kawai et al., 2000; Satoshi, Kiyoji, Hiroyo, & Fumio, 1989). Despite advantageous findings supporting vitamin C and vitamin E supplementation, some studies have indicated null findings (Bryant, Ryder, Martino, Kim, & Craig, 2003; Gaeini, Rahnama, & Hamedinia, 2006). Bryant et al. (2003) reported possible differences in vitamin C and vitamin E supplementation following independent dosage phases, where vitamin E supplementation was superior for reducing MDA concentrations after 60 min and 30 min cycling tests. In contrast, one study demonstrated vitamin E supplementation was insufficient for reducing muscle damage, protein, and lipid oxidation (Gaeini et al., 2006). Moreover, both

antioxidants in high dosages may be associated with adverse metabolic adaptations to exercise (Gomez-Cabrera et al., 2008; Picklo & Thyfault, 2015). Paulsen et al. (2014) examined vitamin C and vitamin E ingestion in 54 young men and women. This study utilized a double-blind, randomized, controlled trial, where 1000 mg of vitamin C and 235 mg of vitamin E or placebo were randomly assigned during 11 weeks of an endurance training program. Paulsen and colleagues reported decreased metabolic adaptations via decreased PGC- α activation, which is known to stimulate mitochondrial biogenesis. However, the treatment group exhibited a greater $\dot{V}O_{2max}$ than the control group (Paulsen et al., 2014). Conversely, previous literature indicated no adverse effect on exercise-induced metabolic adaptations through high doses of vitamin C (Wadley & McConell, 2010). In all, there are mixed findings that both support and dismiss beneficial reductions for exercise-induced OS along with enhanced exercise performance following exogenous antioxidants. Future research should investigate these antioxidant effects in humans due to the unclear effects and other dependent variables such as training status and sex.

Curcumin

Description/Mechanism

Curcumin, the active ingredient of the spice turmeric, is a found within the *Curcuma Longa* plant (Hamidie et al., 2015). Curucmin is a lipophilic polyphenol, suggesting it can penetrate all cell membranes (i.e. blood brain barrier, cell wall, and cell membrane) (Goel, Kunnumakkara, & Aggarwal, 2008). Evidence suggests curcumin may enhance the redox environment, and induce mitochondrial biogenesis (Hamidie et al., 2015), along with inhibiting inflammatory cytokines (Gupta et al., 2011; Kim et al., 2007;

Kondamudi et al., 2015). Several mechanisms are thought to explain curcumin's effect of inflammation and OS. One mechanism is indirect inhibition of Nf-kB (Singh & Aggarwal, 1995), which is responsible for the production of many inflammatory cytokine such as TNF-α and IL-6 in muscle. Curcumin may also decrease other inflammatory cascades such as COX-2 (Chun et al., 2003). Curcumin, may up-regulate endogenous antioxidants through direct activation of the transcriptional factor, Nrf2, which then activates and binds to the antioxidant response element (ARE) (Shehzad & Lee, 2013). Additionally, the chemical structures of polyphenols, such as curucmin, provide a direct mechanism for scavenging ROS. The disassociation of the hydroxyl group may potentially decrease the scavenging of superoxide radicals through donation of electrons in vitro (Singh, Barik, Singh, & Priyadarsini, 2011). Further, due to the chelation properties, curucmin may inhibit the Fenton reaction where hydrogen peroxide is oxidized to a more unstable radical, hydroxyl radical, in the presence of iron (Perron & Brumaghim, 2009). The decreased ROS production may also lead to indirect inhibition of Nf-kB (Biswas, McClure, Jimenez, Megson, & Rahman, 2005) in the aforementioned mechanisms. Many studies have examined the effects of curcumin on the modulation of inflammation and ROS production in animals, with promising results regarding human trials. In one study, Hamidie et al. (2015) reported increased expression of AMP-activated kinase (AMPK) from curcumin supplementation in rats. AMPK in turns, phosphorylates and primes PGC-1α for deacetylation via sirtuin 1 (SIRT1). The increased activity of PGC-1α results in greater levels of mitochondria biogenesis. These adaptations improve the redox environment within the cell and may lead to reduced oxidative strain.

Supporting Evidence

Along with curcumin's ability to enhance mitochondrial biogenesis in conjunction with endurance training, curcumin may improve recovery, thus, potentially improving exercise performance indirectly (Davis et al., 2007). Davis et al. (2007) reported reductions in markers of muscle damage with down-hill running (predominately eccentric muscle contractions). Furthermore, curcumin blunted the inflammatory cytokines, TNF-α and IL-6. Another study demonstrated reductions in blood markers of inflammation such as C-reactive proteins (CRP) through curcumin supplementation (Sarker et al., 2015). Sarker et al. (2015) also reported an increase in GSH and improvements in the redox environment. Earlier studies support improved antioxidant status (preventing reduction of SOD and CAT) and reduction of inflammatory cytokines in diabetic rats with curcumin supplementation (Gupta et al., 2011). Likewise, another study reported decreases in CRP and increased nitric oxide with chronic inflammation during curcumin ingestion (Banerjee, Tripathi, Srivastava, Puri, & Shukla, 2003). However, Banerjee et al., (2003) found no change in the inflammatory cytokine TNF-α. Given the association of improved antioxidant capacity and reduced OS, curcumin may attenuate factors accompanied with cardiovascular disease. Most importantly, the aforementioned studies investigated these effects of curcumin supplementation in mice. Literature that investigates curcumin supplementation in human subjects is limited and inconsistent. Although a few studies reported slightly lower levels of CK, they demonstrated no significant decreases in markers of OS, inflammation, and endogenous antioxidants (Drobnic et al., 2014; Nicol et al., 2015; Sciberras et al., 2015). Some limitations to the above studies are inadequate dosage (i.e. reduced bioavailability) (Nicol et al., 2015) and absence of an eccentric

muscle damage protocol (Sciberras et al., 2015). In contrast, other studies showed significant decreases in OS, muscle damage, and inflammation, as well as significant increases in antioxidants (McFarlin et al., 2016; Soni & Kuttan, 1992; Takahashi et al., 2014; Usharani, Mateen, Naidu, Raju, & Chandra, 2008). Most recently, McFarlin et al. (2016) indicated minimal supplementation of curcumin (i.e., 400 mg/day for 5 days) effectively lowered levels of TNF-α and CK following an eccentric muscle damage protocol. As little as 90 mg of curcumin two hours before and after exercise, is shown to effectively reduce OS and increase antioxidants (Takahashi et al., 2014). Nonetheless, curcumin may lower OS, inflammation, and increase antioxidant status.

Summary/Conclusion

Although OS is essential to induce antioxidant and mitochondrial adaptations, excessive and/or chronic OS is detrimental to exercise performance and health; moreover, elevated ROS and inflammatory cytokines may adversely affect recovery and performance. Despite increases in endogenous antioxidants due to exercise, the demand for dietary or supplemental alterations including exogenous antioxidants may be inevitable. Specifically, McBride and Kraemer (1999) suggested supplemental antioxidants may be beneficial at the start of a new training program. Ultimately, curcumin may provide benefits, which include, but not limited to, increased exercise performance, enhanced recovery post exercise, and overall better health. More research is necessary to further solidify curcumin supplementation and its effect on oxidative stress, inflammation, and muscle damage.

CHAPTER III

MATERIALS AND METHODS

IRB Approval

This investigation was approved by the Institute of Biosafety Committee (IBC) at Mississippi State University, as well as the Institutional Review Board (IRB) at Mississippi State University. All participants reported to the J.A. Chromiak Applied Research Laboratory in the Department of Kinesiology at Mississippi State University between 5-9 a.m. for testing. Subjects were recruited via word of mouth, as well as email. Recruitment flyers were also posted throughout the Department of Kinesiology at Mississippi State University.

Design and Subjects

The study design was a randomized, double-blinded, placebo-controlled trial to compare curcumin supplementation and a placebo on markers of OS, inflammation, antioxidant capacity, muscle damage, and muscle soreness. Twenty apparently healthy non-smoking males aged 18-39 years were selected for this study, with 19 subjects completing all phases. One subject was dropped due to compliance. Subject characteristics can be found in Table 1. Participants were randomly assigned to either the treatment or placebo group. Subjects must be participating in light or moderate exercise activity (≥30 min of moderate intensity of physical activity at least three days/week for at least three months or ≥ 150 minutes) (American College of Sports, 2000). Exclusion

criterion included: regular ingestion of any anti-inflammatory drugs/foods (NSAIDS) (at least 3 of 7 days) (McFarlin et al., 2016), pre-existing medical diagnoses of cardio metabolic disorders (e.g., hypertension, diabetes), musculoskeletal disorders (e.g. arthritis, osteoporosis), known allergy to curcumin, taking blood thinners (e.g., coumadin), and any medical condition which could hinder completion of the exercise protocol (e.g., lower body extremity injury). Participants underwent an 8 hour fast, and refrained from any alcohol or caffeine consumption 48 h before each session. Subjects were also instructed not to perform any exercise 72 h before testing and remain inactive until the last blood analysis was taken, 48 h post exercise. A priori, sample size was chosen based on previous studies that analyzed similar blood markers (McFarlin et al., 2016; Nicol et al., 2015; Soni & Kuttan, 1992) and G*Power (Faul, Erdfelder, Lang, & Buchner, 2007). An effect size of $f^2 = 0.25$ and an α error probability of 0.05 were set for a power of 0.95.

Testing Procedures

Participants completed two testing sessions along with follow up blood draws (BD) (Figure 1). During the initial visit, the participants provided written informed consent and completed a health history report. Additionally, the general testing procedures were explained. During each testing session, anthropometric measures were taken before the exercise protocol. An initial BD for baseline measurements was taken immediately before the exercise protocol. Participants performed an exercise-induced muscle damaging (EIMD) protocol before and after 28 days of supplementation. Blood was sampled pre exercise, immediately post exercise, as well as 60 min, 24 h and 48 h

post exercise. Upon each BD, perceived muscle soreness was taken using a visual analog scale (VAS).

Supplementation

Participants were randomly assigned to three, 500 mg capsules of curcumin once a day for a total of 1.5 g of curcumin/69 mg of curcuminoids or a cellulose placebo capsule daily for 28 days. This study implemented a newly-enhanced absorption and pharmacokinetics of fresh turmeric derived from curcuminoids in comparison with the standard curcumin from dried rhizomers (Krishnakumar, Kumar, Ninan, Kuttan, & Maliakel, 2015). Subjects were instructed to consume two capsules of either the placebo or curcumin upon waking in the morning with breakfast and one capsule in the afternoon with dinner. To ensure compliance, subjects were reminded weekly to ingest the suggested amount of their provided supplement. Further, each subject was required to maintain a 90% supplement uasge rate, which was calculated using the following equation:

$$Amount\ of\ Capsules\ (End\ of\ 28\ days) \div Total\ Amount\ of\ Capsules \times 100$$

Eccentric Muscle Damage Protocol

Participants completed 15 minutes of continuous sitting with one leg (i.e., while the opposite leg is used for balance) at a bench height of 16.5 inches. A metronome was set at a pace of 30 beats/min, and the participants were instructed to follow a cadence of 15 sit-stand repetitions/minute for a total of 225 repetitions. The participants were allowed to stand with both legs. The lower phase of the sitting protocol consists primarily of eccentric muscle actions. Previous literature has utilized various modalities to initiate

muscle damage via eccentric muscle actions (Davis et al., 2007; McFarlin et al., 2016; Nicol et al., 2015). A single session of eccentric muscle actions can elicit OS and muscle damage (Mair et al., 1995). The participant's body weight was used for resistance making the load relative for each participant. The exercise leg was randomized for both pre and post supplementation.

Instrumentation

An aerobic step bench (Step Fitness & Recreation Inc, Marietta, GA) was used for the EIMD protocol. An appropriate bench height was selected based on each participant's height. A heightronic digital stadiometer (Quick Medical 235-D Digital Stadiometer; Issaquah, WA) and digital scale (DEFENDERT 5000; Parsippany, NJ) was used to assess anthropometric measures for height and weight. A VAS was also used to measure muscle soreness and weakness (Boonstra, Preuper, Reneman, Posthumus, & Stewart, 2008). The VAS consisted of a horizontal line, 20 cm in length, with endpoints of "no soreness" to "unbearable soreness".

Blood Sampling and Analysis

A 14 mL sample of blood was collected at each BD. On the two exercise testing sessions, an intravenous catheter was inserted into the antecubital vein, where an initial 3 mL of blood was drawn into a syringe and discarded before the sample blood volume was transferred into the appropriate vacutainer. On non-testing days, venous blood samples were harvested from the antecubital veins for each sample using a butterfly needle, a while subjects were in a supine position. Subsequently, blood was transferred into non-coated vacutainers for serum and sodium heparin vacutainers for plasma and centrifuged

for 15 minutes at 2500 rpm at 4ºC. Plasma samples were stored at -80º C. Blood samples were drawn: pre exercise, post exercise, 60 min, 24 h, and 48 h post exercise for both conditions (i.e., before and after supplementation) for a total of 10 BDs. Blood samples were used to analyze markers of OS (thiobarbituric acid reactive substances, (TBARS), muscle damage (creatine kinase, (CK)), inflammation (tumor necrosis factor alpha, (TNF-α)), and antioxidant capacity (total antioxidant capacity, (TAC)) expressed in units of Trolox equivalents. All blood analysis except for CK concentrations, which was analyzed using a spectrophotometer (Pointe Scientific; Canton, MI), was analyzed in duplicates and absorbance was measured using an iMark Bio-Rad Microplate Absorbance Reader (Life Science Research, Hercules, CA).

Plasma was used to measure TBARS, expressed in units of malondialdehyde (MDA) and TAC using a commercial available assay kit (Cayman Chemical, Ann Arbor, MI). Serum was used to analyze TNF-α via end-point method (R & D systems, Minneapolis, MN). Additionally, CK concentrations were measured via kinetic assay (Pointe Scientific; Canton, MI).

Statistical Analysis

Data are reported as mean ± SD. All statistical procedures were conducted using SAS version 9.4 (SAS Institute, Cary, NC). A 2 X 2 X 5 RMANOVA (Treatment X Condition X Time) was used to measure the changes in TBARS, CK, TNF-α, and TAC. A 2 X 2 X 4 RMANOVA was used to measure perceived muscle soreness between time points and treatment. A Fisher's least significance different (LSD) post-hoc analysis was used in the instances of significant main effect ($p < 0.05$).

CHAPTER IV

RESULTS

Inflammation

Mean TNF-α levels are shown in Figure 2. There were no significant interactions (treatment X condition [$F = 0.00$, $p = 0.975$]; time X condition [$F = 0.58$, $p = 0.675$]; or treatment X time X condition [$F = 0.36$, $p = 0.938$]) regarding plasma TNF-α concentrations. However, there was an overall significant main effect for time ($F = 3.89$, $p < 0.005$) at 24 h post exercise where plasma levels were significantly lower than all other time points.

Total Antioxidant and Oxidative Stress

As for TAC plasma concentrations, there were no interactions (treatment X condition [$F = 0.92$, $p = 0.339$]; time X condition [$F = 0.81$, $p = 0.52$]; treatment X time X condition [$F = 0.40$, $p = 0.919$]) or main effects for treatment ($F = 1.92$, $p = 0.1679$), time ($F = 1.43$, $p = 0.228$), or condition ($F = 1.33$, $p = 0.252$) (Figure 3).

Moreover, for plasma MDA concentrations, there were no interactions (treatment X condition [$F = 1.73$, $p = 0.191$]; time X condition [$F = 0.96$, $p = 0.431$]; treatment X time X condition [$F = 1.08$, $p = 0.384$]). Additionally, there were no significant main effects for treatment ($F = 1.29$, $p = 0.278$)), time ($F = 0.04$, $p = 0.834$), or condition ($F = 1.22$, $p = 0.271$) (Figure 4).

Muscle Damage and Soreness

Mean CK plasma levels can be found in Figure 5. There were no interactions for treatment X time X condition ($F = 0.54$, $p = 0.826$) or time X condition ($F = 0.923$, $p = 0.23$). However, there was a treatment X condition interaction ($F = 4.69$, $p = 0.032$) for CK plasma concentration. CK plasma concentrations for both the curcumin (211.75 U/L) and placebo (234.32 U/L) group were not significantly different before supplementation ($p = 0.318$). In contrast, CK concentration were significantly lower, overall, post supplementation in the curcumin group (199.62 U/L) compared to the placebo group (287.03 U/L) ($p < 0.0001$). In addition, CK concentrations significantly increased in the placebo pre (234.31 U/L) to post (287.03 U/L) exercise ($p = 0.0181$).There was a significant main effect for treatment ($F = 13.09$, $p = 0.0004$) where CK levels where significantly lower in the curcumin group (205.69 U/L) compared to the placebo group (260.67 U/L), overall.

Lastly, post supplementation VAS muscle soreness means are demonstrated in Figure 6. There were no interactions for: treatment X condition ($F = 0.89$, $p = 0.514$), location X condition ($F = 1.25$, $p = 0.271$), treatment X condition ($F = 2.26$, $p = 0.133$), time X condition ($F = 1.75$, $p = 0.137$); or treatment X time X condition ($F = 1.44$, $p = 0.178$). However, there was a time X location interaction ($F = 4.20$, $p < 0.0001$). Additionally, there was a significant main effect for treatment ($F = 6.34$, $p = 0.012$). Curcumin resulted in significantly lower soreness (2.88) compared to the placebo (3.36) ($p = 0.0120$), overall. Further, there was a significant main effect for both condition ($F = 20.85$, $p < .0001$) and location ($F = 48.95$, $p < .0001$), where soreness was significantly

lower post supplementation (2.68) compared to pre supplementation (3.56) ($p < .0001$), and the exercises leg was reported more sore compared to the non-exercised trained leg.

Table 1

Subject Characteristics

Descriptive Data	Mean ± SD
Age (years)	21.7 ± 2.9
Height (cm)	177.7 ± 7.4
Mass (kg)	83.7 ± 12.4

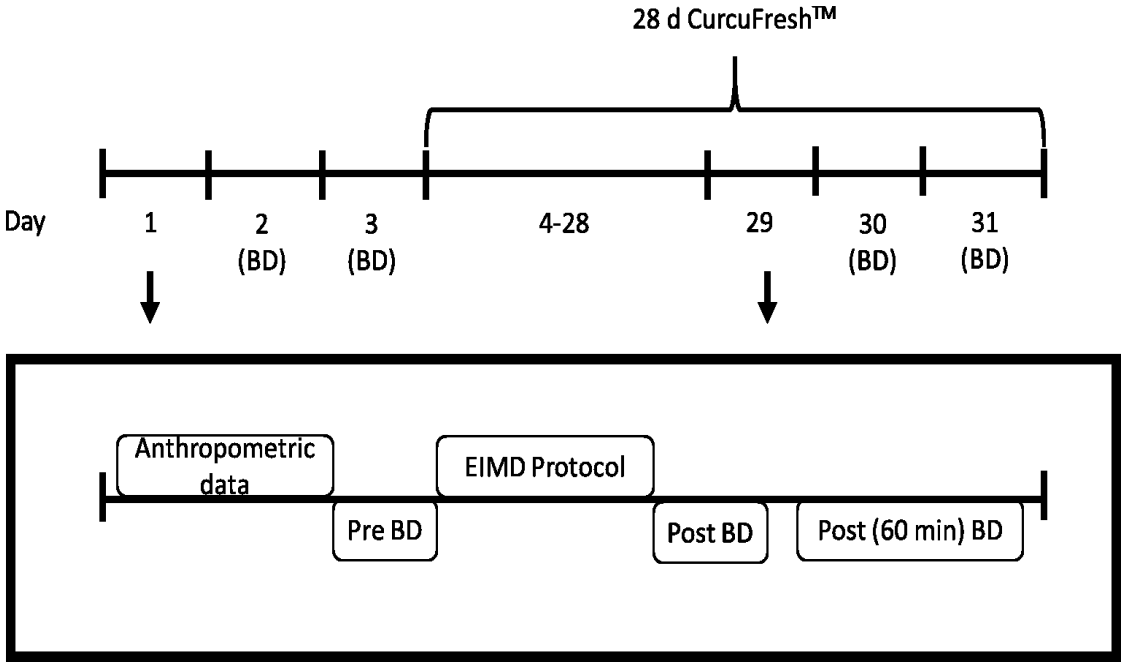

Figure 1. Timeline of testing procedures.

BD = blood draw; EIMD = Exercise Induced Muscle Damage

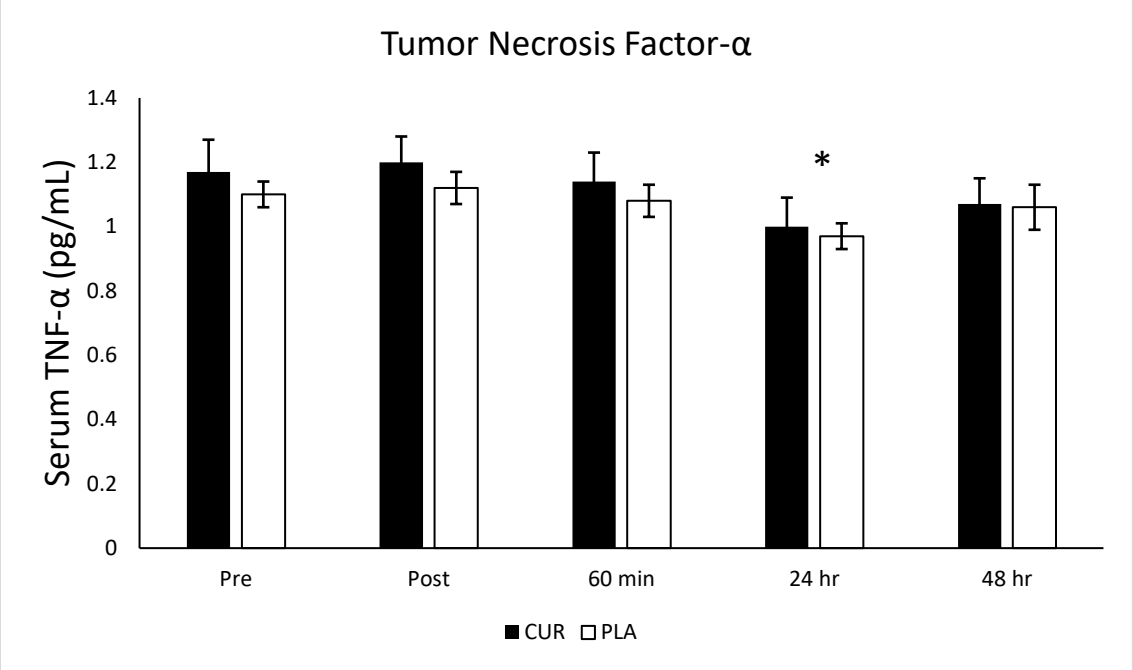

Figure 2. TNF-α concentrations, overall.

Concentrations reported as Mean ± SE
*Indicates significant difference in TNF-α concentrations at 24 hrs post exercise compared to all other time points ($p < 0.05$). CUR = Curcumin; PLA = Placebo; Pre = Before exercise; Post = Immediately After exercise; min = minute; hr = hour.

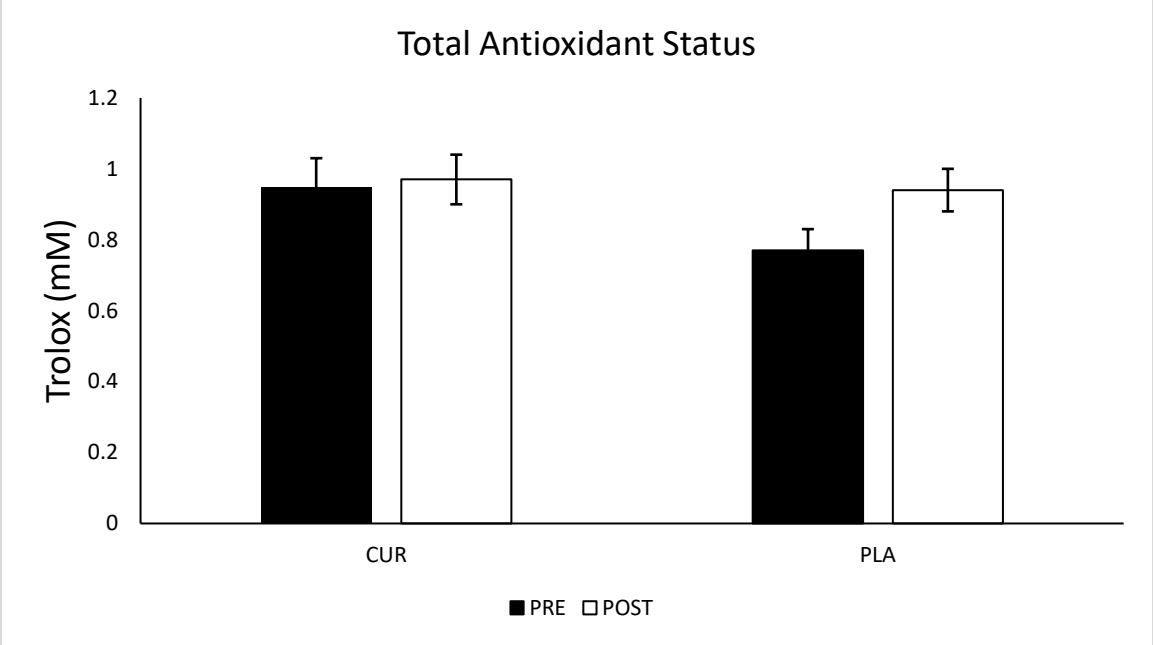

Figure 3. Total Antioxidant Capacity concentrations, overall.

Concentrations reported as Mean ± SE
Pre versus Post supplementation. CUR = Curcumin; PLA = Placebo; PRE = Before Supplementation; Post = After Supplementation.

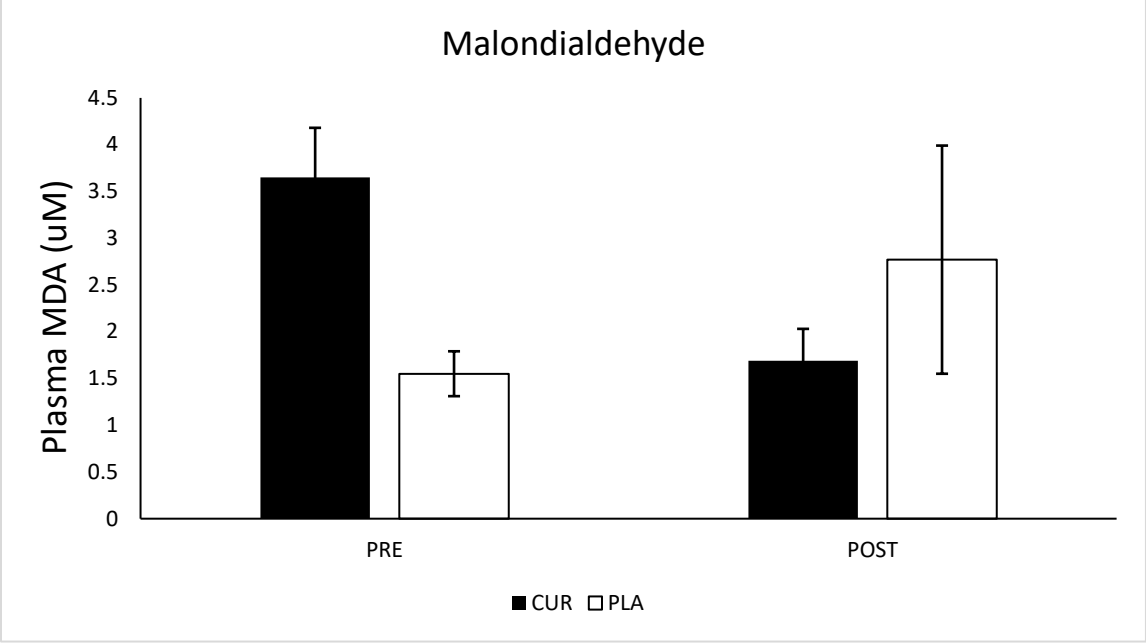

Figure 4. Malondialdehyde concentrations, overall.

Concentrations reported as Mean ± SE
Pre versus Post supplementation. CUR = Curcumin; PLA = Placebo; PRE = before supplementation; Post = after supplementation.

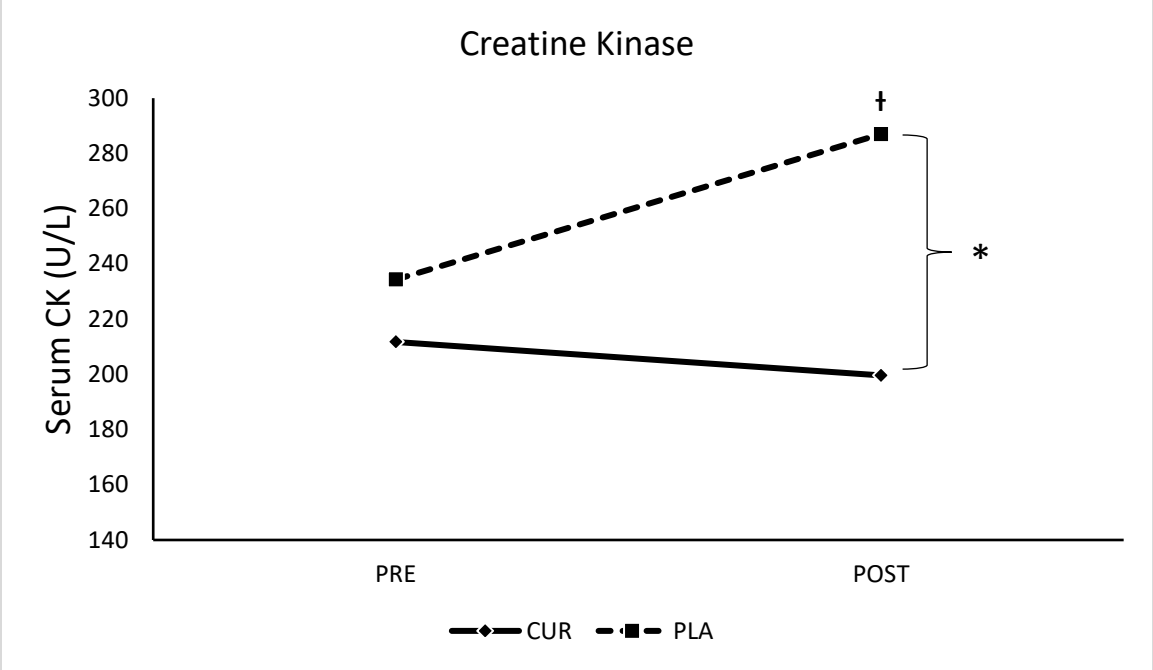

Figure 5. Creatine Kinase concentrations, overall.

Concentrations reported as Mean ± SE
Pre versus Post supplementation. *Indicates significant difference between treatments ($p < 0.0001$); †Indicates significant difference from pre to post supplementation ($p = 0.0181$). CUR = Curcumin; PLA = Placebo; PRE = Before Supplementation; Post = After Supplementation.

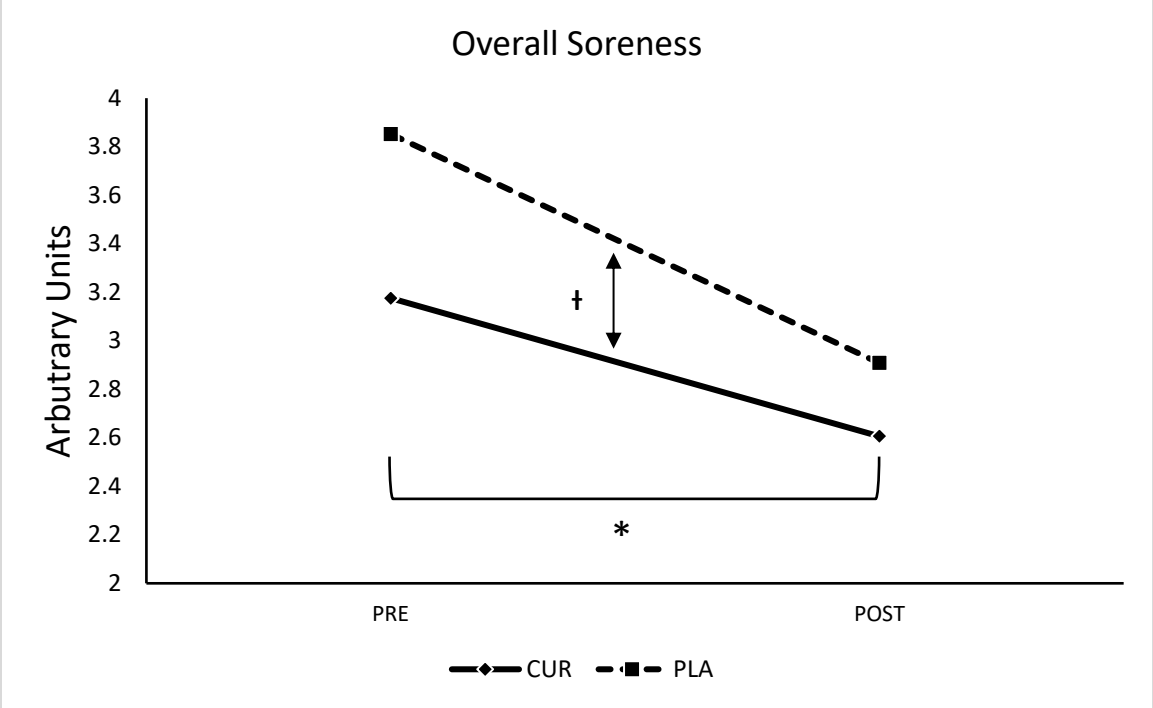

Figure 6. Perceived Muscle Soreness, Overall.

Units reported as Mean ± SE
*Indicated significant difference in perceived soreness from pre to post supplementation, overall ($p < 0.0001$). †Indicates significant difference between treatments, overall ($p = 0.0120$). CUR = Curcumin; PLA = Placebo; Pre = Before exercise; Post = Immediately After exercise; min = minute; hr = hour.

CHAPTER V

DISCUSSION

Summary

This study examined the effect of a newly formulated curcumin (CurcuFresh^{TM}) in humans on biomarkers of inflammation, OS, muscle damage, and muscle soreness. Secondly, this is one of only two studies to implement a 28-day supplemental period or greater (Jäger et al., 2017; Oliver et al., 2017), while others have only examined acute ingestion (Davis et al., 2007; Drobnic et al., 2014; Kawanishi et al., 2013; McFarlin et al., 2016; Nicol et al., 2015; Sciberras et al., 2015; Takahashi et al., 2014). The main findings from our study were that curcumin ingestion resulted in significantly lower plasma concentrations of CK and perceived muscle soreness following the eccentric muscle damage protocol, despite any changes in biomarkers of inflammation and OS. Decreased muscle damage and muscle soreness support along with null findings both support and disagree with our initial hypothesis. Further, these finding agree (decreased muscle damage and muscle soreness) with and disagree (unchanged inflammation and OS) with earlier studies examining similar biomarkers (Davis et al., 2007; Drobnic et al., 2014; Kawanishi et al., 2013; McFarlin et al., 2016; Nicol et al., 2015; Sciberras et al., 2015; Takahashi et al., 2014).

Total Antioxidant and Oxidative Stress

Exercise-induced OS has been associated with fatigue along with muscle damage resulting in decreased performance (Powers & Jackson, 2008). Hypothetically, if OS is reduced along with inflammation, performance may be improved. Therefore, given the strong anti-inflammatory and antioxidant effect of curcumin (Shehzad & Lee, 2013), we sought to examine markers of inflammation (TNF-α), antioxidant capacity (TAC) and OS (MDA). Previous mechanistic work has shown that curcumin works directly to increase endogenous antioxidants via activation of NrF2, which subsequently binds to downstream targets and up-regulates antioxidants (Goel et al., 2008; Shehzad & Lee, 2013). Other direct mechanisms have been shown as well in that curcumin may potentiate its' ROS scavenging abilities via dissociation of the OH group (Singh et al., 2011). Given the aforementioned, it is difficult to explain the lack of change in MDA and overall antioxidant status in the present study. Takahashi et al. (2014) recruited participants with similar training statuses as the present study, and instructed subjects to run at 65% $\dot{V}O_{2max}$ for 60 min. Subsequently, Takahashi et al. (2014) reported increased biological antioxidant potential and reduced glutathione, along with reduced derivatives of reactive oxygen metabolites following curcumin supplementation. From the same study, MDA was unaffected via curucmin treatment, which agrees with our data. One possible reason for these inconsistent findings may be the difference between exercise modality/intensity. Further possible explanations in confounding results concerning antioxidant status may lie in the measurements used in our study, where Takahashi et al. (2014) analyzed specific antioxidant enzymes (GPX, SOD, CAT, and glutathione reductase) (Takahashi et

al., 2014). Lastly, differences have been found in individual antioxidant enzymes (i.e. SOD, CAT) rather than overall antioxidant status following curcumin supplementation in mice in tissue specific analysis (Avci et al., 2012; Kalpana, Sudheer, Rajasekharan, & Menon, 2007), suggesting that curcumins' anti-inflammatory affects may potentially be tissue and/or enzyme specific.

Inflammation

Additionally, curcumin also modulates inflammatory pathways/cascades of both NF-kB (Kang et al., 2009) and COX-2 (Kang et al., 2004), which are both activated following muscle damage. TNF-α was chosen due to its' role as an activator of these pathways, along with its role in the degenerative processes of muscle tissue (Townsend et al., 2015). While one study demonstrated similar findings in TNF-α (Nicol et al., 2015), other studies have reported dissimilar results (Davis et al., 2007; McFarlin et al., 2016). Two studies using similar protocols to elicit muscle damage, Nicol et al. (2015) and McFarlin et al. (2016), recruited non-resistance trained males, but reported differences in TNF-α levels. However, an underlying factor between the two and in the present study, is the disparity of aerobic training status found in our participants (< 30 min of moderate intensity of physical activity at least three days/week for at least three months or ≤ 150 minutes) and those in Nicol et al. (2015) (2-4 hours of endurance training/week; 1-2 hours of team training/week), thus potentially explaining the lack of observed differences in inflammation. Further, it is pertinent to note that unlike the present study, Nicol et al. (2015) reported increased IL-6 concentrations immediately following exercise. Although we did not measure IL-6, the rise IL-6 may mediate TNF-α levels following exercise

(Pedersen et al., 2001), which could also explain the significant reduction found in the present study at 24 hours post exercise. In contrast, other potential dissimilarities found in inflammation in the present study may lie in the sample selected, where active individuals were examined in the present study, whereas, untrained participants were recruited by McFarlin et al. (2016). This can be seen when comparing the pre-exercise values of TNF-α concentrations in the present study (1.2 pg/mL) to McFarlin et al. (2016) (4.0 pg/mL). One might speculate that the aerobic training status seen in our subjects provided enough stimulus for adaptations resulting in lower resting levels of TNF-α, thus, negating any potential anti-inflammatory benefits of curcumin supplementation. Another possible reason for discrepancies in inflammatory responses could be the intensity of the protocol. The increased musculature may have exemplified a greater metabolic demand causing greater oxidative damage, thus, leading to greater inflammatory response (McFarlin et al., 2016).

It is pertinent to mention that for proper muscle regeneration, there must be a balance of both an inflammatory and anti-inflammatory environment following muscle damage (Philippou et al., 2012). Previous works have identified TNF-α as a main pro-inflammatory cytokine secreted following muscle damage (Figarella-Branger et al., 2003). However, if concentrations remain elevated for extended periods, TNF-α may inhibit muscle regeneration (Townsend et al., 2015). Independent of curcumin supplementation, our data show a slight (non-significant) rise in plasma TNF-α concentrations, along with a significant decrease 24 h post exercise ($p = 0.005$). Therefore, our findings suggest that curcumin may aid in decreased muscle damage (i.e.

decreased CK) without hindering the natural inflammatory response needed for adequate muscle growth.

Muscle damage and Soreness

Our results are consistent in regards to muscle damage (CK) with previous data in humans (Jäger et al., 2017; McFarlin et al., 2016; Nicol et al., 2015) and animal models (Davis et al., 2007; Huang et al., 2015). Independent of reduced inflammation, curcumin significantly blunted a rise in CK post supplementation (199.62 U/L) compared to the placebo (287.03 U/L) ($p < 0.0001$), overall. When compared to a variety of eccentric muscle damage protocols, our mean peak levels of plasma CK post exercise (211.7 U/L) resembles those reported by Nicol et al. (2015) (≈ 200 IU/L), both of which are substantially less than other studies. These differences could be attributed to substantially less musculature involvement during the exercise modality compared to other studies (Drobnic et al., 2014; McFarlin et al., 2016) due to the nature of the uni-lateral, sit/stand protocol which involved only one leg. Our reports of reduced CK concentrations following supplementation in the absence of noticeable changes in inflammation both agree (Jäger et al., 2017; Nicol et al., 2015) and conflict with previous studies (Davis et al., 2007; McFarlin et al., 2016). As mentioned previously, curcumin may act upon multiple inflammatory signal transduction pathways (i.e. Nf-kB, COX-2). However, curcumin may act upon other unidentified pathways to support muscle cell integrity other than these inflammatory/ROS mediated pathways (Nicol et al., 2015).

In respect to VAS muscle soreness, there was a significant reduction in perceived muscle soreness following curcumin supplementation. Additionally, there were no

differences in location of muscle soreness in the lower extremity following the exercise These findings support our initial hypothesis, while agreeing with several human studies (Drobnic et al., 2014; Jäger et al., 2017; Nicol et al., 2015) and disagreeing with others (McFarlin et al., 2016). Previous works have shown curcumin may reduce prostaglandins via the COX-2 pathway (Kang et al., 2004), which in turn should decrease muscle soreness (Ferreira, 1972). Curcumin may act through other pathways such as inhibition of transient receptor potential ion channels (Yeon et al., 2010), which are responsible for the acute reduction in pain. The possible differences between the present study and that of McFarlin et al. (2016) may lie assessment techniques of muscle soreness. In retrospect, decreased perceived muscle soreness from curcumin supplementation may have substantial effects on mediating greater subsequent exercise performance along with increasing exercise training volume.

Limitations

Some of the limitations are presented in the techniques used for subsequent analysis of biomarkers of antioxidants and OS. Previous research has shown that specific enzymes related to both endogenous antioxidant status and OS are more subject to noticeable changes with antioxidant supplementation (Sarker et al., 2015; Takahashi et al., 2014). Other limitation can be found in the exercise modality chosen. The lack of overall musculature used in our protocol may have resulted in insufficient muscle damage and inflammation. Further, the purpose of this study was to examine the effects of chronic curcumin supplementation. However, the lack of a cross-over design along with

the absence of allocating training status among participants in each group (placebo and treatment), may pose potential limitations.

Conclusion

In conclusion, our data suggests curcumin supplementation of 28 days may result in decreased muscle damage and muscle soreness following exercise. Although there were no differences inflammatory/OS markers, a consensus is still evident in support of curcumin's anti-inflammatory and antioxidant capacities (Kawanishi et al., 2013; McFarlin et al., 2016; Takahashi et al., 2014). Inevitably, curcumin supplementation may be a viable source for enhancing exercise performance in trained individuals. Future research should investigate long-term (> 28 days) curcumin supplementation and its effect on the redox environment and muscle growth as it pertains to aerobic exercise and RT.

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