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Effect of EpiCor supplementation on delayed onset muscle soreness and inflammation after unaccustomed, eccentric resistance exercise

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Effect of EpiCor supplementation on delayed onset muscle soreness and inflammation after unaccustomed, eccentric resistance exercise

by

Justin Scherff

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Kinesiology (Biological Basis of Physical Activity)
Program of Study Committee:
Rick Sharp (Major Professor)
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Ames, Iowa
2010

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ABSTRACT

Unaccustomed eccentric exercise has been well documented to elicit muscle damage and an inflammatory response to help fix and restore the impaired muscle fibers. Supplements, including anti-inflammatories and antioxidants have been used to help alleviate the damage and decrease the inflammation but positive results are slim. It is proposed that EpiCor, a yeast metabolite with suggested anti-inflammatory and antioxidant characteristics, would decrease the amount of muscle soreness and inflammation that results from unaccustomed eccentric exercise.

Thirty-two male subjects (ages 18-35) ingested a once daily 500mg dose of either EpiCor or placebo 4 weeks prior to the eccentric exercise bout. This matched pairs, double blind study randomly assigned subjects to either group and were matched based on a peak, pre-exercise maximal isometric contraction. Subjects then performed 50 maximal eccentric contractions of the elbow flexors at 30 degrees per second on each arm. Blood samples were taken at pre-, 24, 48, and 72 hours post-exercise, in addition to psychological and illness surveys.

Muscle damage, soreness and circumference all had a significant effect of time throughout the 72 hour recovery period (P<0.001). Elevated levels of CK and LDH were also present with a significant effect of time (P<0.05); however, there were no significant differences between treatments for muscle damage indicators. Results for inflammatory markers were similar in that no statistically significant difference between groups existed for IL-1ra, IL-6, IL-10, and CRP, but some non-significant trends (P<0.11) did exist.
These results do not support the hypothesized effects for EpiCor in reducing the muscle damage, soreness, and inflammation after an unaccustomed eccentric exercise bout of the elbow flexors. This supplement needs further research into the effectiveness of its use in an exercise setting.
CHAPTER 1. INTRODUCTION

Performing unaccustomed, eccentric-based muscular contractions has been well-documented to cause muscle fiber damage that results in delayed-onset muscle soreness (DOMS) and inflammation (Nosaka & Newton, 2002; Croisier et al., 1996; Clarkson & Tremblay, 1988; Kyrolainen et al., 1998). When a muscle is under tension and forced to stretch, micro-tears occur in the muscle fibers (Nosaka & Newton, 2002), which is followed by local inflammation (Stupka et al., 2001; Mackey et al., 2004; Lapointe et al., 2002) and ultimately results in a decrease of force production (Folland et al., 2001). Sources, including Armstrong (1991) and Clarkson et al. (1992), have indicated that delayed onset muscle soreness is maximized at 48-72 hours after the eccentric bout, but researchers have not been able to pinpoint the exact cause for the delayed response. After the micro-tears occur in muscle fibers, cell membranes are thought to be damaged, and the intracellular calcium homeostasis is disrupted, which leads to fiber damage and cell death (Kyrolainen et al., 1998; McBride et al., 1998; Beaton et al., 2002).

As the body repairs the damage done by the first eccentric bout, it adapts from the previous damage in what is known as the repeated bout effect, which suggests the idea that a second bout of similar eccentric movements will cause less damage and stress to muscle fibers (Golden & Dudley, 1992; Brocket et al., 2001; Sorichter et al., 1997; Nosaka et al., 2005; Chen & Hsieh, 2001; Lapointe et al., 2002). Possible mechanisms behind the repeated bout effect include a sustained shift in the optimal contraction angle of the muscle (Brocket et al., 2001), repair or strengthening of various muscle components (Lapointe et al., 2002; Mackey et al., 2004), or a change in the neurological pathways (O’Connor et al., 2002).
Researchers have investigated numerous supplements that may help protect muscles from the severe inflammation and soreness of an unaccustomed eccentric bout of exercise, including non-steroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen (Hasson et al., 1993), ketoprofen (Sayers et al., 2001), and aspirin (Francis & Hoobler, 1987), as well as calcium-channel blockers (Beaton et al., 2002), diclofenac sodium (O’Grady et al., 2000), and antioxidants such as vitamin E (McBride et al., 1998). Hayward et al., (1999) also indicated that a possible link exists between elevated protein intake and diminished markers of muscle damage.

The supplement we propose to study is EpiCor, an immunogenic product based on the fermentation of baker’s yeast in anaerobic conditions. EpiCor is known to have high amounts of metabolites that include vitamins, polyphenols, sterols, and phospholipids. Additionally, Jensen et al (2007, 2008) demonstrated the components of EpiCor possess significant antioxidant and anti-inflammatory properties and act as an immune modulator. Specifically, Jensen et al., (2008) provided results of up-regulation of IL-10 production in EpiCor treatment groups that display anti-inflammatory aspects of the supplement. Specific research with the EpiCor supplement in an exercise setting has not been performed, but with the suggested anti-inflammatory and anti-oxidant properties EpiCor should help reduce the effects of unaccustomed exercise on muscle damage and inflammation.

Due to the suggested benefits of EpiCor supplementation, a prolonged supplementation of EpiCor before and during a maximum effort eccentric bout of exercise should significantly lower markers of inflammation and muscle damage as well as return muscle function to normal more quickly than with placebo. Therefore, the purpose of this study is to evaluate the effectiveness of EpiCor in limiting the amount of muscle soreness,
inflammation, and muscle damage as well as returning normalized muscular functions within 48-72 hours after a maximal eccentric strength training bout. We hypothesize that the EpiCor supplement will reduce muscle soreness and damage after an unaccustomed bout of eccentric exercise and aid in a quicker recovery than a placebo.

Statement of the Problem

Does EpiCor decrease the amount of muscle soreness, inflammation, and muscle damage as well as return normalized muscular functions within 48-72 hours after a maximal eccentric strength training bout?
CHAPTER 2. REVIEW OF LITERATURE

Muscle Damage

One of the first studies of exercise-induced muscle damage came from Hough in 1902, who suggested that delayed muscle soreness was a result of micro-tears in the muscle fibers. Almost 80 years later, Friden et al. (1981) provided some of the first evidence of exercise-induced muscle damage through biopsies of soleus muscle tissue taken two and seven days after exercise. Major findings in this study were myofibrillar disturbances and z-line streaming. Friden and others (1983) continued to research this phenomenon and discovered many observed ultrastructural changes after eccentric exercise, which included z-line streaming and z-lines out of register, loss in thick myofilaments, loss in mitochondria in areas of abnormalities, and disturbed arrangement of A-band filaments. These findings have been supported by many other research studies over the last 30 years, including Armstrong et al. (1983), Newham et al. (1986), Schwane et al. (1983), and Stupka et al. (2001). This research provides support for the notion that exercise stresses skeletal muscle fibers and that if the intensity or duration provides a tensile strength stimulus greater than that of the muscle yield strength, damage to the muscle fibers occurs.

Since a definite pathway for injury has not been established, a possible sequence of events that describe muscular damage after exercise was proposed by Morgan (1990). Lengthening of the active muscle, or eccentric exercise, causes some sarcomeres in each myofibril to extend to long lengths from which some do not fully return to the interdigitating pattern on relaxation. The repeated stretch-contraction would quickly stretch those sarcomeres and place extra tension on neighboring myofibrils at those sarcomeres, which
results in sarcomere tears. If sufficient tearing occurs, the sarcoplasmic reticulum or sarcolemma can become damaged, which leads to intracellular calcium release, contractures, clots, and eventual destruction of the fiber in days following exercise (Morgan, 1990).

Contraction properties of fibers have also played a role in determining which fiber type is more prone to exercise injury and what speeds elicit the most exercise-induced muscle damage. Jones et al. (1986) provided data that type II muscle fibers are more severely affected than type I fibers by eccentric exercise, specifically in the severely affected calf muscles of this study. It is now understood that because type II muscle fibers are the narrowest fiber type and contain the weakest z-lines, they are more susceptible to damage. Results from Friden et al. (1983) provided additional evidence when they recorded that z-band streaming and broadening occurred more in type II than type I muscle fibers. A concurrent decrease in force output during maximal knee extension exercises was seen, an expected result if type II motor units were damaged. Similar results were observed in eccentric exercise of the elbow flexors (Gibala et al. 1995). A study in 2005 by Paddon-Jones et al. examined different expressions of muscle damage following fast or slow velocity eccentric exercise and discovered that fast eccentric contractions resulted in a longer period of muscle soreness, whereas slow eccentric contractions were associated with a greater increase in swelling and greater decrease in the ability to generate torque.

**Indicators of Muscle Damage**

*Creatine Kinase (CK)*

Researchers have generally accepted CK as a primary, indirect indicator for muscle damage because the enzyme is mostly found in muscle tissue. The release of CK into serum or plasma is a result of membrane disruption from exercise, most notably eccentric exercise
as shown by Newham et al. (1983) and Miles et al. (2008). Studies have been performed to examine the CK release rate, and results show that modes of exercise that include eccentric running (Armstrong et al., 1983) and isometric exercises elicit a larger initial CK response that returns to normal within a few days. Local eccentric muscular exercises, however, cause a much larger CK response that does not begin until two to three days after exercise with a peak at four to six days (Jones et al., 1986).

The size and timeline of the CK release is dependent upon the type, intensity, and duration of eccentric exercise. Nosaka et al. (2002) showed that through 12 maximum eccentric exercises compared to two hours of concentric submaximal exercises (approximately 3600 contractions) there was a delayed but sustained elevation in indirect muscle damage markers in maximal compared to submaximal exercise. Chen et al. (2007) provide evidence that eccentric exercise at 100% intensity results in a much larger CK response than exercise at 40, 60, and 80% intensity. Results from Brown et al. (1997) showed that subjects displayed muscle damage with 30 and 50 repetitions, but the 10 repetition group showed no significant CK increase. The latter group, however, did have a decrease in force generation, which suggests that force loss and elevated CK levels are not related. It is possible that 10 reps was not a great enough stimulus to elicit damage to susceptible fibers but, as previously mentioned, statistically significant muscle damage was present after 8 repetitions (Warren et al., 1993). The magnitude of the CK response is highly variable. Newham et al. (1983) discovered that, during stepping exercises, participants all showed an early rise in CK (<400IU/L) but half of subjects, both male and female, showed levels up to 34,500 IU/L and took four to five days to reach peak levels.
**Lactate Dehydrogenase (LDH)**

Lactate dehydrogenase (LDH) is an enzyme used in the glycolytic energy pathway in the production of ATP and has been considered an indicator of tissue breakdown (Mikkelsen et al., 2004). In terms of eccentric exercise, LDH has been used as an indirect indicator of muscle damage whether using endurance exercise (Armstrong et al., 1983) or eccentric muscle contractions (Costa et al., 2007, Lowe et al., 1995, Brown et al., 1997).

The exercise stimulus does have an influence on the release timeline post-exercise with eccentric exercise as seen in Armstrong et al. (1983). Researchers found that all exercise protocols resulted in elevated LDH immediately after running, but only eccentric exercise resulted in a delayed elevation approximately two days post-exercise. Other studies have shown significant increases in LDH post-exercise lasting up to 72 (Brown et al., 1997, 1999) and 96 hours (Milias et al., 2005). In addition to the type, the muscle group used has been shown to have an effect on the size of the LDH release. Jamurtas et al. (2005) provided results directly comparing elbow flexors and knee extensors in the same subjects with the same workload intensity that saw a significant LDH increase from 24-96 hours post-exercise in the elbow flexors but not in the knee extensors.

The above research indicates that LDH is an indirect indicator of muscle damage but can not alone provide quality results for muscle damage as LDH is an indicator of tissue breakdown in general; however, when elevations in LDH are also shown with CK, another accepted indirect indicator of muscle damage, the applicability of the results increases (Costa et al., 2007, Brown et al., 1997, Milias et al., 2005).
**Eccentric versus Concentric Exercise as Cause of Exercise-Induced Muscle Damage**

Research has shown that eccentric exercise is a primary stimulus for causing muscle damage during exercise, which results in soreness, reduced muscle function, inflammation, and a decreased range of motion.

When comparing eccentric to concentric muscle action as a mode for muscle damage, research generally shows that eccentric exercise causes more muscle fiber damage than concentric exercise. Armstrong et al. (1983) directly compared eccentric to concentric exercise through downhill (eccentric) or uphill (concentric) running with mice at a 16° slope. The researchers found that all exercise protocols, including uphill running, resulted in elevations of plasma enzymes immediately after running; however, only eccentric exercise resulted in late phase elevations 1.5-2 days post-exercise. Additional findings from the study indicate that eccentric exercise produces more muscle fiber damage than concentric exercise of equal power. Since then, further research has been performed with humans, looking at downhill versus uphill exercise with similar results that suggest eccentric exercise (downhill) causes more damage to muscle fibers for a longer duration than concentric exercise (uphill). Newham et al. (1986) used indirect measures of muscle damage and discovered that eccentric exercise was responsible for significantly increased and delayed plasma CK release, as also seen by Balnave et al. (1993). Lynn and Morgan (1994) added to the discussion that, through the adaptable capacity of muscle, examining the fiber lengths and sarcomere numbers after exercise can also be an indicator for muscle damage. The researchers discovered that eccentrically exercised muscle fibers contained a higher number of sarcomeres per muscle fiber compared to concentrically exercised muscle fibers, which
indicate that an adaptation occurred. Research from Macpherson et al. (1997) provided evidence of a 9-11% force deficit in stretched muscle fibers, and a majority of the damage was focused in the areas with the longest sarcomeres. More support is provided by Child et al. (1998), whose subjects performed eccentric exercises at two different knee extensor muscle lengths, 160-80 degrees and 120-40 degrees, with results indicating that greater muscle damage occurred after the long muscle lengths protocol (120-40°).

Apart from downhill running, there are other forms of eccentric exercise, most notably through maximal force generation. Friden and others (1986) discovered that muscles concentrically contracting resulted in less accumulation of intramuscular pressure than those muscles that are stretched while contracted. Later, Jones et al. (1989) showed that eccentric exercise resulted in more muscle damage, greater fatigue, and greater delayed onset of pain than concentric or isometric muscle actions, also seen by Croisier et al. (1996). A study conducted by Warren et al. (1993) showed that decreases in maximal isometric strength and muscle function did not result from the first eccentric movement but rather eight repetitions was the beginning of statistical significance.

Gibala et al. (1995) had each subject perform concentric contractions with one arm and eccentric contractions with the other arm and examined the differences in muscle damage. Not only were there significant decreases in isometric peak torque for the eccentrically exercised elbow flexors, but approximately 80% of the total muscle fibers displayed some type of disruption. Nosaka & Newton (2002) also provided evidence that, at an absolute workload equal to 50% of the maximal isometric force of elbow flexors, the eccentric exercise elicited greater muscle damage than concentric exercise. The researchers did note that eccentrically contracted muscles require less energy to contract, yet elicited the
most damage; however, it is known that eccentrically contracted muscles can generate more force than isometric or concentric contractions, which could suggest that eccentric contractions should show less damage than concentric or isometric contractions.

**Knee Extensors versus Elbow Flexors to Elicit Muscle Damage**

When it comes to muscle isolation exercises to elicit muscle damage through eccentric contractions, the two main groups of muscles utilized are knee extensors (Hasson et al., 1993, Brown et al., 1997, Sorichter et al., 1997, Child et al., 1998) and elbow flexors (Newham et al., 1987, Nosaka & Clarkson, 1996, Chen et al., 2001, 2007, Lavender et al., 2008). Based on information presented above about muscle fiber type, it is known that type II fibers are less resistant to exercise-induced muscle damage, so isolating muscles with a higher distribution of type II fibers would be ideal. Klein et al. (2003) provided evidence through magnetic resonance imaging that the biceps brachii were composed of 61.5% type II fibers in young men while Travnik et al. (1995) showed a higher type I fiber distribution in knee extensors, specifically the vastus medialis longus and vastus medialis obliquus; however, none of these studies compared knee extensors and elbow flexors in the same subjects, and it is possible that similar fiber distributions exist in all muscle groups for any given subject.

It was not until 2005 that Jamurtas et al. compared elbow flexors and knee extensors during eccentric exercise to see which muscle group elicited the greatest damage from the same relative intensities in the same subject. Researchers used data that included maximum eccentric and isometric peak torques, range of motion, delayed onset muscle soreness, and indirect blood markers of muscle damage including CK, LDH, and myoglobin (Mb) to view the differences between 6 sets of 12 repetitions at 75% maximal eccentric peak torque in
each muscle group. The results provided evidence that the elbow flexors induced a larger
decrease and slower recovery in strength and a larger increase in the blood markers of muscle
damage than knee extensors. These findings were later verified in 2009 when Saka et al.
provided additional evidence that greater muscle damage and a slower recovery occurred
with elbow flexors than knee extensors.

**Inflammatory Response**

Research has concluded that exercise-induced muscle injury occurs following
eccentric exercise. When the muscle damage occurs, typically there is capillary rupture,
which sends blood-borne inflammatory cells and cytokines directly to the damage. The size
of the inflammatory response depends on the size of the muscle injury and the amount of
vascularization at the injury site (Tidball, 2005, and Merrick, 2002). This rapid inflow of
inflammatory cells can last from several days to several weeks with the primary purpose to
promote the clearance of damaged tissue and prepare the tissue for repair (MacIntyre et al.,
1995).

**Trigger of Inflammatory Response**

No one has been able to pinpoint the exact trigger of the inflammatory response
following exercise-induced muscle damage. Researchers have suggested the release of
prostaglandins (PG) as one of the main causes of the inflammatory response. These are
produced from local inflammatory cells that are in the damaged area, which then leads to the
recruitment of other inflammatory cells (Issekutz and Movat, 1982). Other research suggests
that potentially humoral and cardiovascular factors, more than muscle damage via exercise
(Brenner et al., 1999), and yet Blais et al. (1999) provided evidence that bradykinin release
could mediate the inflammatory response. Cytokines have also been considered as the trigger for the inflammatory process (Pedersen et al., 1998). This conflicting evidence provides support that the events that lead to the inflammatory response as a result of muscle damage is quite complex; however, we can speculate that since the innate inflammatory response is activated by damaged cells and tissues, not just infectious material, the exercise-induced muscle damage is enough to trigger the inflammatory response.

**Role of Cytokines**

One of the more researched chemotactic substances released during the inflammatory process is cytokines, as Cannon and St. Pierre (1998) indicated that almost all cells with nuclei synthesize cytokines and have cytokine receptors on their cellular membranes. When cells such as vascular endothelium are stimulated, they release cytokines which, in turn, regulate the release of adhesion molecules that attract neutrophils. Milias et al. (2005) suggested that leukocytosis is an indicator of muscle damage after endurance exercise as a positive correlation between leukocyte numbers and plasma CK levels existed, with similar positive correlations between leukocyte response, CK, and LDH reported by Kayashima et al. (1995) after strenuous exercise. Additionally, significant correlations between delayed leukocytosis and indices of muscle damage suggest that exercised muscles generate the inflammatory mediators that result in the increased release of leukocytes (Paulsen et al., 2005).

Generally, the inflammatory response after tissue injury results in early release of cytokines IL-1β and TNFα (Miles et al., 2008). Ostrowski et al. (1999) showed that, with strenuous exercise, TNFα and IL-1β levels are substantially increased during the examined four-hour period post-exercise. These researchers also suggested that cytokine inhibitors and
anti-inflammatory cytokines (IL-1ra, sTNF-r1 and sTNF-r2) restrict the magnitude and
duration of the inflammatory response to exercise. These factors, such as interleukins (IL-),
are thought to initiate the onset of circulating neutrophils into the muscle tissue. Many
studies have provided results that specify whether certain cytokines are related to exercise-
induced muscle damage or if specific cytokines control the release of other cytokines.
Typically, research has shown that endurance exercise (Brenner et al., 1999, Ostrowski et al.,
1999, Suzuki et al., 2003, Nieman et al., 2005) is a better cytokine stimulant than eccentric
exercise. Additionally, Louis et al. (2007) present evidence that up-regulation of specific
mRNA resulted from a cardiovascular run at 75% for 30 minutes compared to 30 repetitions
of concentric knee extension at 70% of a 1-repetition maximum. These findings could be
explained by a greater overall muscular stress in the endurance events or by a diminished
cytokine disposal into the systemic circulation and ultimately into the urine during endurance
exercise. During endurance exercise, blood flow through the renal system is significantly
reduced when compared to exercise of specific muscle groups (e.g., elbow flexors). This
decrease in renal function could lead to a slower clearance of cytokines (Hirose et al., 2004).
For the purposes of the present study, the influx of IL-1 receptor antagonist(ra), IL-6, and IL-
10 will be examined in response to exercise. In addition to these cytokines, C-reactive
protein (CRP) will be analyzed after unaccustomed eccentric exercise.

\textit{IL-1(ra)}

IL-1(ra) is an antagonistic inhibitor of IL-1, an important pro-inflammatory cytokine
in the immune response, binding to the receptor and preventing the actions of IL-1
(Ostrowski et al., 1999). Typically, research has shown greater release of IL-1ra after
endurance exercise, and the release after eccentric exercise is quite minimal. Nosaka &
Clarkson (1996), however, did not report any increase in IL-1(ra) after local muscular eccentric exercise in the elbow flexors, possibly due to a smaller muscle mass used during the eccentric training protocol.

**IL-6**

IL-6 has been discussed as a main regulatory cytokine in the inflammatory response, as it has been shown to have both pro- and anti-inflammatory properties (Bruunsgaard et al. 1997). Tomiya et al. (2004) have specifically indicated IL-6 is a cytokine that is observed in myofibers after eccentric exercise and preceding myofiber disruption, suggesting that IL-6 is active in the muscle degeneration process after muscle injury. Other researchers agree with Tomiya et al. and provide evidence that IL-6 is a sensitive marker of acute inflammation and one of the first responses following eccentric exercise. MacIntyre et al. (2001) and Willoughby et al. (2003) showed that a peak saturation of IL-6 occurred approximately six hours after exercise and that early increases in IL-6 predicted a component of delayed onset muscle soreness. Miles et al. (2008) viewed peak levels of plasma IL-6 approximately eight hours after exercise, and Tomiya et al. (2004) presented peak levels between eight and 12 hours; but all levels have been shown to return to baseline approximately 24 hours post-exercise. These results show IL-6 as a pro-inflammatory cytokine, which suggests that elevated plasma levels of IL-6 can be an indication of the amount of inflammation occurring in the damaged tissue. Nieman et al. (2005) presented a statistically significant correlation between IL-6 (125x increase between pre- and post-race) and CK after a 160km run, which suggests that IL-6 is released as a part of the response to muscle damage.

**IL-10**
IL-10 is an anti-inflammatory cytokine with a main function of suppressing pro-inflammatory cytokines such as TNF-alpha, IL-1, IL-6 and IL-8 (Hirose et al., 2004). Released after strenuous exercise, IL-10 has also been shown to govern the release of IL-1ra, a known inhibitor of the pro-inflammatory cytokine IL-1 (Cassatella et al., 1994, Jenkins et al., 1994). Ostrowski et al. (1999) also showed that IL-10 plays a role in the release of IL-1ra, as high levels of IL-10 were present immediately after a marathon and subsequently decreased as IL-1ra levels had a significant increase. Smith et al. (2000) showed a significant increase in IL-10 between 48 and 144 hours post-eccentric exercise, findings consistent with results provided by Silva et al. (2008). IL-10 has been a prime anti-inflammatory cytokine in past research which provides reason to examine the changes in IL-10 in the present study.

**CRP**

C-reactive protein (CRP) is another generally accepted marker of inflammation and is made by the liver in response to elevated plasma levels of IL-6 in the body (Phillips et al., 2003). Subjects in this study also displayed a decrease in CRP after eccentric exercise with the combined use of tocopherols, docosahexaenoate, and flavonoids. Milias et al. (2005) found no increase in CRP after eccentric exercise of the elbow flexors, even with significant increases in muscle damage indicators, with similar results in Nosaka & Clarkson (1996). Malm et al. (2000) did see an increase in CRP following exhaustive eccentric cycling, which has more of an endurance component than the exercise protocol of Milias et al. (2005) or Nosaka & Clarkson (1996), but the increase was not significant when compared to the control group or rest. On the other hand, Paulsen et al. (2005) showed a statistically significant increase in plasma CRP after 300 maximal eccentric contractions of the
quadriceps both at 24 and 48 hours after exercise. These mixed results indicate that more research is needed to fully understand the effects of the plasma CRP response after eccentric exercise.

**Cellular Response**

Previous research has not narrowed down the exact mechanism for how muscle tissue damage, potentially caused by eccentric exercise, elicits the innate immune response; however, research shows that, after muscle damage occurs, one of the first inflammatory cells that reach the damaged muscle are neutrophils. This influx can begin within one hour of exercise, and amounts can remain elevated as long as five days (Fielding et al., 1993). The increased concentrations of neutrophils in the damaged area are indicators of muscle damage and release proteolytic enzymes (proteases) that break down and dissolve the injured muscle tissue and cells. Both Nguyen and Tidball (2003) and Tiidus (1998), on the other hand, suggest that neutrophils can release high concentrations of cytolytic and cytotoxic molecules, including reactive oxygen species, that are capable of damaging surrounding muscle tissue, which exacerbates the already present damage. Brickson and others (2003) indicated that, through the use of antibodies that block a specific reactive oxygen species, less muscle damage occurred. Other research, including Lowe et al. (1995), provided evidence that the rate of protein degradation was not enhanced through the presence of excessive neutrophils when compared with normal levels of neutrophils. Differences did exist in the training protocols of each study as subjects in the Brickson study only performed
a single eccentric contraction to elicit muscular tissue damage while Lowe had subjects perform 150 eccentric contractions.

After the first response of neutrophils begins to subside, macrophages begin to migrate towards the damaged tissue area approximately 8-12 hours after exercise has ceased. Macrophages have been shown to be detrimental to the recovery process (Lapoint et al., 2002) as they elicit extensive damage after the initial injury occurs through a nitric oxide-dependent lysis of muscle cell membranes (Nguyen and Tidball, 2003). On the other hand, research has also shown that reducing the macrophage concentration does prolong the recovery time and other evidence has been presented that macrophages aid in the recovery process (Chazaud et al., 2003). The general consensus is that neutrophils and macrophages make up a significant portion of the inflammatory response; however, the exact contribution from neutrophils, macrophage, and other inflammatory cells is unknown, and the contributions from each during the inflammatory response is quite complex.

The sensation of pain or soreness 24-48 hours post-exercise is a phenomenon called delayed-onset muscle soreness (DOMS). During the inflammatory response, the neutrophils and macrophages release bi-products that have been theorized to sensitize muscle afferent nerve fibers, but no one bi-product has been established as the main cause of pain. Prostaglandins have received much attention as the trigger for the pain response, but research is mixed. Uchida et al. (1999) showed a weak but significant correlation between peak soreness and PG concentration, and Schwane et al. (1983) suggest that PGs, specifically PGE$_2$, sensitizes the type III and IV pain fibers. Conflicting evidence is presented by Kuipers et al. (1985) and Croisier et al. (1996) as PGs were not directly related to muscle soreness. Bradykinins have also been hypothesized as a mediator of the pain response (Blais
et al., 1999 and Murase et al., 2010), but no undisputable evidence is present to support these hypotheses. Free radicals, produced by both neutrophils and macrophages (Nguyen and Tidball, 2003; Malm et al., 1999) have also been examined as the initiator of DOMS. Like PGs and bradykinins, evidence has been mixed, but evidence has been presented for (Maughan et al., 1989) and against (Close et al., 2004) free-radicals as the trigger for the pain response. It could be hypothesized that a combination of the above-mentioned substances interacts in ways not currently understood to create a combined pain response, but further research is needed to understand this phenomenon.

**Muscle Tissue Recovery**

After the initial exercise bout elicited damage to muscle tissue, researchers have discovered that a training effect occurs and provides a ‘protective effect’, which is described as a decrease in muscle damage after a subsequent exercise bout of the same nature. Current research has not established a definitive process through which muscle tissue returns to normal nor an adequate timeline for the process, but many theories do exist. Howell et al. (1993) provided evidence that supports total regeneration of muscle strength following an initial bout of exercise-induced damage that lasted up to 12 weeks. At the end of day 10, muscle had only recovered approximately 70%, and measurements performed by the researchers during weeks five and six post-exercise suggested that the half-life for muscle strength recovery could be up to five weeks. Others, including Chleboun et al. (1998) reported results that indicated a gradual return of muscular strength 11 days after initial exercise. Clearly, with such a wide discrepancy as to the return to normal force generation
and protocols that range in duration, intensity, and muscles worked, more research is needed to continue to discover the underlying cause and timeline of muscle tissue recovery.

One suggested adaptation is a shift in the length-tension relation, which provides a new optimal angle of contraction. Chen et al. (2007) showed that a significant shift in the optimal angle was evident two to three weeks after the initial maximal eccentric exercise bout in the elbow flexors, with the suggested explanation that the exercise stimulus used induced greater muscle damage when compared to others who show a smaller duration for the change in optimal angle. Brockett et al. (2001) went even further and offered a hypothesis that suggests there are two shifts in length-tension relation. The first shift is a direct result of disruption of sarcomeres in muscle fibers and was evident immediately after exercise but only lasted for a day or two. The second shift involves the development of a more sustained shift in length-tension relation ($7.7^\circ \pm 2.1^\circ$ shift) ($P<0.01$), which represented a training effect in the muscle.

**Repeated Bout Effect**

Significant amounts of literature exist that suggest a repeated bout effect is a part of the adaptation of muscle tissue following an unaccustomed bout of exercise. There is confusion, however, as to the length of the repeated bout effect or “protective effect,” which some studies have shown to be dependent upon the intensity of the original bout of exercise. Byrnes et al. (1985) examined repeated bouts of downhill running in different time increments, including three, six, and nine weeks apart with the same exercise protocol. The researchers found significantly lower levels of CK, myoglobin, and perceived muscle soreness between bouts performed three and six weeks apart but approached baseline levels
after nine weeks. Also, they discovered that patterns of response were similar between bout 1 and 2, suggesting that the mechanisms responsible for soreness and enzyme release are the same but that differences exist in the magnitude brought on by the first bout. Additionally, Balnave and Thompson (1993) had subjects perform downhill walking once a week for eight weeks with a 25% grade at 6.4km/hr and found that muscle soreness and indicators of muscle damage saw significant increases during the first week with subsequent decreases each week thereafter.

Looking at maximal eccentric contractions, Clarkson and Tremblay (1988) compared 70 and 24 maximum eccentric contractions in different arms of the same subjects and provided results suggesting that an adaptation takes place such that the muscle can lower more weight during repeated exercise. The results also showed that muscle is more resilient to exercise-induced muscle damage through the significantly lower indicators of damage, and any damage that does occur can be repaired at a faster rate due to a suggested strengthening of the surrounding connective tissue or membrane hypothesis. Newham et al. (1987) and Brockett et al. (2001) found similar results but offered a hypothesis that force loss was a result of changed contractile elements, which lends support to a possible removal of stress-susceptible fibers as the protective effect. Brown et al. (1997) looked at differing numbers of repetitions (10, 30, or 50) followed by 50 three weeks later and found increases in muscle soreness, force, and CK in the 30 and 50 repetition groups, followed by less or no increase after the second bout of 50 eccentric exercises. This showed that skeletal muscle changes can be elicited by relatively few contractions, but increasing repetitions did not result in an increased prophylactic effect.
There is also evidence that the intensity of the exercise or length of muscle during contraction plays a role in the magnitude of the repeated bout effect. Chen et al. (2007) provided evidence that the larger the intensity of eccentric bout 1, the greater the magnitude of the protective effect and even showed protective effect at 40% intensity. Nosaka and Clarkson (1995) looked at repeated bout effect of short and long eccentric contractions and showed that the magnitude of eccentric exercise-induced muscle damage is dependent on the muscle length, such that eccentric exercise at long lengths induces greater muscle damage than at short lengths.

**Nutritional Supplements**

*Carbohydrates*

Studies have shown that the effect of altered carbohydrate levels on exercise-induced muscle damage has yet to be established. Close et al. (2005) discovered that, despite altered carbohydrate levels for two days prior to eccentric exercise, there was no effect on the loss or recovery of muscle function, delayed-onset muscle soreness, or the production of reactive oxygen species. The study was performed under the assumption that the previously mentioned effects of eccentric exercise were possibly caused by invading phagocytes into muscle tissue, which depends on plasma glucose levels.

*Protein*

In a study that examined the effect of dietary protein levels on enzyme activities after eccentric exercise in mice, researchers discovered that a high protein diet (50% of calories) resulted in higher levels of CK and AST when compared to a normal or low protein diet, 12% and 4% calories from protein respectively. Protein content in this diet was primarily
from casein protein. The researchers suggested the additional amounts of protein in a high protein diet were a primary cause of the increased amounts of enzyme expression after exercise due to the highly controlled diet and exact exercise protocol between subjects. There is some confusion as to the mechanism behind the additional enzyme expression; some research points towards an increase in the enzyme synthesis or an increase in the absolute amounts of enzyme mRNA levels, but generally research shows that the effects of dietary protein must be considered in the context of exercise-induced muscle injury.

*Combined CHO and Protein*

The combined effects of carbohydrates and proteins in a supplement have been shown to attenuate exercise-induced muscle damage more than carbohydrates and proteins individually. Baty et al. (2007) showed that a combined CHO-protein supplement appeared to reduce muscle damage via reductions in myoglobin and CK when compared to placebo, and Bird et al. (2006) demonstrated a reduction in cortisol and 3-methyl-histidine levels, which suppressed protein degradation through a supplementation of liquid carbohydrates and essential amino acids. Additionally, Cockburn et al. (2008) indicated that milk and milk-based protein-carbohydrate supplementation resulted in a smaller influx of plasma CK and myoglobin.

*Cherry Juice*

Research has looked at many other food and plant extracts in reducing the muscle damage and inflammatory response after eccentric exercise. Connolly et al. (2006) evaluated the effectiveness of a fresh tart cherry juice in reducing the effects of eccentrically induced muscle damage. Tart cherries contain flavonoids and anthocyanins that have high anti-
oxidant and anti-inflammatory properties that inhibit the effects of cyclo-oxygenase produce biological mediators that cause inflammation and pain (Wang et al., 1999). Subjects in Connolly’s study reported significantly less strength loss when supplemented with tart cherry juice compared to placebo, four percent compared to twenty-two percent, respectively.

**Allicin**

Allicin, a compound that results from crushing garlic, has been supplemented to reduce muscle damage that results from eccentric exercise. Amagase et al. (2001) suggested that, depending on its ingested form, garlic can have immune-enhancing or anti-oxidative capacities that typically result from many different biologically active compounds. It is suggested that allicin is the compound most responsible for garlic’s biological activity and is typically the most researched garlic compound. Su et al. (2008) suggested that the anti-inflammatory and anti-oxidative capacities of allicin would decrease the inflammatory response and muscle damage after eccentric exercise. Subjects were required to run downhill on a treadmill to elicit the muscle damage, to which allicin supplemented subjects had a diminished release of CK, LDH, IL-6 compared to placebo subjects. Additionally, subjects in the allicin group reported a decrease in soreness after the exercise bout.

**Honokiol**

Other research has utilized honokiol, a bioactive component of the Chinese herb Magnolia officinalis, to reduce the effects of unaccustomed eccentric exercise (Chiang et al., 2009). Honokiol, known for its potential anti-inflammatory and anti-oxidant properties, was supplemented in the form of a 1mg dose for 5 days prior to exercise or one 5mg dose one hour before exercise. Researchers discovered that honokiol supplemented mice showed significantly less muscle damage compared to the placebo group. Chiang suggested that
honokiol suppressed both the COX-2 enzyme expression and pro-inflammatory cytokines, both of which decrease the inflammatory response.

Curcumin

Curcumin is a constituent of turmeric and has been evaluated by Davis et al. (2007) for its effect at reducing exercise induced muscle damage. Previous research has indicated turmeric has anti-inflammatory properties that reduce the activation of COX-2 and remove free radicals (Huang et al., 1991, Kang et al., 2004). Davis and others provided results of a decreased CK and inflammatory cytokine concentrations (IL-6, TNF-alpha, IL-beta) in rats supplemented with curcumin compared to placebo.

Supplementation

The effect of anti-inflammatory drugs on exercise-induced muscle injury has been researched with mixed findings. When the inflammation response is triggered, as it is via exercise-induced muscle damage, prostaglandins and prostacyclin are both generated in the arachidonic acid pathway via the cyclo-oxygenase (COX) enzyme. The production of prostaglandins, or PGE2, sensitizes the type III and IV pain afferents that are now responsive to chemicals like bradykinins and result in the muscle soreness. The basis for why anti-inflammatory drugs work follows.

Aspirin

In a study conducted by Francis and Hoobler (1987), the effect of aspirin was examined following extensive muscle-damage induced by eccentric exercise. Along with another study by Ferreira et al. (1973), this study provided evidence that aspirin does not
eliminate hyperalgesia but rather nullifies the potentiating effect produced by prostaglandins via inhibiting prostaglandin synthesis. Also, these studies provided evidence that aspirin does not improve muscular function, which shows that muscle injury occurred with and without aspirin.

**NSAIDs**

There are two distinct inconsistencies among studies that evaluate the effectiveness of NSAIDs at reducing the effects of unaccustomed, eccentric exercise on muscle tissue: timing of drug administration and the dose of drug administration. Ibuprofen is a well-known anti-inflammatory and has been studied for its effects in reducing exercise-induced muscle damage and soreness. Hasson et al. (1993) evaluated the effect of prophylactic versus therapeutic administration of ibuprofen on muscle soreness, damage, and performance through equal 400 mg doses at four hours before exercise or 24 hours after baseline. Results indicated that the prophylactic group reported 40-50% less soreness in addition to demonstrating a smaller decline in maximal muscle torque generated at 24 hours post-exercise but did not reduce CK levels. Tokmakidis et al. (2003) did observe that six 400 mg doses of ibuprofen during the 48 hours post-exercise resulted in a significant decrease in CK levels when compared to the placebo group, whereas Rahnama et al. (2005) observed no CK or pain decrease with a similar dosing regimen. Tokmakidis et al. (2003), however, generated a very small CK response (~200IU) from the exercise. Gulick et al. (1996) used a much larger dose (1800 mg) of ibuprofen as the load dose and followed with 1200 mg oxaprozin after the exercise-induced damage with no decrease in DOMS symptoms.
Other NSAIDS have been studied, including ketoprofen and diclofenac sodium. Sayers et al. (2001) performed a study with the use of ketoprofen, a NSAID like ibuprofen but with additional pathways of action. In this study, subjects experienced adequate muscle damage and were instructed to take a therapeutic dose of either 0, 25, or 100mg 36 hours after the exercise bout and continue taking the same dose each hour for eight hours total. Results showed that subjects who ingested ketoprofen showed a significantly lower perception of soreness and an increased ability to generate muscle force when compared to the placebo group. Further analysis showed that maximal EMG activity did not change with supplementation and provided evidence that the increased force capacity is from a reduced inflammatory response and not enhanced motor unit activation. Where NSAIDs have been shown to inhibit primarily the cyclo-oxygenase (COX) pathway, ketoprofen also inhibits the lipoxygenase (LIPOX) pathways and had activity at both the spinal cord and peripheral sites of injury (Herrero et al., 1997). These authors suggest that because of the dual action on both COX and LIPOX pathways, ketoprofen has displayed a more potent response in decreasing muscle damage.

O’Grady et al. (2000) looked at the systemic administration of diclofenac sodium on indices of muscle damage during a 27-day trial with an unaccustomed stepping exercise program on day 15. Looking at CK as an indicator for muscle damage, the researchers discovered that post-exercise muscle samples exhibited less muscle tissue damage and suppression of subsequent inflammation in the treatment group than the control group. Also using muscle histological evidence for support of diclofenac sodium, researchers found only one abnormal muscle sample in the treatment group, compared to 11 in the placebo group. Abnormal and normal were defined as presence of edema, inflammation, or necrotic fibers.
**Calcium-Channel Blockers**

The exact mechanism for why intracellular levels of calcium are elevated within muscle cells following a contraction-induced injury is uncertain, but some proposed hypotheses have been studied. There could be a loss of sarcoplasmic reticulum membrane integrity, a possible rupture of the sarcolemmal membrane, opening of stretch-responsive channels, or alterations in triad and t-tubule orientation resulting in calcium entry via voltage-sensitive channels. Duan et al. (1990) showed that after downhill walking there was disruption of the sarcolemma that allows for increased intracellular calcium concentrations that could contribute to the progression of muscle damage. This study provided evidence that, through the administration of a calcium-channel blocker, there was a reduction in the severity of z-band streaming, indicating less muscle cell damage. Beaton et al. (2002) provided parallel results in that a calcium-channel blocker had no effect at reducing CK activity or slowing the inflammatory response but rather displayed a reduction in z-band streaming and desmin disruption, which provides evidence of less damage to sarcomeric proteins. These studies provided a basis of understanding that reducing the amount of intracellular calcium that results from unaccustomed exercise could have an effect on reducing the amount of cellular damage.
CHAPTER 3. METHODS

Thirty-two male volunteers (24 ± 5 years old, 178.9 ± 6.3 cm tall, 81.5 ± 15.5 kg, and a BMI of 25.4 ± 4.5) were recruited for this matched-pairs, double-blind study with subjects being matched on initial isometric strength levels. All were healthy, not currently participating in strength training nor having participated in strength training for the past six months. The subjects participated in physical activity, on average, 1-2 days a week for less than 30 minutes each session for less than 3 months and maintained an average (self-reported) fitness level. Subjects were not currently taking medications or dietary supplements known to effect exercise tolerance, immune function, antioxidant status, or inflammation, but a daily multivitamin that meets, but does not exceed, the RDA was permitted. Each subject was asked to participate in a resistance training session designed to produce moderate muscle soreness. During the four weeks preceding the trial, participants consumed a daily oral dose of either placebo or 500 mg/day of EpiCor taken with breakfast. After being adequately matched, subjects were randomly placed into a treatment group. The subjects were instructed to avoid exercise for 3 days prior to the resistance training session as well as the four days of data collection during the fifth week.

On the day of the resistance training session, the subjects reported to the laboratory at 8 o’clock in the morning where they completed the visual analogue scale (VAS) for soreness (Cook et al., 1997), the Feelings Scale psychological questionnaire, and an illness survey. Once the surveys were completed, arm circumference measurements were taken before the exercise protocol at a location half way between the top of the shoulder and the tip of the elbow in a 90-degree flexion position with the use of a tension tape-measure. Subjects then provided a 10mL blood and a 5mL urine sample prior to any exercise. Subjects performed a
3-repetition isometric maximal strength test prior to the exercise protocol at 110° of elbow flexion, beginning with the dominant hand. The exercise protocol consisted of performing 50 maximal eccentric contractions at 30° per second and moved through the range of motion of the elbow. Subjects reported to the laboratory at 24, 48, and 72 hours after completing the resistance training to repeat all surveys, 3RM strength tests, and blood and urine sampling. During the 72 hours of recovery, subjects were instructed to refrain from exercise and to continue to consume the supplement.

All blood measures were assessed pre-exercise and at all time-points post-exercise that included 4 time-points total. Clinical biochemical measures included ALT, AST, LDH, bilirubin, uric acid, urea, albumin, as well as complete blood count. Inflammatory measures assessed in our laboratory by ELISA or colorometric assay include IL-1 receptor antagonist, IL-6, IL-10, CRP, and CK.

Statistical analysis was performed using 2-way ANOVAs with repeated measures on time through the SigmaStat analysis software. Statistical significance was established using a significance level of α=0.05 and was examined for differences both between and within groups. For significant results a Holms-Sidak post-hoc analysis was conducted. Group summary data were presented as mean ± standard deviation. Researchers remained blind as to the actual treatment groups until the entire statistical analysis was completed.
CHAPTER 4. RESULTS

After the initial analysis, the data were condensed by removing outliers beyond ±2 standard deviations using the rationale that 95 percent of the data are contained within 2 standard deviations of the mean in a normal distribution. In addition to removing the outliers, two subjects were removed due to reported symptoms of illness and three subjects were removed as a result of reporting insignificant levels of muscle soreness after the exercise protocol for the 72 hours after exercise. VAS soreness data were averaged over the four days of data collection, and subjects with mean soreness ± 2 standard deviations were removed from the analysis. Following the removal of the extreme data, the 2-way repeated measures ANOVA was again performed.

Peak isometric strength results showed a significant time main effect (P<0.001) but no statistically significant difference existed between treatment groups (P=0.614) (Table 1).

Table 1. Means and standard deviations for peak isometric strength for each time point and treatment (N·m).

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<tbody>
<tr>
<td>Overall</td>
<td>68.1 ± 17.5</td>
<td>44.1 ± 14.5</td>
<td>42.9 ± 13.7</td>
<td>47.8 ± 13.1</td>
</tr>
<tr>
<td>EpiCor</td>
<td>70.1 ± 18.1</td>
<td>47.7 ± 15.1</td>
<td>41.2 ± 13.0</td>
<td>47.8 ± 12.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>66.0 ± 17.4</td>
<td>40.8 ± 13.5</td>
<td>44.7 ± 14.6</td>
<td>47.7 ± 14.5</td>
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</table>

Both groups experienced a similar decrease in peak strength values 24 hours post exercise, but the placebo group displayed a quicker, but not significant, start to recovery when compared to EpiCor, as this group experienced a continued decrease in strength values for 48 hours post exercise (Figure 1). Results at 24, 48, and 72 hours post-exercise all had lower peak strength data when compared to baseline (Table 1). The soreness variable resulted in no
significant difference between treatments (P=0.869) and a significant effect for time (P<0.001).

Figure 1. Average dominant arm peak isometric strength for each treatment group.

Figure 2 displays that the EpiCor group demonstrated a greater amount of self-reported soreness 24 hours post-exercise when compared to placebo. The EpiCor group, however, displayed a quicker decrease in soreness values when compared to the placebo group between 24 and 72 hours post-exercise (Table 2).
Table 2. Means and standard deviations for dominant arm soreness for each time point and treatment (cm).

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<tr>
<td>Overall</td>
<td>0.3 ±  0.4</td>
<td>6.1 ± 1.9</td>
<td>5.0 ± 1.8</td>
<td>3.4 ± 1.6</td>
</tr>
<tr>
<td>EpiCor</td>
<td>0.4 ±  0.4</td>
<td>6.4 ± 1.7</td>
<td>4.9 ± 2.3</td>
<td>3.2 ± 1.7</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.3 ±  0.4</td>
<td>5.8 ± 2.1</td>
<td>5.1 ± 1.4</td>
<td>3.5 ± 1.7</td>
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</table>

Circumference data displayed no significant difference between treatment groups, as both treatments experienced similar amounts of swelling (P=0.974) and a statistically significant time effect was present (P<0.001) (Table 3). Post-hoc analysis showed that all time-points were significantly higher than pre-exercise measurements (Figure 3).
Table 3. Means and standard deviations of arm circumference for each time point and treatment (cm).

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<tbody>
<tr>
<td>Overall</td>
<td>30.2 ± 2.7</td>
<td>30.8 ± 2.6</td>
<td>30.8 ± 2.8</td>
<td>30.9 ± 2.8</td>
</tr>
<tr>
<td>EpiCor</td>
<td>30.1 ± 1.7</td>
<td>30.8 ± 1.7</td>
<td>30.8 ± 1.9</td>
<td>31.1 ± 1.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>30.4 ± 3.4</td>
<td>30.8 ± 3.3</td>
<td>30.8 ± 3.5</td>
<td>30.7 ± 3.5</td>
</tr>
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</table>

Creatine kinase results displayed in Figure 4 showed no difference (P=0.738) between treatments and a consistent significant difference (P=0.002) between time points 48 and 72 hours when compared to baseline (Table 4). The results for LDH paralleled creatine kinase with no statistical difference (P=0.825) present between treatment groups as displayed in
Figure 5. There was a significant effect of time evident between 0 and 72 hours (P=0.003) (Table 5).

![Graph showing average plasma creatine kinase (CK) for each treatment group.]

Figure 4. Average plasma creatine kinase (CK) for each treatment group.

Table 4. Mean and standard deviation for creatine kinase (CK) for each time point and treatment (IU/mL).

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<tbody>
<tr>
<td>Overall</td>
<td>83 ± 39</td>
<td>170 ± 71</td>
<td>388 ± 503</td>
<td>869 ± 1568</td>
</tr>
<tr>
<td>EpiCor</td>
<td>90 ± 42</td>
<td>157 ± 65</td>
<td>321 ± 454</td>
<td>833 ± 1306</td>
</tr>
<tr>
<td>Placebo</td>
<td>75 ± 35</td>
<td>184 ± 77</td>
<td>451 ± 554</td>
<td>905 ± 1874</td>
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Table 5. Means and standard deviations for lactate dehydrogenase (LDH) for each time point and treatment (IU/mL).

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<tr>
<td>Overall</td>
<td>163 ± 19</td>
<td>174 ± 25</td>
<td>185 ± 32</td>
<td>195 ± 58</td>
</tr>
<tr>
<td>EpiCor</td>
<td>166 ± 18</td>
<td>176 ± 22</td>
<td>182 ± 31</td>
<td>193 ± 44</td>
</tr>
<tr>
<td>Placebo</td>
<td>161 ± 21</td>
<td>173 ± 28</td>
<td>189 ± 34</td>
<td>198 ± 72</td>
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</table>

The inflammatory marker IL-1(ra) (Figure 6) showed no significant changes between treatment groups or over time, (P=0.783 and 0.526, respectively), with means and standard deviations found in Table 6. IL-6 levels did not significantly change between treatments (P=0.570) or over time (P=0.694), but the IL-6 results showed a distinct trend after 24 hours post-exercise: placebo showed a continual decrease while EpiCor showed a substantial increase by 72 hours post-exercise (Table 7 and Figure 7).
Figure 6. Average IL-1ra concentrations for each treatment group.

Table 6. Means and standard deviations for IL-1ra for each time point and treatment (mg/dL).

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<tr>
<td>Overall</td>
<td>0.89 ± 0.34</td>
<td>0.87 ± 0.24</td>
<td>0.82 ± 0.22</td>
<td>0.86 ± 0.25</td>
</tr>
<tr>
<td>EpiCor</td>
<td>0.86 ± 0.32</td>
<td>0.87 ± 0.24</td>
<td>0.84 ± 0.23</td>
<td>0.86 ± 0.30</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.82 ± 0.29</td>
<td>0.86 ± 0.25</td>
<td>0.79 ± 0.20</td>
<td>0.85 ± 0.16</td>
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Table 7. Means and standard deviations for IL-6 for each time point and treatment (mg/dL).

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<tr>
<td>Overall</td>
<td>0.98 ± 0.74</td>
<td>0.86 ± 0.61</td>
<td>0.97 ± 0.67</td>
<td>1.04 ± 0.83</td>
</tr>
<tr>
<td>EpiCor</td>
<td>0.92 ± 0.70</td>
<td>0.85 ± 0.53</td>
<td>1.08 ± 0.58</td>
<td>1.22 ± 0.92</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.05 ± 0.81</td>
<td>0.89 ± 0.72</td>
<td>0.83 ± 0.78</td>
<td>0.67 ± 0.30</td>
</tr>
</tbody>
</table>
The anti-inflammatory cytokine IL-10 displayed in Figure 8 provides evidence that no significant differences exist between treatments (P=0.285) or between time points (P=0.284) (Table 8). IL-10 does show a consistent decrease in the EpiCor group values and an increase in placebo values through 48 hours post-exercise. These trends, however, are not significant. CRP results exhibited similar levels of non-significance for time (P=0.801) and between groups (P=0.814) (Table 9) and can be viewed in Figure 9.

<table>
<thead>
<tr>
<th>Table 8. Means and standard deviations for IL-10 for each time point and treatment (pg/mL).</th>
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<tr>
<td></td>
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<tr>
<td>Overall</td>
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<tr>
<td>EpiCor</td>
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<tr>
<td>Placebo</td>
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Table 9. Means and standard deviations for C-reactive protein (CRP) for each time point and treatment (ng/mL).

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<tr>
<td>Overall</td>
<td>10.40 ± 10.29</td>
<td>12.18 ± 11.25</td>
<td>12.78 ± 13.62</td>
<td>12.41 ± 12.69</td>
</tr>
<tr>
<td>EpiCor</td>
<td>10.04 ± 8.21</td>
<td>13.03 ± 11.16</td>
<td>14.08 ± 12.72</td>
<td>14.96 ± 13.67</td>
</tr>
</tbody>
</table>

Figure 8. Average IL-10 concentrations for each treatment group.
Figure 9. Average CRP concentration for each treatment group
CHAPTER 5. DISCUSSION

The results of the study show that no statistically significant differences were present between EpiCor and placebo treatments for any dependent variable. The final statistical analysis resulted in the removal of two subjects who experienced symptoms of illness (fever, cough, sore throat, etc.) and three subjects who did not report significant soreness during the 72 hours post-exercise. Therefore, a sample size of 27, 14 subjects in the EpiCor group and 13 subjects in the placebo group, was used in the analysis.

Data from the current study are consistent with previous research and indicate that muscle damage did occur from the specific eccentric exercise protocol of the elbow flexors. Isometric strength values showed a maximum decrease of 41.3 and 38.1% for the EpiCor and placebo groups, respectively, which agrees with Jones et al., 1987, Howell et al., 1993, and Gibala et al., 1995. Gibala and colleagues provided muscle biopsy evidence to support their findings that up to 80% fiber disruption was still present after 48 hours of recovery. While we did not perform muscle biopsies, it could be suggested that similar damage occurred from a more taxing exercise protocol (64 repetitions at 80% of one-repetition max in Gibala’s study compared to 50 repetitions at maximum intensity in the present study) in addition to continued decreases in strength at 48 hours post-exercise. Peak isometric strength values were still diminished between 27.7 and 31.8% below baseline after 72 hours, indicating that EpiCor did not aid in the recovery of muscle strength (Figure 1, Table 10). Howell et al. (1993) still reported a 30% decrease in strength 10 days post-eccentric exercise of the elbow flexors which confirms the findings of the present study.

Another indicator of muscle damage in the present study is muscle soreness, which had a significant effect of time (P<0.001) with significantly different levels of soreness at
each time point. Figure 2 displays that peak soreness occurred at 24 hours post-exercise and never returned to baseline. Armstrong (1991) and Clarkson et al. (1992) both provided results that peak soreness occurs between 48-72 hours post-exercise, which could suggest that EpiCor elicits a faster recovery in muscle soreness after exercise; however, there were no differences between treatments (P<0.87), which provides evidence that EpiCor did not significantly reduce muscle soreness.

Other indicators of muscle damage, including CK and LDH, both have a significant effect of time, and their respective figures (4, 5) show that subjects still had elevated levels of both markers after 72 hours of recovery. These continual levels, although not significantly different, do demonstrate both that a significant amount of muscle damage did occur and the EpiCor supplementation did not help to reduce the amount of muscle damage. Additionally, the evidence demonstrates that EpiCor did not enhance the rate or amount of recovery, as demonstrated by both the isometric strength and soreness levels.

Measures of inflammation showed no significant difference between placebo and EpiCor-supplemented groups, but some trends were present. CRP data displayed a near-linearly increasing trend during the entire 72 hour time period post-exercise while the placebo group demonstrated a steady decline in CRP levels after 24 hours post-exercise (Figure 9). A near-significant treatment versus time effect was present (P=0.088), and post-hoc analysis revealed a similar significance level between 0 and 72 hours post-exercise. Generally, research indicates that CRP levels after eccentric exercise are still mixed as neither Milias et al. (2005) nor Nosaka & Clarkson (1996) reported significant increases in CRP while Malm et al. (2000) and Paulsen et al. (2005) demonstrated an increase in CRP levels after eccentric exercise. The first group of researchers utilized elbow flexors for 24-36
maximal eccentric repetitions whereas the second group of researchers utilized leg extensors for 30 minutes of eccentric cycling and 300 maximal eccentric contractions. These brief descriptions highlight an aerobic component in the latter studies and suggest that CRP release is related to muscle damage elicited by eccentric endurance exercise. Additionally, the difference in muscle mass or duration of maximal eccentric exercise could have an impact as damaging a greater amount of muscle fibers for a greater amount of time could lead to a greater CRP release.

The response of IL-10 also showed a trend (P=0.109) throughout the 72-hour recovery time period as subjects with the EpiCor supplement displayed lower levels of IL-10 when compared to subjects with the placebo (Figure 8). The control group saw an increase during the first 48 hours post-exercise whereas the treatment group saw a decrease during the first 48 hours post exercise. This result, although not significant, is similar to results as shown by Smith et al. (2000) and Silva et al. (2008). Silva displayed results for an increased IL-10 response in all subjects 96 hours post eccentric exercise of the elbow flexors while Smith et al. showed significantly elevated IL-10 levels 72-144 hours post-exercise using hamstrings and pectoralis muscle groups. Both Smith and Silva utilized a greater amount of muscle mass than the present study, which could result in increased inflammatory responses from a greater amount of muscle damage elicited and subsequent increased anti-inflammatory response. With the hypothesized anti-inflammatory effects of EpiCor (Jensen et al., 2007, 2008), the treatment group should have continually elevated IL-10 levels that indicate an increase in the anti-inflammatory response that would combat and lower the pro-inflammatory response. Previous unpublished research with the EpiCor supplement provided results that, immediately post-exercise, IL-10 levels increased and returned to normal levels
at 24 hours post-exercise, which suggests a greater anti-inflammatory response mediated by the EpiCor supplement. This study utilized intense cycling for two five-day trial periods with a two-day rest period between weeks. Each trial period included both aerobic and anaerobic cycling for >60 minutes each day that ended with a 90-minute cycling endurance exercise. Comparing the protocols between the studies demonstrates that the endurance exercise elicited a greater anti-inflammatory response compared to the muscular strength exercise.

Results for IL-1ra showed that no statistically significant differences existed between the EpiCor and placebo groups, which do not support EpiCor as having anti-inflammatory capabilities. IL-1ra has been shown as having anti-inflammatory capabilities with pro-inflammatory release patterns as this cytokine is released in conjunction with IL-1, a pro-inflammatory cytokine, to keep the inflammation under control. Nosaka and Clarkson (1996) provided evidence of no increase in IL-1ra levels after eccentric exercise in the elbow flexors, but previous EpiCor research as described above in addition to Ostrowski et al. (1999) demonstrated a significant elevation in IL-1ra post-exercise, 24 hours post-, and immediately post-exercise, respectively. A distinct difference in exercise protocol exists among these studies that show contradicting findings, as the latter two studies utilized intense endurance exercise in the form of a 2-week cycling period and runners in the 1997 Copenhagen Marathon.

The inflammatory measure of IL-6 resulted in no significant differences between the EpiCor and placebo groups during the post-exercise time period. Previous research shows that IL-6 is a regulatory cytokine with both anti- and pro-inflammatory characteristics (Bruunsgaard et al., 1997). Substantial evidence has been provided that IL-6 is a sensitive
marker of inflammation and is one of the first cytokines released after damage occurs. For example, MacIntyre et al. (2001), Willoughby et al. (2003), and Paulsen et al. (2005) showed that a peak saturation of IL-6 occurred approximately six hours after exercise and that early increases in IL-6 predicted a component of delayed onset muscle soreness. The present study did show a significant time effect of delayed onset muscle soreness (Figure 7) but no significant change in IL-6. Although IL-6 had no measurable change, it cannot be ruled out that changes may have occurred since no blood sample was taken before 24 hours.

Limitations did exist to the present study and include the timing of blood measuring, physical conditioning of subjects, and exercise type. Many researchers have indicated that statistically significant changes occur prior to 24 hours post-eccentric exercise (MacIntyre et al, 2001, Willoughby et al., 2003, Tomiya et al., 2004). Adding another time-point prior to 24 hours post-exercise or measuring closer than 24 hours could provide the additional data needed to demonstrate significance for our results. Another limitation of the study was that some subjects had maintained a constant exercise routine prior to the study that could have enhanced the internal recovery process after exercise-induced damage. Untrained individuals are more likely to experience more muscle damage and a greater inflammatory response than individuals with some recent exercise training history. Had the subjects only been drawn from individuals with no previous exercise history in the past 6 months, instead of non-weightlifting experience, there may have been more exercise-induced muscle damage and, therefore, greater stimulus for an inflammatory response. Finally, there were some subjects who self-reported very little soreness post-exercise. If the exercise protocol were enhanced with more repetitions or included more muscle mass through other eccentric movements to help create soreness, the results of the study could be significant.
Overall, the results of this study show that no significant differences were present between the EpiCor and placebo treatment groups in reducing muscle damage and alleviating muscle soreness. A few trends were present that suggests differences may exist between treatment groups for inflammatory markers; however, these results continue to warrant future research with yeast-metabolites in the aid of reducing muscle damage and soreness after unaccustomed eccentric exercise. A suggestion for future research includes using subjects with no recent exercise history. This present study used subjects with no recent weight-lifting history (>6 months), and any adaptations from consistent aerobic exercise could aid in the clearance of inflammatory or muscle damage markers faster than normal.
REFERENCES


APPENDIX A

Original Data
Table 10. Means and standard deviations of isometric strength for each time point and treatment (Nm).

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>68.0 ± 17.6</td>
<td>43.1 ± 14.2</td>
<td>43.8 ± 14.0</td>
<td>47.5 ± 14.0</td>
</tr>
<tr>
<td>EpiCor</td>
<td>68.8 ± 17.3</td>
<td>45.6 ± 15.4</td>
<td>42.8 ± 14.8</td>
<td>46.7 ± 11.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>67.1 ± 18.4</td>
<td>40.7 ± 13.0</td>
<td>44.7 ± 13.5</td>
<td>48.4 ± 16.2</td>
</tr>
</tbody>
</table>

Table 11. Means and standard deviations of soreness for each time point and treatment (cm)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.41 ± 0.54</td>
<td>5.55 ± 2.26</td>
<td>4.80 ± 2.47</td>
<td>3.29 ± 2.25</td>
</tr>
<tr>
<td>EpiCor</td>
<td>0.53 ± 0.61</td>
<td>5.64 ± 2.33</td>
<td>4.62 ± 2.75</td>
<td>3.21 ± 2.45</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.28 ± 0.45</td>
<td>5.46 ± 2.27</td>
<td>4.99 ± 2.23</td>
<td>3.38 ± 2.10</td>
</tr>
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</table>

Table 12. Means and standard deviations of circumference for each time point and treatment (cm)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>30.4 ± 3.8</td>
<td>31.0 ± 3.7</td>
<td>30.9 ± 3.9</td>
<td>31.0 ± 3.8</td>
</tr>
<tr>
<td>EpiCor</td>
<td>30.5 ± 3.9</td>
<td>31.1 ± 3.8</td>
<td>31.2 ± 4.1</td>
<td>31.3 ± 3.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>30.4 ± 3.9</td>
<td>30.8 ± 3.7</td>
<td>30.7 ± 3.9</td>
<td>30.7 ± 3.9</td>
</tr>
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</table>

Table 13. Mean and standard deviations of CK for each time point for each treatment group (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>96 ± 72</td>
<td>184 ± 127</td>
<td>523 ± 861</td>
<td>1310 ± 3472</td>
</tr>
<tr>
<td>EpiCor</td>
<td>102 ± 64</td>
<td>178 ± 133</td>
<td>655 ± 1109</td>
<td>1855 ± 4624</td>
</tr>
<tr>
<td>Placebo</td>
<td>91 ± 82</td>
<td>189 ± 125</td>
<td>388 ± 514</td>
<td>765 ± 1704</td>
</tr>
</tbody>
</table>

Table 14. Means and standard deviations of LDH for each time point for each treatment group (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>165 ± 21</td>
<td>175 ± 28</td>
<td>192 ± 46</td>
<td>209 ± 115</td>
</tr>
<tr>
<td>EpiCor</td>
<td>167 ± 20</td>
<td>173 ± 23</td>
<td>186 ± 42</td>
<td>224 ± 150</td>
</tr>
<tr>
<td>Placebo</td>
<td>163 ± 22</td>
<td>177 ± 33</td>
<td>198 ± 49</td>
<td>194 ± 65</td>
</tr>
</tbody>
</table>

Table 15. Means and standard deviations of IL-1ra for each treatment and time point (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
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<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.94 ± 0.44</td>
<td>1.07 ± 0.66</td>
<td>1.06 ± 0.85</td>
<td>1.03 ± 0.83</td>
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<tr>
<td>EpiCor</td>
<td>0.95 ± 0.40</td>
<td>1.05 ± 0.61</td>
<td>0.90 ± 0.28</td>
<td>0.89 ± 0.31</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.93 ± 0.49</td>
<td>1.09 ± 0.73</td>
<td>1.22 ± 1.17</td>
<td>1.16 ± 1.13</td>
</tr>
</tbody>
</table>

Table 16. Means and standard deviations of IL-6 for each treatment and time point (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1.64 ± 2.13</td>
<td>2.37 ± 4.56</td>
<td>1.79 ± 2.78</td>
<td>1.80 ± 2.19</td>
</tr>
<tr>
<td></td>
<td>EpiCor</td>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
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<td>--------------</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.00 ± 0.70</td>
<td>2.19 ± 5.06</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1.08 ± 0.55</td>
<td>2.51 ± 3.82</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1.22 ± 0.92</td>
<td>2.38 ± 2.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr</td>
<td>2.27 ± 2.84</td>
<td>2.55 ± 4.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>2.51 ± 3.82</td>
<td>2.51 ± 3.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48hr</td>
<td>1.08 ± 0.55</td>
<td>2.38 ± 2.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>1.22 ± 0.92</td>
<td>2.38 ± 2.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hr</td>
<td>1.00 ± 0.70</td>
<td>2.19 ± 5.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>1.08 ± 0.55</td>
<td>2.51 ± 3.82</td>
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</tbody>
</table>

Table 17. Means and standard deviations of IL-10 for each treatment and time point (pg/mL).

<table>
<thead>
<tr>
<th></th>
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<th>24hr post</th>
<th>48hr post</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3.02 ± 4.77</td>
<td>3.15 ± 4.87</td>
<td>2.93 ± 4.84</td>
<td>2.57 ± 4.69</td>
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<tr>
<td>EpiCor</td>
<td>1.96 ± 0.99</td>
<td>2.02 ± 1.09</td>
<td>1.59 ± 0.85</td>
<td>1.56 ± 1.26</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.08 ± 6.61</td>
<td>4.28 ± 6.72</td>
<td>4.26 ± 6.63</td>
<td>6.45 ± 3.57</td>
</tr>
</tbody>
</table>

Table 18. Means and standard deviations of CRP for each treatment and time point (ng/mL).

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>24hr post</th>
<th>48hr post</th>
<th>72hr post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>12.76 ± 16.64</td>
<td>15.32 ± 16.59</td>
<td>16.73 ± 20.69</td>
<td>16.35 ± 20.10</td>
</tr>
<tr>
<td>EpiCor</td>
<td>10.04 ± 8.21</td>
<td>13.03 ± 11.16</td>
<td>14.08 ± 12.72</td>
<td>14.96 ± 13.67</td>
</tr>
<tr>
<td>Placebo</td>
<td>15.43 ± 22.12</td>
<td>17.61 ± 20.81</td>
<td>19.38 ± 26.61</td>
<td>17.74 ± 25.38</td>
</tr>
</tbody>
</table>
APPENDIX B

Informed Consent Form
INFORMED CONSENT DOCUMENT

Title of Study: Effectiveness of EpiCor in improving immune function, inflammation, and performance after intense exercise.

Investigators:  
Rick L. Sharp, PhD  
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Email: risharp@iastate.edu

Marian L. Kohut, PhD  
Department of Kinesiology  
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Iowa State University  
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Ph: 515-294-8364  
Email: mnkohut@iastate.edu

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

Previous research has examined ways unaccustomed exercise, particularly resistance training, leads to delayed onset muscle soreness (DOMS) that usually peaks at 48-72 hr after the exercise session. There is considerable evidence that such exercise causes damage to the muscle tissue, producing local inflammation as the root cause of the soreness. Oxidative stress also accompanies muscle-damaging exercise, and it is thought that oxidative stress contributes to the inflammatory response. Coupled with the soreness, there is generally reduced range of motion and impaired strength for several days after the exercise. For these reasons, dietary supplements which minimize inflammation and enhance anti-oxidant defenses may attenuate the soreness and preserve performance capacity in both untrained and trained individuals engaged in physical training. The purpose of this proposal is to evaluate the effectiveness of EpiCor in modifying the inflammatory response, oxidative stress response, and muscle performance responses after heavy resistance exercise that leads to muscle soreness.

DESCRIPTION OF PROCEDURES

Potential participants will contact a study representative to arrange a time to be given informed consent. The subjects will participate in one trial lasting 5 weeks. After the informed consent is documented, subjects will be screened as potential participants prior to enrollment in the study. The screening process will include the following: height, weight, vital signs (blood pressure and heart rate), and a Medical History Questionnaire. If deemed safe after the initial screening (following American College of Sports Medicine guidelines), subjects will then participate in a short training session on the Biodex exercise machine used in this study. This training session will allow them to understand what the machine feels like and prepare them for the actual experiment. Once these tests are completed and subjects meet the entrance criteria, subjects will be asked to participate in the study. Specific dates and times will be arranged for trial visits.

Subjects’ participation will begin with preliminary testing and will include the following: upper arm circumference (both arms), muscle soreness survey, feeling scale survey, and one maximal
repetition of isometric contraction on both arms. After the preliminary testing, a four week dosing period will follow (EpiCor or placebo) during which the participant will resume their normal daily activities. At the end of the 4 week dosing period, participants will return to the laboratory to repeat the testing for isometric elbow flexion strength, muscle soreness, feeling scale. In addition, one blood sample will be obtained from a forearm vein for later analysis of inflammatory markers, oxidative stress response, cortisol as a stress marker, and a standard clinical chemical screen / complete blood count. A clean urine sample will also be obtained. This testing will be followed by an eccentric exercise testing session which is shown in prior research to induce delayed onset (24-72 hr later) muscle soreness and systemic inflammation. The eccentric exercise session will consist of 50 maximal effort contractions of elbow flexors attempting to resist the Biodex lever arm as it moves the elbow joint from full flexion to full extension (similar to letting a heavy weight down slowly). Each contraction lasts about 2 seconds and 12 seconds are allowed between contractions. This protocol will be performed on the right arm followed by the left arm. The soreness, feeling scale, isometric strength, arm circumference, urine and blood tests will then be repeated at 24 hr, 48 hr, and 72 hr after performing the eccentric exercise protocol.

During the 4 week dosing period, participants will consume a daily dose of 500 mg/day of either placebo or EpiCor taken with breakfast. The treatments will be randomized among participants so that half of the participants will receive EpiCor and half will receive placebo in a double-blind fashion. Pill-counts will be monitored to ensure compliance. Supplements will be provided by the study sponsor and labeled with a non-identifying code.

The maximal one repetition testing will involve an isometric contraction of the elbow flexors at 110 degrees of flexion, performed 3 times for 5 seconds. Both the one repetition maximal isometric contraction and 50 maximal eccentric contractions will be performed on a BIODEX exercise machine.

To minimize the risks of hematoma (bruising), local discomfort, and infection associated with venipuncture, universal precautions will be used by a trained professional.

Urine samples will be analyzed for creatinine, cortisol and iso-prostanes (a urinary marker of inflammation).

TIME TABLE & POINT OF DATA COLLECTION:

Baseline Testing
- Measurements of upper arm circumference and one repetition maximum for isometric elbow flexion will be taken.
- Psychological surveys of Muscle Soreness and Feeling scale (FS)
- Medical history questionnaire.

Dosing period:
- Four weeks in which participants will consume a daily dose of a nutritional supplement (EpiCor) or placebo at breakfast. Participants will maintain their usual pattern of exercise and daily activities.
Day 1 of exercise testing:
- Survey measures of muscle soreness/feeling scale; arm circumferences, blood draw, urine sample, one repetition isometric maximal test on each arm, and 50 maximal eccentric contractions on each arm.

Day 2: Survey measures of muscle soreness/feeling scale, arm circumferences, blood draw, urine sample, and one repetition isometric maximal test on each arm.

Day 3: Survey measures of muscle soreness/feeling scale, arm circumferences, blood draw, urine sample, and one repetition isometric maximal test on each arm.

Day 4: Survey measures of muscle soreness/feeling scale, arm circumferences, blood draw, urine sample, and one repetition isometric maximal test on each arm.

On each of the four days that require blood sampling, approximately 10 milliliters (mL) of blood will be taken (equivalent to about 2 teaspoons). Over the entire study, blood sampling will therefore total about 40 mL which is less than one tenth the amount normally taken during blood donation.

RISKS

While participating in this study you may experience the following risks:

Blood sampling from forearm veins using straight needle is associated with a risk of infection, hematoma (bruising), and transmission of communicable diseases. These risks are minimized by using sterile procedures, applying adequate pressure over the puncture site following withdrawal of the needle, and the use of disposable equipment including needles and sterile latex gloves.

The intensified exercise regimen may result in slight muscle soreness, localized to the muscles used in the exercise program. This soreness should not prevent subjects from performing normal daily activities. The exercise program may produce light headedness, dizziness, and in extremely rare instances, myocardial infarction. These risks will be minimized by selecting young healthy subjects with little risk for a cardiovascular incident, and by the careful supervision of all exercise sessions by knowledgeable and CPR-trained individuals.

The risk during the administration of the nutritional supplemental includes contamination of the supplement and/or by participants handling of glassware with dirt, bacteria, or blood is minimal. In order to prevent this possibility, we will properly sterilize all glassware/containers provided to participants and separate the supplements from contaminated materials. Also supplements use may contain yeast base products. Individuals allergic to yeast should not participate in this study. In the event that there is evidence of adverse side effects, the subject will be immediately informed and the trial will be halted. The subject will be immediately referred to the student health center for follow up treatment to ensure that the situation is reversed.
BENEFITS

Society may benefit from this research if we find that the dietary supplement alters the course of the inflammatory or oxidative stress process following intense weight training exercise. Another benefit is that the results are highly likely to provide a better understanding of how inflammatory response and oxidative stress damage are associated with muscle soreness symptoms and performance.

COSTS AND COMPENSATION

You will not have any costs from participating in this study. You will be compensated for participating in this study. Compensation for completion of the nutritional supplementation for the full month is $20. Compensation for completing the exercise protocol, blood/urine sampling, and surveys on day 1 is $20. Compensation for completing the blood/urine sampling and surveys on days 2, 3, and 4 are $20 per day. Therefore, the total compensation for completion of the study is $100. If the total study is not completed, the subject will be paid for the portion of the study that was completed on a pro-rated basis. For example if a subject only completes 2 weeks of nutritional supplementation, he will be paid $10.

For the process of receiving compensation you will be asked to provide your social security number (SNN) on a Research Participant Receipt Form. The purpose of this form are to serve as a documentation of the receipt of compensation associated with participation in a research study conducted by ISU personnel and to obtain information relating to IRS Form 1099 requirements. Federal and state law protects the privacy and security of your SNN and Iowa State University will not disclose your SNN without your consent for any other purposes except as allowed by law.

PARTICIPANT RIGHTS

Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

At any time during the study, you may withdraw your consent to participate without prejudice toward you. Such withdrawal can be for any reason you choose. Constant monitoring of all experiments will be performed by knowledgeable and CPR trained individuals in an attempt to prevent any complications. Emergency first aid supplies and equipment will be immediately available.

CONFIDENTIALITY

Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, federal government regulatory agencies, Food & Drug Administration (FDA), and the Institutional Review Board (a committee that reviews and approves human subject research studies) may inspect and/or copy
your records for quality assurance and data analysis. These records may contain private information.

To ensure confidentiality to the extent permitted by law, all data and samples will be coded numerically by subject and no names, initials, or other identifying characteristics will be reported in publication or presentation. Although your individual results will be reported to the study's sponsor (Embria, Inc.), your identity will not be disclosed to the sponsor. Hardcopies of all data will be kept in a locked file cabinet in the primary investigator's office. Computer files of data will be stored on a password protected computer in the primary investigator's office.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study. For further information about the study contact Dr. Rick Sharp (294-8650) or Dr. Marian Kohut (294-8364). If you have any questions about the rights of research subjects or research-related injury, please contact the IRB Administrator, (515) 294-4566, IRB@iastate.edu, or Director, Office of Research Assurances, (515) 294-3115, 1138 Pearson Hall, Ames, IA 50011.

******************************************************************************

SUBJECT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Subject's Name (printed) ____________________________________________

(Subject's Signature) ___________________ (Date) ___________________

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

(Signature of Person Obtaining Informed Consent) ___________________

(Date) ________________
APPENDIX C

Medical History Questionnaire
MEDICAL HISTORY QUESTIONNAIRE

Please respond to the following items as accurately as possible. This information will be used by the investigator to ensure a safe exercise environment and to determine if there are any contraindications to exercise or participation in this study. All information will remain confidential unless further professional consultation is warranted.

A) Personal Information

Name ___________________________ Date of Birth ____________ Tel.# _____________________
Height ______________ Weight ___________ Male or Female (circle)

B) Medical Information

1. How would you describe your recent general health?
   ______ Excellent ______ Good _______ Fair _______ Poor

2. Place an X in those boxes which describe symptoms or disorders which you have been diagnosed to have. If possible, also indicate the date of the diagnosis.
   ______ high blood pressure ______ arthritis ______ chest pain
   ______ irregular heart beat ______ epilepsy ______ heart attack
   ______ heart murmur ______ anemia ______ migraine
   ______ asthma ______ back trouble ______ headaches
   ______ hay fever or other allergies ______ dizziness/ ______ diabetes
   ______ other _______ _______ fainting spells

3. Describe any surgery that you have had within the last two years:


4. Have you ever sustained an injury or experienced any type of chronic pain which has been diagnosed as due to physical activity or sports participation?
   ______ Yes ______ No

   If yes, please describe __________________________________________
   How long ago?

5. Do you smoke cigarettes? ______ Yes ______ No

6. Are you presently taking any of the following medications or dietary supplements?
   ______ drugs to control blood pressure ______ drugs for asthma
   ______ drugs to regulate heart rate ______ drugs for diabetes
   ______ drugs for allergies ______ thyroid hormone
   ______ cortisone ______ prednisone

   Indicate the name(s) of those drugs or supplements __________________________________________________
   Also note the dosage and frequency of use ___________________________________________________________

7. What allergies do you have? __________________________________________

   Are you allergic to yeasts? ____________________________________________

8. How long has it been since your last physical examination?
   ______ less than 1 year _______ 1-2 years ______ 2-3 years
   ______ more than 3 years
9. Have any of the above symptoms, disorders or injuries limited your physical activity in the past? __________________________________________________________
   In what way? _______________________________________________________

C) Family Medical History

1. Have any of your blood relatives been diagnosed as having any of the following symptoms/disorders? (Include grandparent, parents, brothers, sisters)
   ___ heart attack, under age 50
   ___ stroke, under age 50
   ___ high blood pressure
   ___ hyperlipidemia (high cholesterol)
   ___ obesity
   ___ other __________________________________________________________

D) Exercise Information

1. List and give the date of any supervised exercise or sports program that you have participated in recently ____________________________________________

2. Are you currently participating in a regular program of physical activity?
   ___ Yes ___ No.
   If yes, how often do you exercise per week (on average)?
   ___ 1-2 days/wk  ___ 5-6 days/wk
   ___ 3-4 days/wk  ___ every day

   For how long do you exercise each day?
   ___ < 30 min/day  ___ 30-60 min/day
   ___ 60-90 min/day  ___ 90-120 min/day
   ___ > 120 min/day

   What types of activities are regularly included in your program?
   ___ jogging  ___ calisthenics
   ___ cycling  ___ swimming
   ___ weight lifting  ___ aerobic dance
   ___ recreational sports (basketball, racquetball, volleyball, tennis, etc)
   ___ other _________________________________________________________

   How long have you been in your present program?
   ___ less than 1 month  ___ 6 months to 1 year
   ___ 1-3 months  ___ more than 1 year
   ___ 3-6 months

3. How would you categorize your current physical fitness level?
   ___ superior  ___ good  ___ average  ___ below average  ___ poor

4. Is there any reason why you think your activity should be limited in this research project? ________________________________________________
   ___________________________________________________________________
   ___________________________________________________________________

I attest that all of the above information is accurate to the best of my knowledge
Signed __________________________ Date __________________________
APPENDIX D

Muscle Soreness Survey
Muscle Soreness
via VAS (Cook et al., 1997)

(no soreness) 0

10 (extremely sore)
APPENDIX E

Feelings Survey
Feelings Scale

While participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasurable. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses.

+5    Very good
+4
+3    Good
+2
+1    Fairly good
0     Neutral
-1    Fairly bad
-2
-3    Bad
-4
-5    Very bad
APPENDIX F

Illness Questionnaire
Illness Questionnaire

Date ______________ Name/ID no ______________________

Note to participants: If you are filling out this survey on a daily basis, please respond such that the word “week” is replaced by the word “day”

1. Have you experienced fever, chills, or aches (not aches associated with typical exercise conditioning) within the past week?
   YES  NO
   IF yes, how many days with these symptoms? ______

2. Have you experienced runny or stuffy nose, cough, sore or scratchy throat within the past week?
   YES  NO
   IF yes, how many days with these symptoms? ______

3. Have you experienced any other symptoms that might suggest you have been ill?
   YES  NO
   IF yes, how many days with these symptoms? ______
   IF yes, please describe symptoms:
APPENDIX G

Subject Recruitment Email
Research Participants Needed

Healthy, 18-35 year old, active men are needed to participate in a research study investigating changes in muscle performance and immune response during exercise. Participants should be able to extend and flex arms at a moderate to high intensity. The study will involve a familiarization session involving an initial screening and paperwork (informed consent and medical history), and any questions will be answered by the researchers. The start of the study will include a preliminary testing session and 1 trial consisting of a month of consuming a nutritional supplement/test exercise training/24hr-48hr-72hr post-testing. Small blood and urine samples will be collected on planned days of testing/training. Compensation will be provided for your participation.

Note: Individuals allergic to yeast should not participate in the study!

Food and beverages will be available at exercise sessions.

Contact Information: For more information about this study please contact:

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APPENDIX H

Subject Recruitment Flyer
Research Participants Needed

Healthy, 18-35 year old, active men are needed to participate in a research study investigating changes in muscle performance and immune response during exercise. Participants should be able to extend and flex arms at a moderate to high intensity. The study will involve a familiarization session involving an initial screening and paperwork (informed consent and medical history), and any questions will be answered by the researchers. The start of the study will include a preliminary testing session and 1 trial consisting of a month of consuming a nutritional supplement/test exercise training/24hr-48hr-72hr post-testing. Small blood and urine samples will be collected on planned days of testing/training. Compensation will be provided for your participation.

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