

ANTIOXIDANTS AND RECOVERY FROM EXERCISE INDUCED MUSCLE DAMAGE.

Kara A. Stone

The University of Central Missouri

An Abstract

of a thesis submitted in partial fulfillment
of the requirements for the degree of
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Abstract

by

Kara A. Stone

Recent research suggests that ingesting antioxidants during training may reduce exercise induced muscle soreness (EIMD), therefore the purpose of this study is to examine the effect of antioxidant supplementation on the performance related symptoms of EIMD in female participants. **Methods:** Participants were 10 non-resistance trained females, 21.6 ± 2.8 years. Subjects underwent 12 days of supplementation with an antioxidant capsule and 12 days of supplementation with a placebo capsule. To induce muscle damage, subjects performed an eccentric leg press protocol followed by measurements for range of motion (ROM), resting blood lactate, 5-RM testing, Wingate power output testing, and perceived muscle soreness. Assessments occurred prior to supplementation, immediately after exercise protocol, 48 and 96 hours post-exercise. **Results:** There were no significant changes in ROM, resting blood lactate, muscular strength, power output, or perceived muscle soreness between trials over the 96 hours following the exercise protocol. **Conclusion:** Antioxidant supplementation appears to have no effect on the performance related symptoms of EIMD in female participants.

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APPROVED:

Thesis Chair: Jennifer Case, Ph.D.

Thesis Committee Member: Steve Burns, Ph.D.

Thesis Committee Member: Mike Godard, Ph.D., FACSM

ACCEPTED:

Chair, Department of Nutrition and Kinesiology: Dennis Docheff, Ph.D.

UNIVERSITY OF CENTRAL MISSOURI

WARRENSBURG, MISSOURI

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Chapter 1

Introduction

Exercise Induced Muscle Damage (EIMD) has long been a topic of exploration in the fitness community. EIMD is characterized by varying severities of muscular pains, that develop 24-48 hours post exercise and can last 3-4 days, as well as by a decrease in strength that can last up to two weeks. More symptoms of EIMD include decreased muscular endurance, swelling, stiffness, decreased force production, and reduced range of motion (Pettitt et al., 2010). Often blood markers such as lactate dehydrogenase (LDH), creatine kinase (CK), and erythrocyte membrane malonyldialdehyde (MDA) are used to indicate EIMD. There are a number of known causes and symptoms for EIMD, but further investigation is necessary to determine a treatment for these symptoms. Researchers have examined anti-inflammatory medication, rehabilitation modalities, such as heat and cold therapy, myofascial release, supplementation of various antioxidants, and even static stretching as possibilities for reducing the effects of EIMD, but have yet to deduce an effective treatment. Through data collection and research, this study elucidated the effects that antioxidant supplementation has on performance-related symptoms of EIMD.

Purpose of the Study

The purpose of this study was to examine the effect of antioxidant supplementation on the performance related symptoms of EIMD.

Significance of the Study

Little information is available on the treatment of EIMD with antioxidants. Limited knowledge of the effect of antioxidants on EIMD makes it difficult for individuals to understand why they become sore, experience decreased ROM, strength, power output, and other performance decrements after beginning a new exercise program or returning to exercise after a period of physical inactivity. This limited understanding leads new exercisers to creative

“theories” that often give way to fitness myths, or can cause them to spend a great deal of money on supplements that may or may not be helpful. More knowledge on the subject of antioxidants and EIMD may make it easier to explain what is occurring in the muscles during strength training, as well as, why it is occurring, and if there are possible ways to limit the symptoms of EIMD. It is possible that supplementing with antioxidants may allow people to experience the benefits of strength training with minimal pain and decreased recovery time which in turn could lead to a healthier, more active society.

Delimitations

The following points delimitate the study:

1. Ten female volunteers, ages 19-28 years of age, who have not competed in any type of resistance training within the six months preceding the study.
2. A purposive sample consisting of undergraduate students of the University of Central Missouri during the 2014-2015 academic year.
3. The use of a plate-loaded leg press to measure 5-RM, PAR-Q for assessment of participants safety during exercise, a Wingate bicycle ergometer for power output assessment, a goniometer to determine range of motion (ROM), portable lactate analyzers, and a pain scale ranging from 0-10 to allow researchers to pinpoint variations in delayed onset muscle soreness (DOMS) symptoms.
4. The subjects were randomly assigned to supplement with either 15 Nature's Way Antioxidant Formula supplements or 15 placebos made of natural gelatin capsules filled with flour. The study was of cross-over design. After a two-

week period in which the participants refrained from supplementation and resistance training, subjects began taking the opposite capsules.

Limitations

The study is limited to the following:

1. The sample size of the study was small (N=10) requiring caution in extrapolation of the data to a greater population.
2. It was advised that subjects not partake in any physical training and/or exercise other than that discussed in the study for the duration of the study (8 weeks).
3. Subjects were advised not take any other supplements during the duration of the study
4. Subjects were also advised to log daily food intake for the entire duration of the study.

Assumptions

The study was completed under the following assumptions:

1. All subjects completed the entire fitness assessment, training regimen, and assessments to the best of their ability.
2. The testing instruments used were reliable and valid measures of the above mentioned constructs.
3. The subjects complied with the researcher's requests for procedures and methods.
4. The subjects were a representative sample of the non-exercising population

Hypotheses

The information presented has led to a development of the following hypotheses: 1) Subjects will obtain full range of motion of the knee joint sooner when supplementing with Antioxidant Formula when compared to supplementing with a placebo capsule; 2) Subjects who supplemented with Antioxidant Formula will have reduced ratings of perceived soreness and resting blood lactate post exercise; 3) Subjects will regain peak power output sooner when supplementing with antioxidants than when supplementing with placebo capsules; 4) Subjects supplementing with antioxidants will have a decreased fatigue index sooner than when supplementing with placebo capsules; 5) Subjects supplementing with antioxidants will have recovery of anaerobic capacity sooner than those supplementing with placebo capsules, and 6) Subjects supplementing with Antioxidant Formula will regain strength sooner than when supplementing with placebo capsules.

Definition of Terms

1. **Physical Activity.** Any bodily movement produced by the skeletal muscles that requires energy.
2. **Exercise.** Planned, structured, and repetitive movement intended to improve or maintain physical fitness.
3. **Perceived Muscle Soreness.** Soreness to during activity, based on a scale of 0-10, where 0 is no soreness and 10 is debilitating soreness which is determined by the participant.
4. **Antioxidant.** A molecule that inhibits oxidation of other molecules.

Chapter 2

Introduction

Exercise Induced Muscle Damage (EIMD) has become a highly debated topic in the world of exercise science, particularly in regard to muscular adaptations. More specifically, can optimum muscular adaptation occur while limiting the side effects of EIMD? Exercise Induced Muscle Damage typically occurs after exhaustive or unaccustomed exercise, including heavy eccentric training; for example downhill running, bench stepping, and the lowering of weights.

There are two phases of injury resulting from EIMD: primary injury, from mechanical damage to the muscle, and secondary injury, which results from reactive oxygen species (ROS) that are formed as free radicals by phagocytic leukocytes and lipid peroxidation. The secondary injury results in side effects, such as reduced range of motion (ROM), muscle soreness and stiffness, increased blood lactate concentration, increased ratings of perceived exertion (RPE) during exercise, and reduced strength and power output that can last five to ten days (Jeukendrup & Gleeson, 2010).

Although balanced oxidation-reduction (Redox) is essential for functioning organisms, it is possible for an imbalance, known as oxidative stress, to diminish antioxidants and cause an increase in cell damage, hindering normal physiological functioning of the organism. Oxidative stress has been linked, in combination with exogenous sources, to human conditions including inflammatory, metabolic, cardiovascular, neurodegenerative diseases, cancer, and even aging (Peternej & Coombes, 2011). It is thought that disease risk can be reduced through increasing antioxidants in exercising individuals by reducing EIMD and promoting recovery. Antioxidants, either enzymatic or non-enzymatic, can “scavenge” ROS and reduce them to less active molecules, prevent radicals from forming, repair damage done by radicals once they have

formed, and combine with other anti-radical agents to create a better environment for reducing agents (Goldfarb, 1999).

Vitamins C and E are some of the most commonly used supplements taken in large doses to protect against muscle damage. This may be due to the fact that vitamin C is the most important water soluble antioxidant scavenger and may assist in recycling vitamin E in its radical form. However, in its non-radical form, Vitamin E has the ability to scavenge lipid peroxy radicals, superoxides, and hydroxyl, thus preventing lipid peroxidation (McGinley, Shafat, & Donnelly, 2009). Research supporting the reduction of oxidative stress indices via both vitamin C and E but little support in regards to reducing EIMD is discussed in the following section. Studies have shown that supplementation interferes with ROS signaling functions which might, in turn, inhibit muscular performance (McGinley, Shafat, & Donnelly, 2009).

Some other antioxidants to be discussed include tart cherry juice, which is high in niacin, New Zealand blueberries, containing high anthocyanins, grape seed extract, containing anthocyanins and flavonols, omega-3 supplements, green tea polyphenols, purslane extract, quercetin, sulforaphane, and methylsulfonylmethane. Various antioxidants and their effectiveness or ineffectiveness in reducing the effects of common markers of EIMD and oxidative stress are further discussed in the next section.

Review of Literature

The review of literature is presented in the following organization: (1) Antioxidants and Blood Markers, (2) Antioxidants, Blood Markers, and Performance (3) Antioxidants and Performance, and (4) Summary.

Antioxidants and Blood Markers

Previous studies have examined the effects that antioxidant supplementation has on blood markers and found that increasing antioxidants through supplementation, whole foods in the diet, and juice extracts can be beneficial in reducing EIMD. Cavas and Tarhan (2004) explored the relationship between enzymatic activities of cardiac indices, MDA, and the antioxidant defense system in young swimmers. Fifteen female and fifteen male swimmers, ages 11-13 years that had attended the national swimming competition, participated in the one month long study.

Participants were divided into two groups, one receiving a placebo and one receiving a One a Day for Junior vitamin-mineral supplement containing 60mg of vitamin C and 30IU of vitamin E per tablet. The swimmers training plan consisted of training four days/week at two hours/day. Blood analysis for CK, LDH, Aspartate Aminotransferase (AST), Creatine Kinase-MB (CK-MB), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) was completed in the afternoon post-exercise. Vitamin-mineral supplementation significantly affected CK activities, CK-MB activities, and LDH. There was no significant difference between the male control group and male supplemented group in regards to AST. Superoxide dismutase activities were significantly higher in supplemented groups than in control groups as were CAT and GSH-Px activities. Supplementation was found to significantly affect MDA levels. The data suggested that increased antioxidants, such as vitamins A, C, and E, and increased enzyme activity could reduce ROS-based lipid peroxidation and reduce EIMD.

In another study assessing the effect of antioxidant supplementation on blood markers of muscle damage, Chan, Lin, Liu, and Hsu (2008) investigated the effects of a fructose-electrolyte-antioxidant sports drink on muscle damage and metabolic response. Eight untrained males, ages of 19 to 23 years, were randomly assigned to a placebo group or supplement group. The

supplement contained a mixture of fructose, electrolytes, vitamins C, E, and β -carotene. Each subject completed a $\text{VO}_{2\text{max}}$ test followed one week later by exhaustive cycling in which they were given either the supplement or placebo every 15 minutes during exercise. Following another one week period, the groups then repeated the exercise while receiving the opposite treatment. Blood analysis was completed prior to exercise (Pre-Ex), 30 minutes into the exercise (Ex-30), and immediately after the exercise protocol (Post-Ex) to evaluate SOD, blood antioxidant status (TAS), GSH, CK, and CK-MB, blood lactate (BL), and glucose. No significant treatment effects were observed on MDA, SOD, or GSH. Creatine kinase activity was significantly lower in the supplement group at Post-Ex as was CK-MB. The supplement had an effect on BL as the placebo group was significantly higher during Ex-30 and the supplemented group had returned to baseline. Glucose levels were significantly higher in the supplement group at Ex-30 than at Pre-Ex, indicating that sports drink supplementation with added vitamins may decrease lactate accumulation and sustain blood glucose during exercise. Also, the study concluded that the sports drink used may limit muscle damage and alter metabolic response during endurance exercise, however, it should be noted that more research is needed as cycling did not have the eccentric component to significantly increase oxidative stress.

Similarly, Atashak et al (2013) studied the effect of omega-3 fatty acid supplementation on muscle damage, inflammatory markers, and oxidative stress after acute resistance exercise in young athletes. The subjects included 20 collegiate handball players who were split a control and supplement group. Supplements were taken for seven days prior to a fasted anthropometric measurement session. On the eighth day subjects completed an intense leg exercise consisting of 120% of their predicted 1RM for each of the three exercises. Blood collection and analysis was immediately before exercise and 24 hours after the exercise session. In the placebo group MDA

was significantly higher 24 hours after exercise and C-reactive protein (CRP) and CK increased significantly. The study indicated that short-term omega-3 supplementation may improve the rise in antioxidant markers and minimize EIMD and inflammation post-exercise.

Diaz et al (2010) detailed the effect of nutrition on antioxidant enzymes, cortisol, and cell damage during a two-day skiing competition. Twenty-one male skiers ages 30-44 were used in the study. Participants skied 12km with a drop of 1350m one day and 7km with an 850m drop the next day and were required to keep nutrient diaries from the competition days. Blood analysis was completed for CK, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ -glutamyl transpeptidase (GGT), alkaline phosphatase (AP), and cortisol and C-reactive protein (CRP) the day before and at the end of the competition. Performance was measured by total race time from both days. Results from the study showed that skiers met most of the recommended vitamin and mineral requirements aside from folate, potassium, magnesium, Vitamin D, zinc, and iodine. Enzymes related to muscle and cell damage (AST, LDH, and CK) increased significantly after the competition as did CRP and cortisol levels. Negative correlations were shown between muscle damage indices and total energy, fat, and protein intake. A negative relationship also existed between the minerals and muscle damage. A positive correlation existed between CRP and vitamin B₁₂ intake, and a positive correlation was seen between cortisol and participants with the lowest intakes of vitamins B, C, and niacin. The study showed no relationship between oxidative stress indices and performance time. The authors concluded that the skiers with the lowest energy intake were the ones who exhibited: greater muscle damage, lower cortisol, and lower antioxidant enzyme activity. Thus, giving way to greater demand for research in antioxidant and nutrient needs in regards to muscular damage and recovery.

Jówko et al (2012) evaluated the green tea polyphenols (GTP) effect on EIMD and oxidative stress markers in soccer players. Subjects included sixteen club soccer players that performed a muscular endurance protocol of bench press and back squat. The subjects were asked to limit certain foods to minimize their polyphenol content for two days before performing the protocol. Subjects consumed a standardized breakfast three hours prior to the test; next, they were randomly assigned two capsules in a double blind fashion. An hour and a half later they performed a muscular endurance protocol. Blood analysis occurred at the time of ingestion, five minutes post-exercise, and 24 hours post-exercise. Superoxide dismutase, CK, thiobarbituric acid reactive substances (TBARS), total antioxidant status (TAS), total plasma catechin concentration, and lactate levels were recorded. A single dose of GTP did not affect lactate concentration, SOD, TBARS, or TAS in either group, but did increase plasma total catechin level prior to exercise, but decreased after 24 hours of recovery. After 24 hours, the placebo group had a significantly higher CK activity than the pre-exercise values, where the GTP group was not higher than the pre-exercise values. As TAS was not affected by a single dose of GTP, researchers concluded that GTP failed to suppress EIMD when administered prior to exercise, but it should be noted that a higher dosage may be necessary to exert any positive effect on EIMD.

Malaguti et al (2009) observed the protective properties of sulforaphane (SF), an isothiocyanate found in cruciferous vegetables, against skeletal muscle damage in rats. Thirty- two male rats were randomly divided into a control group (C), a group subjected to exercise (E), a SF group (S), and a group subjected to exercise and SF (SE). The S and SE groups were supplemented 24, 48, and 72 hours before exercising until exhaustion with 25 mg/kg body weight. Immediately after exercise, blood and muscle samples were taken from the anesthetized

animals. Lactate dehydrogenase and CPK activities were similar in the SE group and C group, showing that the treatment counteracted EIMD. The E and SE groups were similar to the C group in TBAR activity as well. SF treatment was also able to counteract the effect of exhaustive exercise on NAD(P)H:quinine oxidoreductase (NQO1) expression. This data showed that SF acts as an indirect antioxidant by increasing NQO1 expression and activity in skeletal muscle. It is important to take note that 25mg/kg body weight is not easy to achieve in human trials so there is no clinical application from this particular study.

The results from the previous studies showed that supplementing with various antioxidants, such as, Vitamins A, C, E, beta carotene (a precursor for Vitamin A), niacin, Omega-3 fatty acids, and combinations of vitamins and minerals lead to a significant reduction in indices of EIMD, particularly blood markers like CK and LDH. There were also correlations between increased vitamin supplementation and a reduction in lactate accumulation during exercise. Studies have been completed using supplements and dietary logs examining vitamin intakes among athletes, concluding that a decrease in nutrient intakes relates to an increase in muscle damage and further research is necessary to determine whether that relationship exists due to a lack of vitamins and minerals, a carbohydrate deficiency, or a negative nitrogen balance. Green tea polyphenols were also examined, but the dosage was inappropriate to see significant results.

Antioxidants, Blood Markers, and Performance

Previous studies have examined the effects that antioxidant supplementation may have on indirect markers of EIMD without exploration of performance indices; the following studies examine blood markers as well as performance markers. Luden, Saunders, and Todd (2007) investigated effects of a post-exercise carbohydrate-protein-antioxidant (CHO+P+A) supplement

on muscle soreness, performance, and plasma creatine kinase (CK) in cross-country runners. The subjects were 23 NCAA Division 1 cross country runners (12 females and 11 males). The participants took part in physical fitness assessments, including measuring body mass and two VO_{2max} tests prior to two interventions. The participants performed normal training procedures prescribed by the head coach during the first six-day intervention period and consumed either CHO+P+A or carbohydrate only (CHO) beverages within 30 minutes after exercise. Three weeks later the athletes completed a second intervention in which they ingested the opposite beverage. The two beverages were matched in carbohydrate content of 1.46g/kg of body weight, but additional 0.365 g/kg body weight of vitamins C, E, and whey protein were added to beverage CHO+P+A. Muscle soreness was obtained before the first long run of each intervention and after five days of training and treatment using a scale of 0-6. Blood draws were obtained prior to the first long run and six days into training and treatment to test CK. To measure performance all athletes completed a 5km race at the end of each six-day treatment period. The authors concluded CK and muscle soreness were significantly lower in the CHO+P+A group, but there were no differences in performance between treatments. According to this study, “postexercise CHO+P+A beverage consumption significantly attenuated markers of muscle damage” (Luden, Saunders, & Todd, 2007, p. 120). Although their results were significant, more research is needed to ensure that the significance was due to the timing of adequate protein intake.

Maximum isometric force (MIF) and range of motion (ROM) are among some other indices of EIMD that have been examined in previous studies. Bloomer, Goldfarb, McKenzie, You, and Nguyen (2004) examined the effects of antioxidant treatment, vitamins C and E, on CK and muscle soreness, after eccentric exercise. Eighteen healthy women, ages 19-31 years, who

were not resistance trained, were used in the study. Subjects ingested 400IU of vitamin E, 1g of ascorbic acid, and 90 µg Selenium for 14 days prior, the day of, and three days following eccentric exercise. Subjects went through initial assessments including, muscle soreness, ROM, blood testing, and maximal isometric force (MIF) in that order. Subjects then completed an eccentric exercise program, totaling four sets and 12 repetitions using their nondominant elbow flexors. Post assessments were given in the same order 0hours, 2hours, 6hours, 24hours, 48hours, 72hours, 96hours, and 14 days after protocol. Creatine kinase activity was significantly lower in the supplement group from 24-96 hours post-exercise and muscle soreness was statistically higher in the placebo group at both 48 and 72 hours post-exercise. No significant interaction occurred for MIF. No interaction between groups was evident in regards to ROM. The study showed that vitamin C and E supplementation was beneficial in reducing some blood markers, such as CK, but carried no impact on ROM or MIF.

The effect of purslane, the richest vegetable form of omega-3 fatty acid, on muscle soreness was examined by Meamarbashi and Abedani (2011). Twenty non-athlete male college students performed one session of bench-stepping exercise for five minutes to induce delayed onset muscle soreness (DOMS) using their right leg to lead and descending with their left leg. Blood analysis, ROM, pain perception, thigh circumference, MIF, and anthropometric parameters were measured three days before exercise, immediately after, and 24 and 48 hours post exercise. Participants were given 1200mg of purslane per day or a lactose placebo; they were to take their capsule starting three days prior to exercise and continued until two days after exercise. In regards to CK and LDH activity, the experimental group peaked at 24 hours and exhibited a reduction at 48 hours whereas the placebo group peaked at 48 hours. Cortisol reduction, as well as, peak IgA concentration occurred at 24 hours and 48hours for the experimental and control

group respectively. Right leg pain perception was 64% lower for the experimental group than the control group and no difference was obtained for the left leg. Right knee ROM was significantly higher at 48 hours and MIF remained significantly lower for 24-48 hours in the experimental group. From the above data it can be concluded that omega-3 fatty acids, in purslane extract, is responsible for diminishing the side effects of DOMS, however, more research is needed to determine dose-response and mechanisms of action.

O'Fallon et al (2012) investigated the attenuation of indirect markers for EIMD (MIF and blood markers) by the flavonoid quercetin supplementation after eccentric exercise. The study used 30 subjects, aged 18-25 years, in a double-blind placebo-controlled study. Subjects arrived at the laboratory in a fasted state and were randomly assigned a block design for exercise with their dominant arm after 10-15 minutes of rest. Six days after exercise the subjects ingested either a placebo First Strike bar or a quercetin containing First Strike bar twice daily for seven days in 12 hour increments. Isokinetic and isometric strength was measured and blood samples were obtained. Both groups responded similarly to all tested indices indicating that quercetin had no effect on EIMD.

Howatson et al (2010) investigated the efficiency of tart cherry juice in reducing EIMD, oxidative stress, inflammation and recovery in marathon runners. Thirteen male and seven female runners participated in the study by completing the 2008 London Marathon. Subjects were assigned a placebo or cherry juice mixture five days prior to the marathon, during, and two days post-marathon. Indirect markers of EIMD, excluding muscle soreness and MIF prior to activity, total antioxidant status (TAS), inflammation, and oxidative stress were measured six days prior to the marathon, the day prior, immediately after marathon completion, and at 24 and 48 hours post-marathon. Apple juice was used in both groups to limit the possibility of vitamin C

effect. Recovery of strength based on MIF was significantly faster following 48 hours in the cherry juice group. Significantly lower levels of inflammation indices were apparent in the cherry juice group versus the placebo group respectively and TAS increased significantly in the supplemented group and remained elevated 24 hours post-race. Although optimal dosage remains undetermined, the study concluded that when eight ounces of tart cherry juice is taken twice per day for five days prior to, during, and two days after endurance exercise, such as running a marathon, cherry juice accelerated strength recovery, increased TAS, and minimized lipid peroxidation and inflammation.

Another study looked solely at the effect of antioxidants on blood analysis and DOMS. Kalman, Feldman, Scheinberg, Krieger, and Bloomer (2012) reported the influence of methylsulfonylmethane (MSM), a naturally occurring nutrient found in meat, dairy, and many fruits and vegetables that are made of oxygen, sulfur, and methyl groups, on recovery markers and performance in men as a pilot study. Eight moderately exercise-trained men came in for screening visits in which blood was taken, a 1-RM test for knee extension was completed, and anthropometric measurements were taken. The subjects were randomly assigned MSM at either 3.0 g/day or 1.5 g/day for 28 days prior to and two days following exercise testing. Subjects were required to complete two exercise test days following an overnight fast. Forty-five minutes before exercise subjects were given a standardized breakfast consisting of a bagel, one tablespoon of low fat cream cheese, water *ad libitum*, eight ounces of orange juice and an assigned MSM dose. They then completed an exercise test consisting of 15 sets of 10 repetitions at 30%, 45%, and 60% of their 1-RM and three sets until failure at 70% of their 1-RM. Blood analysis, muscle soreness questionnaires, and heart rate and blood pressure were taken two and 48 hours post exercise. Heart rate and blood pressure were not affected by the MSM dose. The

3.0 g/day group experienced 1.5 percent greater reduction in muscle soreness and the 1.5 group experienced 0.5 percent greater reduction. Total antioxidant status (TAS), or antioxidant capacity, significantly increased immediately post-exercise in the 3.0g/day group. Exercise performance was not affected by dose of MSM. The data shows that MSM supplementation may have boosted selected markers of exercise recovery, especially with a 3.0 g/day dose of MSM, but had no effect on performance.

The previously mentioned antioxidants have shown to be efficient in reducing blood markers, like CK, as well as muscle soreness, inflammation, and lipid peroxidation. It has also been observed that with an increased dose of antioxidants, such as MSM, subjects experienced greater reductions in muscular soreness. Antioxidants have also shown, based on the studies reviewed, to increase ROM and strength recovery after eccentric exercise.

Antioxidants and Performance

When assessing the effect of antioxidants on EIMD the performance based benefits the results have been inconclusive. Studies have been completed examining the effect on muscle soreness, ROM, MIF, and peak power derived from vertical jump with much variation in type of antioxidant, dosage, and variables per study. Connolly, McHugh, and Padilla-Zakour (2006) tested the efficacy of tart cherry juice using sixteen men, ages 18-26 years, participated in a crossover study using an eccentric exercise protocol and were either given cherry juice or placebo four days prior to exercise. Unlike Howatson et al (2010) this study included measurement of muscle soreness, pain, ROM, and MIF. Maximum isometric force was reduced significantly in the placebo group; pain peaked at 24 hours and then decreased in the supplemented group but continued to increase and peak at 48 hours in the placebo group. There was no significant difference between groups in ROM or muscle soreness. The study shows that

the cherry juice supplement is efficient in decreasing some symptoms of EIMD, particularly strength loss and recovery of strength. It should also be noted that consumption of cherry juice is more convenient in regards to palatability than some of the other supplements mentioned in this review.

Another study used the same antioxidant supplementation but studied only the effect on muscular pain. Kuehl, Perrier, Elliot, and Chesnutt (2010) also explored the effects of tart cherry juice on the human body, particularly its effect on muscular pain due to the antioxidant capacity and anti-inflammatory properties. Fifty-four healthy runners participated in the Hood to Coast Relay were involved in this study. The study was a placebo-controlled, randomized, double-blind trial in which each participant completed three running segments and three data collection sessions: seven days prior to the race, the beginning of the race, and immediately after the race. The participants ingested two 355mL bottles of the given liquid daily prior to the race and during the race. Participants' pain was assessed using a visual analog scale (VAS) from 0-100. The supplement group reported significantly higher pain scores for day 1 but significantly lower after the race. Tart cherry juice supplementation for eight days reduced the EIMD index of muscle soreness among endurance runners. Tart cherry juice is not the only antioxidant from fruit that has been linked to reducing indices of EIMD and muscle recovery.

McLeay et al (2012) investigated the effectiveness of blueberries on EIMD after eccentric exercise. The study used ten healthy females that participated in recreational resistance and aerobic exercise. The women completed a familiarization session one week prior to the first day of the trial; blood was taken at the beginning of the trial prior to warm-up and pre-damage testing. Next, they were given their assigned beverage (blueberry smoothie or control) and required to return to the laboratory at midday for a standardized lunch and again in the evening

for repeated blood testing and eccentric exercise. It should be noted that the blueberry blend and control smoothie were similar in vitamin C, E, and antioxidant capacity (determined by ORAC) but that the blueberry smoothie contained over five times the amount of polyphenolic compounds that the control contained. Subjects returned to the lab after 16, 36, and 60 hours post- exercise for blood analysis, ratings of muscle soreness, standardized breakfast and their beverage, and performance tests. A faster recovery rate was noted in the blueberry group in the first 36 hours as well as improvements in performance. No overall difference was indicated between groups for muscle soreness. A gradual decrease in ROS-generating potential was observed in the blueberry group after 36 hours and lower CK activity was significant following 60 hours recovery for the blueberry group ($p < 0.01$). Interleukin- 6 (IL-6) showed no significant difference between groups, but TAS proved to be significantly higher in the blueberry beverage over the period of the trial. Much like the Kuehl, Perrier, Elliot, and Chesnutt (2010) study of tart cherry juice, taking in blueberry derived antioxidants before and after eccentric exercise accelerates recovery of MIF.

One study examined specifically the relationship between antioxidants and power output. Lafay et al (2009) researched the concept that grape extract might have an effect on TAS, oxidative stress, and physical performance. The study used 20 elite male power athletes (handball players, basketball players, sprinters, and one volleyball player) in a randomized, placebo controlled, double-blind study of crossover design. Participants ingested two capsules of either placebo or grape extract with breakfast for one month. After one month and two weeks the treatments were switched. After 30 days power output was derived using three successive single vertical Counter Movement Jumps (CMJ). Performance in the handball players increased significantly during the supplemented period. The performance enhancement may, partially, be

caused by grape extract supplementation and supplementation may protect against oxidative stress damage. However, it is important to note that this is the first trial showing results from grape extract flavonols and performance increases could be due to a number of changes occurring during a 30 day period including, but not limited to, variation in macronutrient ingestion, adaptation to practice, learning effects, and strength and power increases due to training.

Antioxidants derived from whole fruits in the form of juice, extracts, or ingesting the fruit have been extremely beneficial in reducing the effects of EIMD on performance measures such as recovery of MIF, muscular pain, and power output. Previously mentioned antioxidants have been noted to decrease the time to recovery for MIF in multiple studies. They have also decreased the time of peak muscular pain using various supplements prior to eccentric exercise. Although research is limited in the area of increased recovery of power output, studies exist promoting antioxidants for faster recovery of strength and increased performance.

Conclusion

There has been a great deal of research in the area of antioxidants, EIMD, and muscle recovery and the data is inconclusive. It can be noted that various antioxidants, particularly vitamins C and E, have significantly reduced indirect indices of muscle damage, specifically CK, LDH, and muscle soreness, with no significant result of the effects on MIF. On the other hand, fruit-derived antioxidants show promising effects on recovery of MIF post-exercise as well as muscle soreness, but more research is needed to determine any effect on other indirect measures of EIMD. Also, with the exception of Omega-3 fatty acids as antioxidants, other commonly advertised supplements have not been proven worthy of supplementation for the prevention of EIMD and recovery and those that have require copious amounts of the free radicals, much to

large for human consumption. Although each promising supplement shows significant evidence of the possibility of EIMD reduction, there is no supplement that fully reduces skeletal muscle damage, only supplements that aid in symptom reduction (i.e., muscle soreness or time to recovery). There is still a great deal unknown about how these compounds interact with the human body. Therefore, more research is necessary to attain results that could be used in clinical settings, athletic settings, and the general population of exercising adults. Further research is needed in regard to specific antioxidants and dose-response, how antioxidants might assist one another in muscle recovery, or pairing vitamin antioxidants with minerals to better facilitate defense against EIMD, or even if defense against EIMD caused by ROS is hindering muscular adaptation.

Chapter 3

Methods and Procedures

The methods of this research study have been designed to determine if Vitamins A, B2, C, and E in combination with Zinc, Copper, and Selenium had an effect on common indices of exercise induced muscle damage including range of motion (ROM), resting blood lactate, muscle soreness, muscular strength, and power output.

Subjects

Subjects were 10 females, who had not participated in any type of regular (two or more times per week for two consecutive weeks) resistance training in the past six months prior to the study. Subjects ranged from 19 to 28 years of age. Participants were selected from volunteers responding to word of mouth, flyers, and/or email. The study was a double blind, randomized, cross-over design. Subjects were randomly assigned to supplement their diet with Nature's Way Antioxidant Formula or a placebo capsule by drawing an envelope containing the first dose of supplements and showing the contents to a laboratory technician, who would record the data, deliver further doses and delete any data that may give way to subject identity. Each subject was required to read and sign an informed consent form approved by the Institutional Review Board of the University of Central Missouri (See Appendix A).

Procedure and Instrumentation

Subjects were advised not to do any exhaustive endurance training or strength training during the duration of the study, to come into the lab for testing euhydrated, not to have eaten within 2 hours of testing, and not to use any anti inflammatory medications or rehab modalities during the duration of the study. Subjects were also required to keep a dietary log, for diet analysis, using myfitnesspal.com during the duration of the study to note any significant changes

in diet. Day 1 involved participants partaking in baseline assessments in the following order: a pre-participation health screening using the PAR-Q, a health history background including listing any medications the subject is currently taking and any known drug allergies (see Appendix B). Next, participants' height and weight were measured. Range of motion of the knee joint was measured using a goniometer. Resting blood lactate measurements were taken using portable lactate analyzers (Scout Lactate+). Subjects were then asked to rate their pain based on a perceive soreness scale of 0-10 (0 being no soreness at all-10 being worst possible, unbearable, excruciating soreness) (see Appendix C). Subjects then completed a five minute warm-up on a bicycle ergometer, a 5-RM in leg press exercise to measure strength (Freemotion Epic), and a power output test using a Wingate bicycle ergometer (Velotron).

After baseline assessments were completed, subjects participated a two week wash-out period to remove any supplements they may have stopped on day one and to recover from any EIMD symptoms that may have occurred from baseline measures. On day 14 subjects started supplementing with 15 (one capsule, two times per day) Nature's Way Antioxidant Formula supplements consisting of: 25,000 IU (15mg/day) of beta-carotene, 500mg of Vitamin C, 400 IU (268mg/day) of Vitamin E, 350mcg/day of riboflavin, 15mg/day of Zinc, 200mcg/day of Selenium, 100mg/day L-cysteine, 100mg/day Quercetin, 14mg/day of grape skin extract, 10mg/day of Green tea polyphenol catechin extract (leaf), 4mg/day of Coenzyme Q10, and 2mg/day of Copper or 15 placebo capsules consisting of natural gelatin capsules filled with all purpose flour. Subjects were instructed to take one capsule twice per day, starting on day 14, with food prior to testing and the last remaining capsule with breakfast on the eighth day prior to post testing. On day 21 subjects performed a five minute warm-up on a bicycle ergometer followed by three warm up sets: the first consisting of 8 repetitions at 40% of the subjects

predicted 1-RM, the second consisting of 6 repetitions at 60% of the subjects predicted 1-RM, and the third consisting of five repetitions at 80% of the subjects 1-RM (NSCA, 2012), followed by an eccentric exercise protocol consisting of four sets of 12 repetitions of the leg press exercise using 130% of the subjects concentric one rep max and the subjects full range of motion (Fleck & Kramer, 2014). On day 21, after exercise, measurements were repeated for 5-RM in leg press exercise, power output, ROM, blood lactate, and asked to rate their pain perceive soreness scale of 0-10 in the previously mentioned order. Prior to leaving the lab subjects were given either eight supplements or eight placebo capsules, based on the portion of testing they were currently in, and were instructed to continue taking the capsules twice a day with food until all capsules were gone. Subjects returned to the lab 48 and 96 hours post exercise, to complete ROM, blood lactate testing, 5-RM testing of leg press exercise, power output testing using the Wingate bicycle ergometer, and asked to rate their pain. Following another two week washout period, the subjects from the supplemented group were switched to a placebo group and vice versa. The above protocol was then repeated. During the weeks of supplementation and testing, participants were given daily reminders, through their preferred method (text message or email), to take the capsules and log their food on my fitness pal.

Statistical Analysis

A comparison between the data of subjects when supplementing with the antioxidant blend and when supplementing with the placebo was made utilizing an ANOVA with repeated measures to determine differences in the following means: ROM, blood lactate, power output, strength, and perceived soreness ratings before exercise, immediately after exercise, and at 48 and 96 hours post exercise. Paired t-tests were used to compare differences in performance

measures, between baseline and each post-exercise assessment, while supplementing with an antioxidant vs. supplementing with a placebo. The level of significance was set at $p < 0.05$.

Chapter 4

Results

The purpose of this study was to examine the effects of 24 days of antioxidant supplementation on the recovery of ROM, resting blood lactate, strength, power, and pain caused by EIMD. An ANOVA with repeated measures was completed for each variable and each trial to determine differences in the following means: ROM, blood lactate, power output, strength, and perceived soreness ratings before exercise, immediately after exercise, and at 48 and 96 hours post exercise. Paired t-tests were used to compare differences in each of the aforementioned performance measures while supplementing with an antioxidant vs. supplementing with a placebo. All data used for ANOVA and t-test comparisons came from the raw scores for subjects from two trials, one during supplementation with the antioxidant and another during supplementation with the placebo capsule. The analysis of the data is presented in this chapter according to the following topics: (1) demographic data, (2) range of motion, (3) resting blood lactate, (4) perceived soreness, (5) strength, (6) Anaerobic measures, and (7) diet analysis.

Demographics

The physical characteristics of the participants can be found in Table 1. The mean age was 21.6 ± 2.8 years, mean height was 164.4 ± 5.0 cm, and mean weight was 72.3 ± 11.6 kg.

Participant Drop Rate

Twenty one subjects participated in this study. A total of 11 participants dropped, due to lack of time to participate (8), perceived side effects of the placebo (1), and excessive pain (2) leaving a total of 10 completing both trials.

Supplement Compliance

All ten subjects completed one trial in which they supplemented their normal diets with Nature's Way Antioxidant capsules and one trial in which they supplemented their normal diets with a flour placebo capsule. Subjects were asked to return any capsules that they did not take. On average participants missed three antioxidant capsules per trial and 4 placebo capsules per trial. It should be noted that the capsules were not identical and a few participants mentioned that they thought one was a placebo. The researcher did not confirm or deny these assumptions until all data was collected and recorded.

Range of Motion

A repeated measures ANOVA was completed for range of motion in the right leg and left leg during antioxidant supplementation (RLS and LLS, respectively) with antioxidants and during supplementation with a placebo (RLP and LLP, respectively) to determine if there was significant change between baseline, immediately (IPEX), 48 hours, and 96 hours post exercise. Eight paired t-tests were used to compare differences in ROMR and ROML during supplementation with the antioxidant (A) vs. the placebo (B) capsules at baseline, IPEX, 48 hours, and 96 hours (See Table 1).

Table 1

Variable ROM (M ± SD)

| Group | Baseline | IPEX | 48 hours | 96 hours |
|-------|-------------|-------------|--------------|-------------|
| RLS | 119.8 ± 8.4 | 119.6 ± 7.3 | 118.1 ± 7.7 | 118.1 ± 7.7 |
| RLP | 119.8 ± 8.4 | 120.9 ± 6.3 | 117.2 ± 11.7 | 120.5 ± 7.6 |
| LLS | 120.2 ± 8.0 | 120.1 ± 8.6 | 118.8 ± 7.6 | 118.8 ± 7.3 |
| LLP | 119.5 ± 8.2 | 121.7 ± 5.8 | 116.1 ± 11.5 | 119.2 ± 7.6 |

Note: RLS = Right Leg during supplementation, RLP = Right Leg during placebo trial, LLS= Left Leg during supplementation, LLP= Left Leg during placebo trial.

Through the analysis of the results from the ANOVAs and t-tests conducted in this study, it was determined that there were no significant changes in ROM during the study and that antioxidant supplementation did not significantly affect the recovery of ROM.

Resting Blood Lactate

A repeated measures ANOVA was completed for resting blood lactate during antioxidant supplementation (BLS) and placebo supplementation (BLP) to determine if there was significant change between baseline, immediately post exercise (IPEX), 48 hours post exercise (48 hours), and 96 hours post exercise (96 hours). The ANOVAs show that there was significant change during both trials. A post hoc test was performed for each trial showing that during antioxidant supplementation change was significant at $p < 0.05$ and $p < 0.01$ when supplementing with the placebo. Again paired t-tests were used to compare differences in resting blood lactate during supplementation with the antioxidant vs. the placebo capsules at baseline, IPEX, 48 hours, and at 96 hours. The t-tests showed no significant effect on resting blood lactate at baseline, IPEX, and 96 hours, but showed significant difference at 48 hours. Through the analysis of the results from the ANOVAs and t-tests conducted in this study, it was determined that there were

no significant changes in resting blood lactate during the study and that antioxidant supplementation had a significant effect on the recovery of resting blood lactate values (Table 2). However, contrary to the hypothesis blood lactate was higher at 48 hours post exercise when supplementing with antioxidants than when supplementing with a placebo.

Table 2

Variable Resting Blood Lactate in mmol/L (M ± SD)

| Group | Baseline | IPEX | 48 hours | 96 hours |
|-------|-----------|-----------|------------|-----------|
| BLS | 1.5 ± 0.5 | 4.0 ± 1.8 | 2.0 ± 0.7* | 1.9 ± 0.9 |
| BLP | 1.5 ± 0.5 | 3.4 ± 2.2 | 1.6 ± 0.4* | 1.8 ± 0.7 |

Note: BLS= Blood Lactate during supplementation, BLP= Blood Lactate during placebo trial.* Denotes significance.

Perceived Soreness

An ANOVA with repeated measures was completed for perceived soreness in the right leg and in the left leg during antioxidant supplementation (RLS and LLS, respectively) and placebo supplementation (RLP and LLP, respectively) to determine if there was significant change between baseline, immediately post-exercise (IPEX), 48 hours post-exercise (48 hours), and 96 hours post-exercise (96 hours). The ANOVAs show that there was significant change in RLP and LLP. Post hoc tests were completed and suggest that RLP was significant at $p < 0.05$, but LLP was not significant. Eight paired t-tests were used to compare differences in pain during supplementation with the antioxidant vs. the placebo capsules at baseline, IPEX, 48 hours and at 96 hours. The t-tests showed no significant effect on pain between the two trials (Table 3). Through the analysis of the results from the ANOVAs and t-tests conducted in this study, it was

determined that antioxidant supplementation did not have a significant effect on the recovery of perceived soreness from EIMD.

Table 3

Variable Pain (M ± SD)

| Group | Baseline | IPEX | 48 hours | 96 hours |
|-------|-----------|-----------|-----------|-----------|
| RLS | 3.3 ± 1.6 | 5.1 ± 1.5 | 5.0 ± 1.5 | 3.1 ± 1.7 |
| RLP | 3.3 ± 1.6 | 5.4 ± 1.6 | 5.0 ± 2.5 | 3.6 ± 1.6 |
| LLS | 3.2 ± 1.5 | 5.0 ± 1.7 | 5.0 ± 1.6 | 3.1 ± 1.7 |
| LLP | 3.2 ± 1.5 | 5.5 ± 1.6 | 5.0 ± 2.5 | 3.6 ± 1.7 |

Note: RLS= Right Leg during supplementation, RLP= Right Leg during placebo trial, LLS= Left Leg during supplementation, LLP= Left Leg during placebo trial.

Strength

An ANOVA with repeated measures was completed for strength using a predicted 1-RM during antioxidant supplementation (S) and placebo supplementation (P) to determine if there was significant change between baseline, immediately post exercise (IPEX), 48 hours post exercise (48 hours), and 96 hours post exercise (96 hours). The ANOVAs show that there was no significant change in strength. Again, paired t-tests were used to compare differences in strength during supplementation with the antioxidant vs. the placebo capsules at baseline, IPEX, 48 hours, and at 96 hours. The t-tests showed that supplementation had no significant effect on recovery of strength or increased strength during the study (Table 4).

Table 4

Variable Strength in kg (M ± SD)

| Group | Baseline | IPE _x | 48 hours | 96 hours |
|-------|--------------|------------------|--------------|--------------|
| S | 121.4 ± 33.4 | 121.6 ± 34.0 | 135.2 ± 47.9 | 137.1 ± 45.3 |
| P | 132.7 ± 47.2 | 124.2 ± 38.7 | 134.5 ± 39.6 | 143.8 ± 38.5 |

Note: S=supplementation trial, P = placebo trial.

The analysis of the results from the ANOVAs and t-tests conducted in this study show that there were no significant changes in strength during the study and that antioxidant supplementation had no significant effect on the recovery of strength post exercise.

Anaerobic measures

An ANOVA with repeated measures was completed for anaerobic capacity, anaerobic power, and fatigue index during antioxidant supplementation (ACS, APS, and FIS respectively) and placebo supplementation (ACP, APP, and FIP respectively) to determine if there was significant change between baseline, immediately post exercise (IPE_x), 48 hours post exercise (48 hours), and 96 hours post exercise (96 hours). The ANOVAs showed that there was no significant change in anaerobic capacity, anaerobic power, or fatigue index. Twelve paired t-tests were used to determine the significance of supplementation with Nature's Way Antioxidant on recovery of power indices including: anaerobic capacity, anaerobic power, and fatigue index. There was no significance of supplementing with antioxidants on any of the aforementioned power indices.

Table 5

Variable Anaerobic Measures (M ± SD)

| Group | Baseline | IPE _x | 48 hours | 96 hours |
|---------------|------------|------------------|------------|------------|
| ACS (W/kg) | 4.8 ± 1.1 | 4.3 ± 0.9 | 4.1 ± 1.1 | 4.4 ± 1.0 |
| APS (W/kg) | 8.2 ± 1.2 | 8.1 ± 1.0 | 8.4 ± 1.2 | 8.4 ± 1.0 |
| FIS (W/s) | 13.2 ± 3.3 | 14.0 ± 2.9 | 14.7 ± 3.7 | 14.9 ± 4.0 |
| ACP (W/kg) | 4.8 ± 1.1 | 4.1 ± 1.0 | 4.1 ± 1.1 | 4.1 ± 1.1 |
| APP (W/kg) | 8.2 ± 1.2 | 8.0 ± 1.1 | 8.2 ± 1.2 | 8.4 ± 1.3 |
| FIP (W/s) | 13.2 ± 3.3 | 14.3 ± 2.8 | 14.9 ± 3.8 | 15.1 ± 4.0 |

Note: ACS= Anaerobic Capacity during supplementation, APS= Anaerobic Power during supplementation, FIS= Fatigue Index during supplementation, ACP= Anaerobic Capacity during placebo trial, APP= Anaerobic Power during placebo trial, FIP= Fatigue Index during placebo trial.

Analysis of the results provided through the ANOVAs and t-tests shown above shows no significant difference in anaerobic capacity, anaerobic power, or fatigue index regardless of the supplement being taken.

Diet Analysis

Paired t-tests were used to analyze the difference in average carbohydrate intake (CHO), average protein intake (PRO), and average fat intake (FAT) for participants during antioxidant

supplementation (S) and placebo supplementation (P) (Table 6). This was done in place of a standardized diet. At $p < 0.05$, there was no significant difference between macronutrient intake during supplementation with the antioxidant vs. when supplementing with the placebo.

Table 6

Diet Analysis

| Group | CHO (g) | PRO (g) | FAT (g) |
|-------|--------------|-------------|-------------|
| S | 192.0 ± 54.4 | 53.9 ± 12.4 | 77.2 ± 41.1 |
| P | 175.7 ± 59.8 | 47.3 ± 22.6 | 54.8 ± 23.0 |

Note: S= supplementation trial, P= placebo trial.

Summary

When examined over a period of 96 hours, the above mentioned results show no significant change in ROM, resting blood lactate, muscular strength, perceived soreness, or in the anaerobic measures. Also, there appears to be no significant differences in average macronutrient amounts consumed during the duration of the study. Antioxidant supplementation with Nature's Way Antioxidant Formula appears to have no effect on the performance related symptoms of EIMD in female participants.

Chapter 5

Discussion

The purpose of this study was to examine the effects of antioxidant supplementation on performance markers of EIMD. Though the study did not give way to a new breakthrough in antioxidant supplementation, it did provide insight that Nature's Way Antioxidant Formula does not significantly reduce ROM of the knee joint, resting blood lactate, or perceived soreness. Furthermore, the current study showed that Nature's Way Antioxidant Formula did not significantly enhance the recovery of anaerobic power or dynamic strength. By eliminating antioxidants that are ineffective in reducing symptoms of EIMD, researchers can begin to study more effective antioxidants, at greater depths. Creating a greater breadth of knowledge will help to determine if there is a supplement that can benefit those who are new to exercise by reducing unpleasant side effects of exercise and possibly increasing the population's health and wellness.

The current study showed no difference in ROM between the participants during supplementation with the antioxidant or during supplementation with the placebo and these findings are supported by previous studies (Connolly, McHugh, & Padilla-Zakour, 2006; O'Fallon et al, 2012). Bloomer, Goldfarb, McKenzie, You, and Nguyen (2004) completed a very similar study examining the effects of antioxidant therapy in 18 untrained women who were exposed to eccentric exercise. The study re-assessed range of motion at 2, 6, 24, 48, 72, and 96 hours, as well as, 14 days post-exercise. The authors showed no significant interaction in ROM after 14 days of supplementation with vitamins C, E, and Selenium.

Meambarbashi & Abedani (2011) showed results that differ from the current study while examining the effects of purslane extract on DOMS. The authors tested knee ROM immediately after exercise, 24 hours after exercise, and 48 hours after exercise in non-athletes who had

supplemented their diets with 1200mg/day of dried purslane leaves. Their results show that right knee joint ROM changed significantly at 48 hours post-exercise. Perhaps the mode of ingestion, synthetic or through a diet rich in antioxidants, is more important than the subsequent testing times as Urso and Clarkson (2003) stated when they examined oxidative stress, exercise, and antioxidant supplementation.

The current study showed no significant difference in resting blood lactate between the antioxidant trial or placebo trail when tested 21 days prior to exercise, immediately after exercise, 48 hours after exercise, or 96 hours after exercise. One study showed participants blood lactate returning to baseline when tested 30 minutes into exercise but no significant effect immediately post-exercise (Chan, Lin, Liu, & Hsu, 2008). It should be noted that Chan et al (2008) used a cycling protocol, with no eccentric component, and more studies exploring the effects of antioxidant supplementation on indirect markers of EIMD are needed as the eccentric component is responsible for causing EIMD (Jeukendrup & Gleeson, 2010). Until further studies are completed examining the effect of EIMD on blood lactate, this difference will remain unexplained.

No significant changes occurred in perceived muscle soreness at any time between the antioxidant supplementation trail and the placebo trial. The findings from the current study are supported by previous studies (Connolly, McHugh, & Padilla-Zakour, 2006; McLeay et al). O'Fallon et al (2012) completed a very similar study examining the effects of quercetin supplementation on markers of muscle damage after eccentric exercise in 30 healthy subjects. Similar to the current study, subjects ingested quercetin and vitamin C twice per day for seven days prior to exercise and five days after exercise. The authors showed no significant interaction in muscle soreness.

Bloomer, Goldfarb, McKenzie, You, and Nguyen (2004) showed results that differ from the current study though the study design was similar to the current study. The authors examined muscle soreness at 2, 6, 24, 48, 72, and 96 hours, as well as, 14 days post-exercise. Their results show that muscle soreness was significantly lower in the supplemented group at 48 and 72 hours post-exercise. It is possible that peak recovery from DOMS occurs at 24-48 hours after exercise and begins to subside at 72 hours post-exercise as concluded by Pettit et al (2010). Kuehl, Perrier, Elliot, and Chesnutt (2010) also showed different results from the current study while examining the efficacy of tart cherry juice in reducing muscular pain. The authors examined pain at the race start and the race end using a visual analog scale of 0-100 (VAS). Perhaps the VAS is a more efficient indicator of significance of muscular pain as concluded by Burnett, Smith, Smeltzer, Young, & Burns (2010) while studying perceived muscle soreness in recreational runners. It should also be noted that Kuehl et al (2010) and Meamarbashi & Abedani (2011) had subjects supplementing with non-synthetic antioxidants periods of time similar to the current study and that further research investigating the mode of antioxidant ingestion is still needed.

In the current study there were no significant changes in anaerobic measures (anaerobic power, anaerobic capacity, fatigue index) between participants during supplementation with the antioxidant formula or during supplementation with the placebo capsules. One study showed a significant increase in explosive power of male handball athletes (Lafay et al, 2009). It should be noted that Lafay et al (2009) used counter movement jumps to determine explosive power, and more studies exploring the effects of antioxidant supplementation on anaerobic measures recorded via Wingate are needed to truly explain this difference.

Changes in dynamic strength were also insignificant between the antioxidant supplementing group and the placebo group in the current study and these findings are supported

by previous studies (Bloomer, Goldfarb, McKenzie, You, and Nguyen, 2004 & Meamarbashi & Abedani, 2011). Connolly, McHugh, & Padilla-Zakour (2006) showed results that differed from the current study when examining cherry juice and the prevention of muscle damage. The results showed that MIF strength loss was significantly greater in the supplemented group than in the placebo group. Howatson et al (2011) also had different results from the current study when observing the influence of tart cherry juice on indices of recovery following marathon running. The authors stated that isometric strength recovered significantly faster in the cherry supplemented group. McLeay et al (2012) also showed results that differed from the current study while documenting the effect of New Zealand blueberry consumption on recovery from EIMD. The previous study showed a significant interaction effect for peak isometric tension. The results for concentric and eccentric strength followed a similar trend. More research testing the efficacy of antioxidant supplementation is needed as maximal contraction is limited by the tendon during isotonic contractions but not during isometric contractions (Taifour, Naiwaiseh, & Khasawneh, 2013).

The above mentioned studies have common differences with the current study as the antioxidants used in the studies appear to be efficient in protecting from or reducing recovery time of exercise induced strength loss. The studies also contain polyphenols that the current studies supplement does not contain. Again, perhaps the mode of antioxidant intake is an important area for exploration as taking in antioxidants through diet, rather than through supplementation, can provide a combined supplement strategy which has proved to be promising for the reduction of EIMD symptoms (McGinley, Shafat, & Donnelly, 2009).

Implications for Future Studies

This study was limited by many factors and difficulties that arose throughout the study. First, it was difficult to gather and retain subjects due to the nature of the study and the associated soreness. It was also difficult to retain subjects as the study was time sensitive and did not allow for major fluctuations in schedules due to personal reasons. The participant drop rate of 52% was much higher than the expected 25% drop rate. This limited the statistical power of the current study due to the small sample size. The present study should be replicated using a larger sample size.

Secondly, there were some issues with compliance. Though subjects were reminded by their preferred method and envelope check lists, they often forgot to take capsules, placebo capsules fell apart, and participants forgot to log their daily food intake. In order to minimize the effect of these limitations, a similar study might be conducted using a standardized diet and identical supplement and placebo capsules instead of handmade capsules. Using identical capsules may also minimize any possible placebo effect associated with the current study.

Thirdly, a similar investigation should be completed examining not only the effect of antioxidant supplementation on blood lactate, but the effects on more direct blood markers of EIMD as well. Future research would also benefit from a similar study that tests a synthetic antioxidant compared to a whole food containing the same antioxidant properties in order to see if the mode of ingestion plays a role in the effects of antioxidant supplementation on EIMD. Overall, further investigation on antioxidants and recovery from EIMD may be more reliable in a clinical setting as opposed to a campus setting where participation may be greater as the subjects may be able to receive greater compensation and diets, supplementation, and population criteria may be better regulated.

Conclusions

The purpose of this study was to examine differences in the following means: ROM, blood lactate, power output, muscular strength, and perceived soreness ratings before exercise, immediately after exercise, and at 48 and 96 hours post exercise. The results for ROM, power output, muscular strength and perceived soreness showed no significant difference, at any time, between participants regardless of supplementation. However, the current study did suggest that resting blood lactate was significantly higher in the antioxidant group at 48 hours post-exercise and more research is necessary to determine the underlying cause of these results. The current study's results might indicate that resting blood lactate is not an efficient indicator of EIMD. Previous studies are indicative of more direct blood markers, like CK and LDH, though more research is needed.

The current study also suggests that synthetic supplementation of antioxidants may not be effective in reducing symptoms of EIMD and that a balanced diet including polyphenols such as purslane, tart cherry, and blueberries. It is possible that a balanced diet could be more effective than synthetic supplementation in providing the combined supplement strategy that may be needed to see effects in reducing symptoms of EIMD, but more research is needed comparing the two types of antioxidants. The current study indicates that more research is needed investigating antioxidant supplementation on anaerobic power when tested via Wingate and dynamic strength. It is possible that anaerobic power can exhibit effects due to learning, neuromuscular development, and other factors when assessed using CMJ. It is possible that measuring power using a Wingate could reduce those effects but more research is needed. It may also be derived from this study and previous studies that VAS is a better indicator of significance of DOMS than a general 0-10 pain scale as it may make it easier for subjects to pinpoint muscular soreness.

Overall, the current study and previous studies yield inconclusive information and more research is needed to determine the effects of antioxidant supplementation on recovery from EIMD.

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Appendix A

Informed Consent

CONSENT FORM

Identification of Researchers: This research is being done by Kara Stone, a graduate student at the University of Central Missouri.

Purpose of the Study: The purpose of this study is to examine the effect of antioxidant supplementation on the performance related symptoms of exercise induced muscle damage in the form of delayed onset muscle soreness.

Request for Participation: You are invited to participate in a study on antioxidants and exercise induced muscle damage in the form of delayed onset muscle soreness. It is up to you whether you would like to participate. If you decide not to participate, you will not be penalized in any way. You can also decide to stop at any time without penalty. You may withdraw your data at the end of the study.

Exclusions: You must be a female 19-30 years of age, not pregnant, and not currently weight training to participate in this study. You must not be taking any other supplements while participating in the study.

Description of Research Method: The total time commitment will include 22 days of supplementation and dietary logging and eight sessions, 45-60 minutes each, of exercise testing including blood draws obtained by a finger stick with a two week rest period in between. The supplements taken in this study are safe and do not exceed the tolerable upper level dietary reference intakes set by the United States Department of Agriculture.

Privacy: All of the information we collect will be confidential. We will not record your name, student number, or any information that could be used to identify you. We will also provide you with a blank sheet of paper so that you can cover your responses as you write them down. This will prevent other research participants from seeing your answers.

Explanation of Risks:

Possible risks associated with resistance training include, but are not limited to: muscular soreness, fatigue, joint pain, and inflammation.

Explanation of Benefits: You will benefit from participating in this study by getting firsthand experience in resistance training. You may also enjoy completing the exercise testing. We will provide you with a coupon that you may use if any of your instructors award extra credit for research participation. Upon completion of the study you may also be entered into a drawing for a free two hours of personal training in which three winners will be randomly selected.

Questions: If you have any questions about this study, please contact Kara Stone at (660) 238-0438. If you have any questions about your rights as a research participant, please contact the Human Subjects Protection Program at (660) 543-4621.

If you would like to participate, please sign a copy of this letter and return it to me. The other copy is for you to keep.

I have read this letter and agree to participate.

Signature: Printed name:

Date:

Person obtaining consent: _____

Approved: 10/16/2014 Expires: 10/16/2015

Appendix B

Health History Questionnaire
Health History Background

Name: _____ Date: _____
Home Phone: _____ Office Phone: _____
Family Doctor: _____ Doctor's Phone: _____
Age: _____

Health History (Check if appropriate)

- Disease of arteries and heart
- Diabetes or abnormal blood sugar test
- Epilepsy
- Stroke
- Anemia
- Abnormal Chest X-ray
- Asthma
- Liver or Kidney disorder
- Other lung disease
- Orthopedic or muscular problems

Level of Physical Activity

Yes / No Are you currently involved in a regular exercise program?
Yes / No Do you regularly walk or run one or miles continuously? If Yes, average number of miles you cover per workout or a day: _____. What is your average time per mile (min:sec)? _____.
Yes / No Do you practice weight lifting or home calisthenics?
Yes / No Do you frequently participate in competitive sports? If Yes, please list

Average number of times per month you participate in these activities: _____
If currently not exercising, how long has it been since you have not exercised?

Family History of Cardiovascular Disease (MI, Hypertension, Hyperlipidemia)

Who: **Problem:** **Age: (at onset or death)**

Smoking

Yes / No Do you currently smoke?
If yes, how much? _____

If you smoked in the past, when did you quit? _____

Tension/Stress

() Relaxed, no tension () Slight Tension () Moderate Tension

Medications and Supplements (Please indicate what, if any, medications you are taking)

Name of Medication/Supplement: **Dosage:** **Taken**
Since:

Strength: **How Often:**







Allergies (Please list any known drug allergies, check the box bellow if no known allergies exist)

NONE

Gluten Sensitivity Do you have any gluten sensitivities?

YES NO

Appendix C
Perceived Soreness Scale

| | Scale | |
|---|-------|---|
| No pain | 0 |  |
| | 1 | |
| Mild, annoying pain | 2 |  |
| | 3 | |
| Nagging, uncomfortable, troublesome pain | 4 |  |
| | 5 | |
| Distressing, miserable pain | 6 |  |
| | 7 | |
| Intense, dreadful, horrible pain | 8 |  |
| | 9 | |
| Worst possible, unbearable, excruciating pain | 10 |  |