THE EFFECTS OF AÇAI (EUTERPE OLERACEA MART) ON DELAYED MUSCLE SORENESS (DOMS) IN COLLEGIATE MALE ATHLETES AND NON-ATHLETES

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Eccentric exercise often results in the production of markers of oxidative stress and an inflammatory response, which leads to delayed onset muscle soreness (DOMS). DOMS is associated with impaired muscular performance. Dietary interventions may reduce inflammation and improve physical performance. The first aim of this investigation was to determine if Açai supplementation reduces markers of oxidative stress and the inflammatory process caused by eccentric exercise. The second aim was to determine if Açai supplementation reduces muscle soreness and improves muscle function. Individuals were counterbalanced/stratified into the Açai group or a placebo group. Supplementation started 48 hours prior to downhill running. Markers of oxidative stress and inflammation, and range of motion, muscle soreness perception, agility, and vertical jump displacement were assessed at baseline, after, 24, and 48 hours after downhill running. Twenty collegiate athletes and non-athletes (21±2 years old) completed the protocol. The Açai group \((N = 10)\) reported significantly less muscle soreness in the quadriceps muscle \((p = .011)\) compared to the placebo group \((N = 10)\). In addition, there was a significant difference \((p = .023)\) in the group by time interaction in the quadriceps muscle soreness. Furthermore, the Açai group scored slightly different on range of motion, agility, vertical jump displacement, creatine kinase, and c-reactive levels.
compared to the placebo group throughout all 4 time periods. Açai has demonstrated to be an effective supplement to decrease quadriceps muscle soreness after downhill running. Furthermore, the consistency of results throughout the 4 time periods may suggest its potential to slightly change performance and levels of oxidative stress and inflammation.
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CHAPTER I

INTRODUCTION

Delayed Onset Muscle Soreness (DOMS)

Both physically inactive individuals, who engage in periodic physical activity (i.e., “weekend warriors”), and athletes, who engage in exercise of high intensity and/or high volume, can experience delayed onset muscle soreness (DOMS). DOMS is characterized by muscle pain and stiffness that may limit physical function for several days after exercise (Drobnic et al., 2014). It may cause decreased range of motion and maximum strength, which limits performance (Baird, Graham, Baker, & Bickerstaff, 2012; Brown, Day, & Donnelly, 1999; Page, 1995). DOMS usually commences 12–24 hours following eccentric or intense exercise (Drobnic et al., 2014). It peaks in 2–3 days, and subsides linearly within 8–10 days (Clarkson, Nosaka, & Braun, 1992). Even though the etiology and the cellular mechanisms of DOMS are not known, potential contributors include skeletal muscle damage and enzyme efflux and inflammation (Armstrong, 1984; Cheung, Hume, & Maxwell, 2003; Peake, Nosaka, & Suzuki, 2005).

Inflammation and Muscle Damage

The sensation of soreness following unaccustomed eccentric muscle action or high intensity and/or high volume physical activity and its association with acute inflammatory response was first proposed during the late 1970s (L. Smith, 1991). Smith proposed that DOMS is a form of acute inflammation. This inflammation is activated in response to connective and/or contractile tissue damage and that the perception of soreness represents pain due to inflammation (L. Smith, 1991). C-reactive protein (CRP)
is a classical acute phase protein, which displays an uncompromising increase during the first few days after severe exercise (Dufaux, Order, Geyer, & Hollmann, 1984; Strachan et al., 1984). According to Beavers, Brinkley, and Nicklas (2010), CRP is the most frequently studied biomarker of inflammation. Creatine kinase (CK) is a sensitive enzyme that has been used as a marker for contractile tissue damage in multiple studies (Deyhle, Sorensen, & Hyldahl, 2016; Köhne, Ormsbee, & McKune, 2016; Magrini, Khodaee, San-Millan, Hew-Butler, & Provance, 2017).

According to Peake, Suzuki, and Coombes (2004), inflammatory responses to muscle damage are dependent on age, type of eccentric exercise, gender, and repeated bouts of eccentric loading. Downhill running and eccentric cycling have shown greater response of circulating neutrophil counts and systemic cytokines than other types of eccentric exercise involving smaller muscle mass. CK levels have shown increase after downhill running in multiple studies (Ormsbee et al., 2015; Peake, Suzuki, Wilson et al., 2004; Pokora, Kempa, Chrapusta, & Langfort, 2014).

**Treatment of DOMS**

Connolly, Sayers, and McHugh (2003) reviewed multiple articles that presented different forms of treatment for DOMS including pharmaceuticals, herbal remedies, acupuncture, stretching, ice, massage, and nutritional supplements. They found an inconsistency on all different forms of treatment as both positive and negative results were reported. However, anti-inflammatory drugs such as ibuprofen, diclofenac, and ketoprofen have shown the potential to alleviate some symptoms of DOMS. A primary concern with the use of nonsteroidal anti-inflammatory is that they have side effects such
as gastrointestinal and renal distress and hypertension (Connolly et al., 2003). Therefore, a natural source of antioxidant and anti-inflammatory compounds such as the fruit Acai (Euterpe oleracea Mart) should be investigated to determine if it decreases the symptoms of DOMS.

**Acai (Euterpe Oleracea Mart)**

Although the human body relies on endogenous defense mechanisms to decrease oxidative stress, exogenous sources of antioxidant are also available (Paz et al., 2015). Acai is an exotic fruit that grows in a palm tree (Arecaceae) in the Amazon flood plain region and it has gained international attention due to its pharmacological properties (Bonomo et al., 2014). The extracts and juices of the fruit Acai are believed to have antiproliferative, anti-inflammatory, antioxidant, and cardio-protective properties (Hogan et al., 2010; Kang et al., 2011; Paz et al., 2015; Xie et al., 2012; Yamaguchi, Pereira, Lima, Veiga-Junior, 2015). The polyphenols and flavonoids present in Acai could provide exogenous (dietary) sources in the fight against free radicals and oxidative stress caused by exercise.

**Rationale**

Although eccentric training is a promising training modality for the optimization of performance and the prevention of injuries in athletes, it frequently results in DOMS (Vogt & Hoppeler, 2014). Therefore, it is important that athletes have recovery protocols to minimize muscle damage and soreness.
Objective

Although there are numerous studies on the promising effects of the fruit Açai, there are no studies available to date that have investigated the effects of Açai on the outcomes of DOMS. Therefore, this study examines the effects of the fruit Açai (*euterpe oleracea* mart) on symptoms of DOMS after a bout of downhill running in collegiate male athletes and non-athletes, and it addresses the following aims and hypotheses:

The **first aim** is to measure the effects of Açai on markers of muscle damage and inflammation. Creatine Kinase (CK) blood levels was used as a biomarker of muscle damage, whereas C-reactive protein (CRP) blood levels were used as an inflammatory biomarker (Drobnic et al., 2014).

**Hypothesis #1:** Volunteers in the Açai group will show significantly lower serum creatine kinase (CK) and C-reactive protein (CRP) blood levels after 24 and 48 hours after downhill running compared to volunteers in the placebo group.

The **second aim** is to measure the effects of Açai on muscle soreness and physical performance.

**Hypothesis #2:** Volunteers in the Açai group will demonstrate significantly lower levels of muscular soreness and significantly higher scores on range of motion vertical jump displacement, and agility test shortly after and 24 and 48 hours after downhill running compared to volunteers in the placebo group.

If the hypotheses are accepted, then this suggests that the anti-inflammatory and antioxidant properties of the fruit Açai can reduce the potential side effects of DOMS and may improve recovery time and muscle function following downhill running.
CHAPTER II

LITERATURE REVIEW

Delayed-Onset Muscle Soreness (DOMS)

Theodore Hough was the first individual to describe delayed onset muscle soreness (DOMS) in 1900. Hough found that repetitive rhythmic muscle contractions could result in soreness 12–24 hours later. He suggested that this muscle soreness was due to either ruptures of the muscle fibers, the connective tissue, or the nerve instead of a fatigue phenomenon. Hough also suggested that muscle soreness might also involve inflammation of the interstitial connective tissue (Hough, 1900). Recent research has demonstrated that vigorous physical activity, such as downhill running (Carvalho-Peixoto et al., 2015), induces inflammation and oxidative stress, which may occur via muscle metabolism and muscle damage. In turn, this process can induce fatigue and impair recovery from exercise (Peake, Suzuki, & Coombes, 2004).

Exercise causes an increase in free radicals and oxidative stress (Cooper, Vollaard, Choueiri, & Wilson, 2002; Powers, Nelson, & Hudson, 2011; Urso & Clarkson, 2003; Vinã et al., 2000). According to Close, Ashton, McArdle, and Maclaren (2005), various forms of contractile activity produce free radicals, which are known to result in skeletal muscle damage. The degree of oxidative stress and muscle damage is associated with the degree of exhaustion of a person who performs exercise and not the absolute intensity of the exercise the person performs (Vinã et al., 2000). Given the link between delayed-onset muscle soreness (DOMS) and contraction-induced muscle damage,
post-exercise free radical production has been associated with DOMS (Close et al., 2005).

Muscle damage is fairly routine for many athletes including soccer, and football players as well as, track and field and cross-country runners. The classical muscle damage process involves the release of chemo-attractive factors, vasodilatation, leukocyte adhesion, neutrophils and macrophages migration, and activation of satellite cells (Malm et al., 2004).

Armstrong (1984) proposed a model centered on three theories to explain the phenomenon of DOMS. The first hypothesis suggests that the structural damage occurs once high tensions are placed on the muscle, particularly those associated with eccentric exercise. The second hypothesis suggests that there is a disruption of Ca ++ homeostasis in the injured fibers of a damaged cell membrane and that the disruption of Ca ++ homeostasis results in necrosis that peaks about two days following exercise. The third hypothesis suggests that the accumulation of chemical substances such as bradykinin, serotonin, and histamine elicit action potentials in free nerve endings of group-IV sensory neurons in the arterioles, capillaries, and at the musculotendinous junctions (Armstrong, 1984).

As reported previously, DOMS can affect athletic performance and cause reduction in joint range of motion and peak torque. Due to the possible alterations in muscle recruitment patterns, stress is placed on ligaments, muscles, and tendons. If athletes attempt to return to sport without proper recovery, this stress has the potential to increase the risk of further injury (Cheung et al., 2003).
**DOMS and Eccentric Muscular Contraction**

Eccentric exercise is required for several physical and sport activities such as downhill running, soccer, and track and field, in which athletes sprint, jump, and run intermittently as they change directions and/or speed (Guilhem, Cornu, & Guevel, 2010). Eccentric contraction is characterized by forceful lengthening of the contracting muscle and it leads to a disruption on sarcomeres in the myofibrils and damage in the excitation-contraction coupling system (Proske & Morgan, 2001).

The most common way to induce eccentric exercise-induced damage (EEIMID) experimentally is downhill running (Drobnic et al., 2014; Peake, Suzuki, Wilson, et al., 2004). According to Brunett, Smith, Smeltzer, Young, and Burns (2010), the quadriceps muscles face a heavy eccentric load during downhill running as they brace forward momentum on the downward grade. It has been shown by Croisier et al. (2003) that eccentric muscular activity produces severe muscle soreness post 30 minutes downhill running. Downhill running has been widely studied in the last decades (Braun & Dutto, 2003; Chen, Nosaka, Lin, Chen, & Wu, 2009). Even a relatively short period of downhill running has resulted in muscle damage (Eston, Mickleborough, & Baltzopoulos, 1995). In addition, downhill running has shown muscle damage of the same magnitude as plyometric or maximal eccentric exercises, which was shown with an increase of plasma creatine kinase (Chen, Nosaka, & Wu, 2008; Hamill, Freedson, Clarkson, & Braun, 1991).

DOMS caused by eccentric contraction is an important part of muscular adaptation to physical exercise. A review by Vogt and Hoppeler (2014) concluded that
eccentric exercise training enhances maximal muscle strength and power. In addition, eccentric training protocols lead to an increase in muscle fascicle length and neuromuscular activation. As a result of eccentric training, the structural changes on the muscle-tendon system and the adaptations of the neuromuscular system enhance the velocity of muscular contraction (Guilhem et al., 2010).

Microscopic structural evaluation and blood tests show that eccentric contractions cause injury to skeletal muscle fibers (Page, 1995). In addition to disruption of the normal myofilaments (myosin and actin) structures in some sarcomeres, an increase of intramuscular proteins (creatine kinase enzymes) release into the plasma occurs following damage to the sarcomere (Page, 1995). Muscle biopsy studies have shown that, after eccentric muscular action, there is an increase in mast cell degranulation, separation between the extracellular matrix and the myofibers, and increased plasma constituents in the extracellular space. Mast cell degranulation is a process that occurs via the activation of tyrosine kinases within the cells. This cellular process releases a mixture of compounds such as histamine and serotonin (Stauber, Clarkson, Fritz, & Evans, 1990; Yu, Liu, Carlsson, Thornell, & Stál, 2013). These chemical compounds action on the free nerve endings of group-IV sensory neurons have been related to muscle pain following exercise (Armstrong, 1984; Franz & Mense, 1975).

**Analysis of Muscle Damage**

Serum creatine kinase (CK) concentration in the blood was used to estimate muscle damage induced by the downhill running activity performed in this investigation. According to Baird and colleagues (2012), the measurement of CK activity is an
important indicator of muscle tissue damage. According to Gagliano and colleagues (2009) the general population shows baseline serum CK levels between 35–175 IU/L. In instances of subclinical disorders, minor injury, genetic factors, physical activity status, and medication, the serum CK levels can vary from 20 to 16,000 U/L. As for clinical diagnosed muscle damage, serum CK levels have been reported anywhere from 10,000 to 200,000 U/L to as high as 3X10^6 U/L. The disruption of striated muscle tissue with associated leakage of intracellular muscle components in the blood stream is seen in these high serum CK levels (Baird et al., 2012).

According to Alardin and colleagues elevated CK level is a sensitive measure of muscle injury (Alardin, Varon, & Marik, 2005), especially in the absence specific myocardial or brain infarction, physical trauma, or disease serum CK levels greater than 5,000 U/L indicates serious muscle injury (Alardin et al., 2005).

**Analysis of Inflammation Biomarker**

In addition to blood levels of CK, blood levels of C-reactive Protein (CRP) concentration were used as a measure of inflammation. Kasapis and Thompson (2005) performed a systematic review of 19 articles on the effects of exercise on C-reactive protein (CRP) and inflammation. All of these articles demonstrated a significant change in maximum mean CRP after strenuous exercise, ranging from 5km run to 6 day-ultramarathon, and acute phase response to exercise that appears to be relative to the amount of activity, type of exercise, muscle mass involved, and muscle injury. Weight, Alexander, and Jacobs (1991) reported that CRP levels significantly increased by 2,000% immediately after and 24 hours after a 42-km marathon race. These findings demonstrate
that muscle injury contributes to this inflammatory response as creatine kinase mean increased from 88.5 prior to the race to 801.7, 24 hours after the race. 

Furthermore Taylor and colleagues (1997) showed that after 160km triathlon (21 km of canoeing, 97 km of cycling, and 42 km of running), C-reactive protein increased 266% 24 h after the race and returned to baseline by 48 hours. Additionally, Siegel and colleagues (2001) investigated the CRP level of 55 runners in the 1996 and 1997 Boston marathons. The researchers noted increases in CRP (122%) within 4 h after the event (p < 0.001; Siegel et al., 2001). Lastly, Strachan and colleagues (1984) investigated the CRP levels of 38 trained runners, before, immediately after, and 24 hours after they competed in 5 different races ranging from 15km to 88km. CRP levels peaked at 24 hours on every race and increased with race duration, reaching levels that are comparable to patients with small myocardial infarction (Strachan et al., 1984).

It is important to note that all articles cited above used competitive athletes. In addition, all articles reported the analysis of CRP before, after, and 24 hours and 48 hours after exercise, besides Siegel and colleagues (2001), who did not perform CRP analysis 48 hours after exercise. Even though the exercise mode chosen by the researchers was long distance, the articles show a variation in both distance (15km–160km) and type of exercise (running, canoeing, cycling).

The Effects of DOMS on Athletic Performance

Delayed onset muscle soreness can result in decreased range of motion (ROM; Bradley, Olsen, & Portas, 2007). According to Bradley and colleagues, athletes who participate in sports involving sprinting increase their risk for thigh injuries if their hip
and knee range of motion (ROM) is reduced. In addition, reduced joint ROM in lower extremities has been highly correlated with decreased movement efficiency (Rabin, Kozol, Spitzer, & Finestone, 2014).

In addition to decreased ROM, several studies have shown that DOMS decreases maximal power during tasks such as vertical jump (Kargarfard et al., 2015; Tokmakidis, Kokkinidis, Smilos, & Douda, 2003). Kargarfard and colleagues recruited 30 experienced male bodybuilders. These bodybuilders performed 5 repetitions, 3 sets of knee extension, and flexion at 75–77% of their respective 1RM. While the massage group received a 30-minute massage after the exercise protocol, the control group rested during a passive recovery. The researchers measured participants’ vertical jump, agility, CK levels, isometric torque, and perception of soreness at baseline, immediately after the DOMS protocol, right after the massage, and 24, 48, and 72 hours after the massage. Participants on both groups showed significant \( (p < .001) \) decrease in vertical jump, agility performance, and isometric torque an increase in CK and muscle soreness levels (Kargarfard et al., 2015).

Tokmakidis and colleagues (2003) recruited 19 subjects to test the effect of ibuprofen on DOMS by performing muscle damage and muscular performance analysis. These subjects performed eccentric leg curl exercise in order to induce muscles soreness. The researchers measured subjects’ vertical jump, maximal strength, and knee ROM before, after, 24 and 48 hours after exercise. The results of muscular performance showed a significant decrease \( (p < .05) \) in vertical jump, maximal strength, and knee
ROM immediately after and 24 and 48 hours after exercise in both the placebo and the ibuprofen groups.

As for Tokmakidis and colleagues (2003), vertical jump was used as an assessment of power by many other investigators (Markovic, Mirkov, Knezetic, & Jaric, 2013; Markovic, 2015; Samozino, Morin, Hintzy, & Belli, 2010). Power is the rate in which we perform mechanical work (Markovic, Dragan, Nedeljkovic, & Jaric, 2014) and it was used as one of the assessments of performance in this investigation.

Agility is also compromised by the presence of DOMS (Kargarfard et al., 2015). Agility is known as the ability to perform multidirectional displacement (Fessi et al., 2016). Throughout practice and competition, collegiate athletes perform activities that include acceleration, deceleration, and multidirectional displacement. During multidirectional displacement activities, athletes move forward, laterally, and backward. These changes in direction must be performed with a minimum loss of balance and speed.

In the search for the best agility test protocol for this study I found the t-test to be the best option. Pauole, Madole, Garhammer, Lacourse, and Rozenek (2000) examined the t-test as described by the National Strength and Conditioning Association in 1994. The investigation concluded that the t-test is a reliable and valid form of measurement for agility and sports performance in nondisabled college athletes. Researchers may use the t-test to measure not only athletes’ agility, but also sprinting speed and lower-body power (Pauole et al., 2000; Semenick, 1990, 1994).
Treatments for DOMS

There are a variety of ways to reduce muscle soreness and enhance muscle recovery after intense exercise including whole-body vibration, cold-water immersion, massage therapy, foam roller, massage, proprioceptive neuromuscular facilitation (PNF), acupuncture, anti-inflammatory drugs (NSAID), antioxidants, and phytochemical supplementation.

In a study with untrained individuals, Timon, Tejero, Brazo-Sayavera, Crespo, and Olcina (2016) investigated whole-body vibration as a form of treatment on muscle soreness and muscle strength recovery after eccentric quadriceps exercises. They showed that a single bout of whole body vibration treatment decreased the release of creatine kinase levels at 24 and 48 hours after the completion of exercise as well as muscle soreness 48 hours post exercise. However, a single bout of whole body vibration treatment did not improve the recovery of muscle strength (peak isometric torque) and blood urea nitrogen post eccentric exercise (Timon et al., 2016). Based on these findings, they concluded that although post-exercise vibration treatment attenuates DOMS, muscle performance could still be reduced 48 hours after the completion of intense eccentric training (Timon et al., 2016).

Hayter, Doma, Schumann, and Deakin (2016) investigated the effects of cold-water immersion (CWI) and cold air therapy (CAT) on maximal cycling performance and recovery markers following strength exercises. The researchers used 20 recreational endurance trained males and females in the study. The participants were endurance-trained, but strength-untrained. The participants performed 5 sets of 6
repetitions of maximum incline leg press and 3 sets of 6 repetitions of leg curl and leg extension. All exercises were performed with a 6RM load. Measurements were taken before, immediately after, and 24, 48, and 72 hours after the exercise protocol. The researchers found that both CWI and CAT were effective in decreasing muscle soreness, as there were no statistical between-group differences (Hayter et al., 2016).

Proprioceptive neuromuscular facilitation (PNF) stretching (hold relax-agonist contraction) has also been shown to decrease the symptoms of DOMS (Hyun-Gyu & Myoung-Kwon, 2015). In this study, DOMS was induced with downhill walking on a 10° declined treadmill at a speed of 4 km/h for 30 minutes. The researchers found that hold relax-agonist contraction technique more effectively increased muscle activity and decreased muscle fatigue (lactic acid levels) than the passive straight leg raising technique (Hyun-Gyu & Myoung-Kwon, 2015). Similarly, Cheatham, Kolber, Cain, and Lee (2015) wrote a meta-analysis, which examined the effectiveness of self-myofascial release (SMR) using a foam roller to enhance post exercise muscle recovery and reduce DOMS. The researchers concluded that no consensus exists regarding the optimal program of SRM for range of motion, recovery, and performance after intense activity.

Kargarfard and colleagues (2015) investigated the use of massage to decrease DOMS. In a total of 30 males with at least 2 years experience in bodybuilding, researchers examined plasma CK level, agility, vertical jump, isometric torque, and perception of soreness after 5 repetition sets of knee extensor and flexor muscle groups. In the massage group, plasma CK levels were significantly lower compared to the control group at 24 and 72 hours after the massage, which indicates better recovery. In addition,
the participants in the experimental group had lower perceived soreness scores and performed better than the control group in less than 72 hours after the massage in all but the agility test. The researchers concluded that massage allowed a better recovery rate and exercise performance on bodybuilders after an intense weight training session (Kargarfard et al., 2015).

Fleckenstein and colleagues (2015) investigated the effect of acupuncture as a treatment for DOMS on the biceps brachii muscle. A total of 60 participants, 22 females and 38 males, were randomly assigned to 5 different groups (needle, laser, sham needle, sham laser acupuncture, and no intervention). Participants’ pressure pain threshold, pain intensity, and maximum isometric voluntary force were examined after the treatment. The results showed that none of the acupuncture interventions significantly improved DOMS compared with the no intervention group. The researchers suggested the use of intramuscular needling (dry needling) for future research as the mechanisms of DOMS takes place in the muscular unit and its innervation (Fleckenstein et al., 2015).

Anti-inflammatory drugs (NSAID) such as aspirin, naproxen, flurbiprofen, ibuprofen, ibuprofen, diclofenac, and keprofen have also been examined as a treatment to attenuate DOMS. Declan, Connolly, Sayers, and McHugh (2003) performed a review on multiple studies on the efficacy of NSAID on DOMS. The researchers found it difficult to compare studies that used such a form of treatment for DOMS due to variation in modes of muscle damage and in NSAID dosage.

Donnelly, Maughan, and Whiting (1990) recruited 16 subjects to participate in a crossover study. In this particular study, 45-minute downhill running caused DOMS.
Subjects took 2,400 mg of ibuprofen 24 prior and for 72 hours after the running protocol. Ibuprofen showed no efficacy on the treatment of DOMS. The results of a study completed by Hasson and colleagues (1993) showed the opposite. The researchers found that 400 mg or 1,200 mg of ibuprofen ingested 4 hours before or 24 hours after eccentric exercise, respectively, significantly reduced DOMS 48 hours after exercise. The protocol used in this study was a 10-minute stepping exercise on a bench with frequency of 15 cycles-min⁻¹ and an additional load of 10% of participant’s body weight. Twenty healthy subjects were recruited for this study (Hasson et al., 1993).

According to Donnelly et al. (1990), the treatment of DOMS with NSAIDs and antioxidants is more promising than other approaches such as massage and stretching. The drawbacks with using NSAID’s on the treatment of DOMS are their side effects. Brooks and Day (1991) have demonstrated that the side effects of NSAIDs include, but not limited to, gastrointestinal and renal distress and hypertension.

He, Hockemeyer, and Sedlock (2015) demonstrated that a combined antioxidant supplementation with Vitamins C and E significantly \((p < .05)\) decreased DOMS after repeated downhill runs. Twenty-two moderately trained males were recruited and randomly divided into supplementation and placebo groups. The participants had to run 40-minute downhill twice on a 10% decline. They ingested 1,000 mg of vitamin C and 400 IU of vitamin E daily for two weeks. Muscle damage, antioxidant status, and DOMS, were tested before, immediately after, and 6, 24, and 48 hours after the exercise protocol (He et al., 2015).
In addition to the forms of treatments discussed previously, there are also several nutritional supplements that have proven to be helpful to decrease the symptoms of DOMS. In a recent meta-analysis, Panza, Diefenthaeler, and Silva (2015) reviewed 14 studies published between 2003 and 2014 that investigated the effect of dietary phytochemical supplementation on muscle damage, oxidative stress, and inflammation markers after eccentric exercise. All studies used eccentric exercise-induced muscle damage (EEIMD) either through the lowering of weight or downhill running. The phytochemical supplementation varied from drinks based on fresh fruit to dried fruit powder or fruit extract, capsules with vegetable and fruit or dried rhizome concentrates among others. In general, the results indicated benefits for most fruit-based and plant extracts/rhizome-based drinks on the biochemical and muscle-damage markers. The authors concluded that the positive results were likely due to the antioxidant and anti-inflammatory properties of the phytochemicals in the supplements (Panza et al., 2015). This suggests that supplements with dietary phytochemicals appear to have the potential to attenuate the symptoms of EEIMD.

Açai (*Euterpe Oleracea* Mart)

Açai (*Euterpe oleracea* Mart), which was originally found in Central and South America, has been used as both a medicinal plant and as a functional food in many parts of Brazil due to its antioxidant phytochemical composition (Dutra, Campos, Santos, & Calixto, 2016). The National Health Surveillance Agency (ANVISA) in Brazil certifies the production of functional food products that are proven to impact individual’s health and, Açai fits under this category (Fujikawa Nes & Fujikawa Nes, 2015).
Numerous studies have been done discussing Açaí antiproliferative, anti-inflammatory, and antioxidant properties, its capacity to reduce pain and muscle stress, and its effect on range of motion and athletic performance (Carvalho-Peixoto et al., 2015; Hogan et al., 2010; Jensen, Ager, Redman, Mitzner, Benson, & Shauss, 2011; Schauss et al., 2006; Sadowska-Krepa et al., 2015).

Polyphenols are the most abundant class of antioxidants available in the diet. The main dietary sources of polyphenols are fruits and plant derived beverages. Polyphenol intake is higher than other antioxidants such as vitamin C (~10 times higher) and vitamin E (~100 times higher; Scalbert, Johnson, & Saltmarsh, 2005). Açaí has exceptional antioxidant properties against the reactive oxygen species superoxide (O$_2^-$) and peroxyl radical (RO$_2^-$; Schauss et al., 2006). These reactive oxygen species are free radicals formed as byproducts of aerobic metabolism and they have the potential to damage cell structures. Açaí contain high levels of proanthocyanidins (1,289mg/100g dry weight), which are a class of antioxidants known as polyphenols, compared to blueberries (255.8mg/100g dry weight; Heinrich, Dhanji, & Casselman, 2010).

The polyphenols and flavonoids (antioxidants and anti-inflammatories) present in Açaí are both important exogenous sources in the fight against free radicals and oxidative stress caused by exercise. Although the human body relies on endogenous defense mechanism against oxidative stress, exogenous (dietary) sources of antioxidants are also necessary (Paz et al., 2015). The lack of antioxidant in our diet in combination with the increased free radicals generated through exercise has the potential to cause damage to skeletal muscle as well as other biomolecules. For example, reactive oxygen species
(ROS), also known as free radicals, attacks polyunsaturated fatty acids cell membranes via lipid peroxidation, which in turn forms reactive aldehydes that diffuses from the original site of damage to other parts of the cell. Consequently ROS have the ability to cause skeletal muscle damage at either the site of origin or elsewhere (Matsuo & Kaneko, 2000).

In addition to its impressive activity as an antioxidant, Açai has the flavonoids luteolin and apigenin, which are potent anti-inflammatories and decrease inflammatory cytokine (IL-6 and TNF-α) production (Xie et al., 2012). Furthermore, Açai pulp has a unique flavone called velutin that can reduce levels of tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6; Xie et al., 2012). Schauss and colleagues (2006) demonstrated that freeze-dried Açai at 250–2500 μg/mL inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO) released by macrophage cells in a mouse model. LPS is one of the most powerful activators of macrophages. In turn, macrophages and monocytes that have been activated by LPS are known to produce inflammatory mediators such as nitric oxide (Yang, Lee, Lee, Ham, & Choi, 2012). Based on the relationship between LPS and NO, Schauss and colleagues (2006) suggested that the freeze-dried Açai may be used as a potent anti-inflammatory substance and may be useful in allergic and autoimmune disorders.

**Açai Antioxidant and Anti-Inflammatory Properties**

Pharmacological studies on Açai have examined the fruit’s antioxidants and anti-inflammatory properties (Hogan et al., 2010; Jensen et al., 2008; Schauss et al., 2006; Ulbricht et al., 2012). Anthocyanins are a group of polyphenols that are found in
Açai in a large concentration. This antioxidant gives the fruit its deep purple color (Ulbricht et al., 2012). The three major anthocyanins found in Açai are cyanidin 3-rutoside, cyanidin 3-diglycoside, and cyanidin 3-glucoside (Jensen et al., 2008). Other polyphenols found in Açai that contribute to its antioxidant capacity are glucuronate, sulfonate, aglycon, methylate, ellagitannin (Pozo-Insfran, Percival, & Talcott, 2006), proanthocyanidins, and phenolic acids (Jensen et al., 2008).

Another important antioxidant and anti-inflammatory polyphenolic compound found in Açai are flavonoids including 2S, 3S-dihydrokaempferol, 2R, 3R- dihydrokaempferol, 3-O-β-D-glucoside, isovitexin, velutin, and trimethoxyflavone (Kang et al., 2011), homoorientin, orientin, taxifolin deoxyhexose, epicatechin, and scoparin (Stoner et al., 2010). Other antioxidant compounds found in Açai are the phenolic acid protocatechuic acid, p-hydroxy-benzoic acid, gallic acid, ellagic acid, (+)-catechin, ellagic acid, p-coumaric acid, ferulic acid, and vanillic acid, Carotenoids (alpha-carotene, beta-cryptoxanthin, lycopene, zeaxanthin, and lutein) and vitamin E (Schauss, 2009; Stoner et al., 2010; Ulbricht et al., 2012).

Study of Açai in Animal Models

Matheus and colleagues (2006) were the first researchers to evaluate the effects of Açai on nitric oxide (NO) production, NO scavenger capacity, and expression of the inducible nitric oxide synthase (iNOS). The water-soluble gas NO is involved in many physiological and pathological conditions including vasodilation, host defense, tumor cell death and, apoptosis. In pathological conditions, NO is a result of the induction of iNOS in response to agents such as IL-1β, TNF-α, interferon-γ (INF- γ), and LPS. Therefore,
many inflammatory processes may be treated with the use of agents such as Açai that inhibit the induction of iNOS (Matheus et al., 2006).

The study by Matheus and colleagues (2006) was performed on mouse monocyte-macrophages. In order to quantify NO production, nitrite concentration in the cells was measured. NO was transformed into nitrite with the use of s-nitroso n-acetyl DL-penicillamine (SNAP) solution. In order to detect iNOS enzyme expression, an extreme long process took place. This process started with a 6 hours incubation of cultures containing the activation of adherent cells and fractions of Açai. Matheus and colleagues concluded that Açai is a potent inhibitory of NO production in macrophage cell line. This inhibition appeared to be due to a reduction of iNOS expression and iNOS activity.

Many other studies on Açai demonstrate the fruit’s medicinal capacity in animal models (Pozo-Isfran et al., 2006; Schauss et al., 2006; Udani, Singh, & Barrett, 2011). Spada and colleagues (2009) studied the antioxidant activity of Açai frozen fruit pulp in the cerebral cortex, hippocampus, and cerebellum of rats. They showed that brain tissue that received pretreatments with Açai was protected against oxidative damage induced by H₂O₂. The free radical H₂O₂ diffuses within and between cells in vivo, and causes damage in membranes, proteins, lipids, and the cellular nucleus (Spada et al., 2009). Spada and colleagues suggested that Açai has potential to be used in the prevention or may slow down the process of neurodegenerative disease such as Parkinson’s and Alzheimer’s.
Oliveira and colleagues (2010) studied the effect of polyphenol-rich Açaí seed extract on adiposity and hepatic stenosis in mice. After 12 weeks of treatment, they concluded that Açaí seed extract significantly reduced obesity and hepatic stenosis through reduced lipogenesis, increased cholesterol excretion, and improved oxidative stress in the liver (Oliveira et al., 2010). Based on these results they suggested that Açaí could provide an alternative nutritional resource for prevention of metabolic syndrome.

Sudo and colleagues (2015) tested the antinociceptive effect of hydro-alcoholic extract from Açaí stones in acute and chronic models of pain. Pain was induced via injection of drugs including formalin and carrageenan into the mice hind paw, intraperitoneal administration of acetic acid, and spinal nerve damage. Researchers found that Açaí stones extract (ASE) inhibited the interaction between the drug formalin and the central nervous system affecting the symptoms of inflammation and pain. ASE also demonstrated antinociceptive effects in inflammatory pain against the drug carrageenan, which induces inflammation and hyperalgesia. In addition, pre-treatment with ASE demonstrated a reduction in the acetic acid-induced writhing response, which suggests that ASE reduces synthesis or release of pain modulators. Lastly, the administration of ASE over 7 days prevented the development of thermal hyperalgesia and mechanical allodynia in rats with spinal nerve damage. According to Sudo and colleagues (2015), the combination of the CNS and anti-inflammatory effects of ASE may underlie the antinociceptive effects in rats subjected to spinal nerve damage. Based on their findings, the authors believed that Açaí extract may be useful in the development
of new analgesic drug, which is important because pain can reduce one’s normal activities and can negatively affect quality of life (Sudo et al., 2015).

Rocha and colleagues (2007) developed a hydro-alcoholic extract, which they obtained from stone of Açai and examined the vasodilation effect of the hydro-alcoholic extract in the mesenteric vascular bed (MVB) of the rat. The rats were sacrificed with CO₂ and the MVB was cannulated and perfused at a flow rate of 4 ml·min⁻¹ with physiological salt solution (PSS). Acetylcoline and nitroglycerin were injected to test the endothelium-dependent and independent responses before dose-response to Açai stone extract (ASE). The vasodilator effects of ASE, Acetylcoline, and nitroglycerin were studied after perfusion with deoxycholic acid dissolved in PSS for 3 minutes. Rocha and colleagues found that ASE induces an endothelium-dependent effect that does not involve the release of prostanoids, receptors activated by Acetylcoline, histamine, adrenaline, and bradykinin along with the opening of ATP-dependent K⁺ and voltage-dependent K⁺ channels. According to the authors, the findings of this investigation suggest that ASE has the potential of being used in the treatment of cardiovascular diseases (Rocha et al., 2007).

Study of Açai in Human Models

The studies in animal models support the medicinal capacity of Açai along with its potent antioxidant and anti-inflammatory properties (Castro et al., 2014; Henning et al., 2014; Jensen et al., 2008), which is further supported by studies in humans (Holderness, Schepetkin, Freedman, Kirpotina, Quinn, Hedges, & Jutila, 2011; Pozo-Insfran et al., 2006; Udani et al., 2011).
Mertens-Talcott and colleagues (2008) evaluated the Açai antioxidant capacity on 14 healthy, nonsmoking college students. The study was designed as an acute four-way crossover clinical trial. Açai pulp and juice were compared to applesauce and non-oxidant beverage. Throughout a 72-hour dietary washout, subjects were directed to abstain from a diet high in antioxidants, excessive exercise, sleep deprivation, and alcohol consumption. Subjects then consumed 7mL/kg of body weight of Açai and blood samples were drawn during 7 different occasions: at baseline prior to consumption and 30 minutes, 1 hour, 2 hours, 4, hours, 6 hours, and 12 hours after consumption. The control beverage was manufactured from deionized water and was free of phytochemical compounds. Through repeated plasma sampling, researchers reported that the plasma antioxidant capacity increased 2.3 fold for Açai juice and 3 fold for Açai pulp (Mertens-Talcott et al., 2008).

Sadowska-Krepa and colleagues (2015) investigated sprint performance, blood pro-antioxidant status, acute exercise-induced muscle damage, and lipid profile following Açai berry-based juice supplementation in seven junior hurdlers. The athletes were tested before and after the completion of a 6 weeks’ training in which they consumed 100mL of Açai MonaVie Active juice blend, which contains Açai berry, 18 other fruits, and glucosamine (amino sugar). The subjects’ performance was measured during a 300-meter sprint test. In addition, blood samples were taken prior to the sprint test, and immediately and 1 hour after the sprint test. The researchers found that 100mL of Açai MonaVie Active juice blend had no effect on sprint performance. However, blood antioxidant capacity and lipid profile had significant improvement (Sadowska-Krepa et
al., 2015). According to Sadowska-Krepa and colleagues, the main limitation of their study was the lack of a placebo group due to the small number of participants ($N = 7$).

In 2011, Jensen and colleagues studied the anti-inflammatory properties of Acai in older adults who displayed some degree of chronic pain that affected range of motion (ROM) in the vertebral column and lower extremities. The 14 subjects consumed 120 mL bottle of MonaVie Active juice, same juice used on the research previous discussed, every 6 days for 12 weeks and were tested at baseline, and after 2, 4, 8, and 12 weeks. Pain was assessed with the subjective survey Visual analogue scale (VAS), questionnaires, and algometry. In addition, blood was drawn during each visit for the measurement of antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione reductase, lipid peroxidation, which is the measurement of the oxidative degradation of lipids, and high-sensitivity C-reactive protein. The subjects experienced reduced pain and improved ROM. Furthermore, the results show a correlation between pain levels and antioxidants status, but no correlation between pain levels and antioxidants status and lipid peroxidation and high-sensitivity C-reactive protein (Jensen et al., 2011).

Most recently, Carvalho-Peixoto and colleagues (2015) investigated the effects of an Acai beverage on muscle and oxidative stress markers, cardiorespiratory responses, perceived exertion, and time-to-exhaustion during maximal treadmill running in 14 elite male athletes. Participants performed three exercises including a ramp-incremental maximal exercise and two maximal exercise bouts at 90% maximal oxygen uptake. The exercises were performed in two conditions, with the intake of the functional beverage
and another without (control). Athletes drank 300mL of the functional beverage immediately after the first exercise bout in the control condition and 3 consecutive days before the next trial. The functional beverage developed by the researchers contained freeze-dried acai, thocyanins, glutamine, and carbohydrates. After consuming the Acai beverage, participants displayed increased time to exhaustion, reduced perceived exertion, attenuated the metabolic stress (measure as oxidative stress biomarkers ammonaemia and malondialdehyde), and enhanced cardiorespiratory responses (mean heart rate). The researchers concluded that Acai could be used as an ergogenic aid for those who intend to increase their performance during high-intensity training (Carvalho-Peixoto et al., 2015).

Conclusion

Human and animal model studies have shown Acai’s capacity as antioxidant and anti-inflammatory agent. In addition, its benefits as an ergogenic aid and in the improvement of performance and range of motion were also shown. As nutritional supplements become increasingly available to help athletes recover from training and racing, those in the category of anti-inflammatory and antioxidant therapy appear promising to promote recovery after intensive training. The antioxidant and anti-inflammatory effect of Acai along with the other benefits shown may make Acai a good candidate for reducing the severity and length of DOMS following eccentric exercises performed by athletes and non-athletes who enjoy being physical active.
CHAPTER III

METHODS

Recruitment and Consent

The recruitment of volunteers for this study was completed through fliers, e-mails, and word of mouth. Fliers were placed on announcement boards throughout the Tiffin University campus, whereas e-mails were sent through the university e-mail listserv. All volunteers were aware of and understood the purposes, risks, and benefits associated with the study prior to signing a letter of informed consent, which was sent to Tiffin University IRB for approval. The letter of informed consent can be found in Appendix A.

Participants

Twenty-two male volunteers, age 17–25 years, athletes and non-athletes undertook an in-person comprehensive cardiovascular pre-screening by completing the AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire during their first visit (Appendix B: Pescatello, Arena, Riebe, & Thompson, 2014). Volunteers who indicated the presence of one or two or more cardiovascular risk factors had to consult their physician or other appropriate health care provider prior to participation. In addition, volunteers’ eligibility for the study was determined with the completion of a Health History Questionnaire (Appendix C). The completion of this questionnaire assisted in establishing volunteers’ medical history of chronic inflammation, muscle disorders, previous lower extremity injury, and/or drug history of immune suppressants
or anti-inflammatories (Appendix C). The volunteer who displayed one of the aforementioned conditions was excluded from the study. See Table 1.

Table 1

*Inclusion / Exclusion From Participation*

<table>
<thead>
<tr>
<th>AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>one, or two or more cardiovascular risk factors / consult physician prior to participation</td>
</tr>
</tbody>
</table>

Health History Questionnaire

Volunteers will forfeit their opportunity to participate if answer yes to one of the following:

- Medical History of chronic inflammation
- Muscle Disorders
- Previous Lower Extremity Injury
- Drug history of Immune Suppressants or Anti-inflammatories
- Allergies of food from the palm trees family

Volunteers had to follow instructions throughout the 5-day protocol and those who failed to follow instructions were dismissed from the study. Volunteers were expected to keep a food log and report any activity outside of the instructions listed in Appendix D.

**Study Design**

This study was conducted as a double blind, counterbalanced/stratified, placebo-control trial, and between-subjects. Data collection started following the Kent State University and Tiffin University Institutional Review Board (IRB) approval. All the data besides the blood samples were collected at Tiffin University Exercise Science Laboratory, Tiffin, OH. The blood samples were obtained by phlebotomists under the supervision of the nurse practitioner Frances Ford, who works at the Tiffin University
Health and Wellness Center. Each volunteer visited the Tiffin University Exercise Science Laboratory 4 times on 5 days and Health and Wellness Center 4 times in 3 consecutive days.

On the first day, volunteers were asked to sign a letter of informed consent, and then baseline data were collected including a health history questionnaire, pre-participation health screening, agility test, vertical jump displacement test, muscle soreness assessment, joint flexibility assessment, 1 repetition maximum (1RM) leg press, and a VO₂max test. In addition, volunteers’ age, anthropometric measurements (height via a stadiometer and body mass with a Health-o-Meter 402KL Physician Balance Beam Scale), resting heart rate, and blood pressure were measured. On the same day volunteers received 5000mg of Açai extract or the placebo. Volunteers took one 1000mg Açai extract capsule or placebo in front of an independent individual prior to leaving the laboratory. The remaining 4 capsules were sent home with the volunteer in a plastic bag. The second capsule was taken 20 minutes before dinner on the same day, the third and fourth capsules were taken before breakfast and dinner on day 2, and the fifth capsule was taken before breakfast on day 3. The empty bag, which had previously held the capsules, was returned to the researcher on day 3, during the second visit, as a confirmation that the supplement or placebo was taken. Volunteers repeated the routine of taking the capsules home and confirming the intake of the capsules by bringing the plastic bag to the researcher on the following day. The ingestion of Açai extract or placebo also took place on days 3, 4, and 5 (Drobnic et al., 2014).
On the third day, volunteers returned to the lab and performed the downhill running protocol. In addition, volunteers performed the agility and the vertical jump displacement tests, and their muscle soreness and joint flexibility were assessed. Blood samples were drawn via a venipuncture from the antecubital space before and after the downhill running protocol.

On the fourth and fifth days, volunteers performed the agility test, the vertical jump displacement test, and their muscle soreness and joint flexibility were assessed. A blood sample was drawn as well. See Table 2 for the 5-day protocol.

**Dietary Intervention and Supplementation**

Volunteers consumed 1000mg of Açai extract or matching placebo, twice a day, 20 minutes before breakfast and 20 minutes before dinner as suggested by The Vitamin Shoppe Açai extract (ConsumerLab.com, 2017). Volunteers initiated the supplementation 48 hours prior to the downhill running protocol and continued for 48 hours after it (Drobnic et al., 2014).

The ConsumerLab report for the supplement the vitamin shoppe Açai extract that was used in this study revealed that the supplement contains Açai Berry 5:1 Extract (*Euterpe oleracea*). In addition, other ingredients found in the Açai extract include vegetable cellulose, dicalcium phosphate, magnesium stearate, and silica. No yeast, wheat, sugar, salt, soy, diary, citrus, fish, animal derivatives, preservatives, artificial colors or flavors added were found in the Açai extract. See Table 3.
# Table 2

## 5-Day Protocol

<table>
<thead>
<tr>
<th>Items &amp; Approximate Time</th>
<th>Day 1 (50-min)</th>
<th>Day 2 (4-min)</th>
<th>Day 3 (58-min)</th>
<th>Day 4 (24 hours after downhill running/23-min)</th>
<th>Day 5 (48 hours after downhill running/21-min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter of Informed Consent (5-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health History Questionnaire (2-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparticipation Health Screening (2-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometric Measurements (2-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discussion of Volunteers Instructions (3-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle Soreness Assessment (2-min)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Joint Flexibility Assessment (10-min)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Vertical Jump Displacement (2-min)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Agility Test (2-min)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>1RM – leg press (5 min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2max Test (20-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplementation / Placebo (2-min)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Blood Sample (10-min)</td>
<td>x / x*</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Downhill Running Protocol (30-min)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pre and post downhill running
Table 3

*Results of Consumerlab.com Testing Used for Açai Supplement*

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Açai Extract Per Capsule</th>
<th>Labeled Amount of Key Ingredient per Daily suggested Amount</th>
<th>Distributer</th>
<th>Caffeine per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Vitamin Shoppe</td>
<td>100mg extract</td>
<td>1,000-2,000mg extract</td>
<td>Vitamin Shoppe</td>
<td>None detected</td>
</tr>
<tr>
<td>Açai Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The placebo was a sugar capsule prepared by the researcher. Empty pharmaceutical-grade gelatin capsules were purchased and it was filled with Domino sugar. These capsules had no additives or preservatives and it was gluten free.

Volunteers were instructed to maintain their usual diet and to abstain from the use of supplements and/or sports drinks containing antioxidants and anthocyanin, a class of flavonoids, rich food such as red, purple or blue fruits, juices, and tea throughout the study (Carvalho-Peixoto et al., 2015). Volunteers reported their nutrient intake each day prior to testing.

**Physical Activity During the Study and Scheduling**

Volunteers were instructed to abstain from any type of physical activity starting 48 hours prior to first blood draw and throughout the entire study. Volunteers’ schedule for testing was set according to their availability in the morning period, at least 1 hour after the consumption of breakfast. Volunteers were able to return to the lab for testing at the same time on 3 consecutive days (third, fourth, and fifth days).
**VO_{2max} Assessment**

Volunteers performed a VO_{2max} test, which was used to determine their cardiorespiratory fitness. The goal was for the volunteers to display a maximal oxygen consumption of at least 38.4ml/kg/min or 36.5ml/kg/min based on their age and normative data for VO_{2max}. This range placed volunteers in the fair category according to the normative data provided by the American College of Sports Medicine (Pescatello et al., 2014). The VO_{2max} test was also used to stratify the Açai extract supplement group and placebo group. For example, the first volunteer who scored fair was placed in one group, while the second volunteer to score fair was placed on the opposite group. The same procedure was repeated with volunteers who scored good, excellent, or superior. Volunteers, who did not fall into the fair or above categories, were not considered for the study. See Table 4.

Table 4

*Normative Data for VO_{2max} - Male (values in ml/kg/min)*

<table>
<thead>
<tr>
<th>Age</th>
<th>Very Poor</th>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-19</td>
<td>&lt; 35.0</td>
<td>35.0 - 38.3</td>
<td>38.4 - 45.1</td>
<td>45.2 - 50.9</td>
<td>51.0 - 55.9</td>
</tr>
<tr>
<td>20-29</td>
<td>&lt; 33.0</td>
<td>33.0 - 36.4</td>
<td>36.5 - 42.4</td>
<td>42.5 - 46.4</td>
<td>46.5 - 52.4</td>
</tr>
</tbody>
</table>

(Heyward & Gibson, 2014)

The standardized maximal treadmill protocol included a fixed grade at 3% and an increase in speed by 1km/h each minute until maximum sustained effort. The treadmill speed was initially set at 6km/h (Drobnic et al., 2014). This protocol is outlined in Table
5. Expired air was sampled using the PARVO Medics TrueOne 2400 Metabolic Cart. The test was completed on a motorized treadmill (MT200 Gait Trainer Treadmill) from Spirit Medical Systems Group. Volunteers walked for 5 minutes prior to the beginning of the test in order to become familiarized with the equipment.

Table 5

$VO_{2\text{max}}$ Protocol

<table>
<thead>
<tr>
<th>Stage</th>
<th>Speed (km/h)</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3%</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3%</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>3%</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
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<tr>
<td>10</td>
<td>15</td>
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</tr>
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</table>

**Eccentric Muscle Injury Protocol – Downhill Running**

The downhill running test was completed on a calibrated motorized treadmill (MT200 Gait Trainer Treadmill) from Spirit Medical Systems Group. Volunteers ran downhill with a treadmill grade of -10% after 5-minute warm-up at grade of 0% at a speed chosen by the volunteer (Drobnic et al., 2014). A pilot study performed in the
Exercise Science lab at Tiffin University demonstrated that 15 minutes of downhill running produced DOMS. Volunteers also completed a 5-minute cool down at a self-selected pace and 5 minute seated passive recovery period. Volunteers ran the 15 minutes interval at 80% of their predicted maximal heart rate. Volunteers’ heart rate was measured with the use of a polar FT7 heart rate monitor. After the 5-minute warm-up, treadmill speed was increased until 80% of predicted heart rate was achieved. The predicted heart rate was calculated with the use of the Karvonen Formula (Powers & Howley, 2015). The volunteer then maintained the new pace for 5 minutes. At this point, the treadmill grade was adjusted to -10% incline (Miller, Bailey, Barnes, Derr, & Hall, 2004). Volunteers were asked to consume approximately 500mL of water from 30 minutes up to the start of the test and they were allowed to drink water throughout the test (Drobnic et al., 2014). The protocol took approximately a total of 30 minutes.

**Sample Size Calculation**

In order to determine an approximate number of volunteers required for the study, the G*Power 3.1.9.2 power analysis software was used. Power was set at 0.80 and alpha was set at 0.05 performing difference between two independent means with an *a priori* analysis. Data reported as mean ± SD group 1 (108.0 ± 3.4), and group 2 (102.5 ± 5.6) for average, determined a sample size of 10 volunteers per group. This calculation reflected the results of the muscle tissue damage biomarker Serum Creatine Kinase (CK). Timon and colleagues (2016) collected blood samples from the antecubital vein pre-exercise, post-exercise, and 24 hours and 48 hours post eccentric exercise. The investigators were evaluating the effects of a single whole-body vibration treatment on
muscle soreness and muscle recovery. A total sample size of 22 volunteers was proposed for this investigation accounting for volunteer drop-off (11 volunteers in the experimental group and 11 volunteers for the control group).

**Outcome Measures**

Outcome measures of muscle damage and inflammatory response, perceived muscle soreness, and range of motion were assessed. In addition, volunteers’ physical performance was measured through an agility test and a vertical jump displacement test.

**Markers of Muscle Damage and Inflammation**

Venous blood samples were drawn from the antecubital vein of the arm of the volunteer’s discretion at baseline, after, 24, and 48 hours after downhill running at Tiffin University Health and Wellness Center. A Serum gel tube was used and 5mL of blood was collected for analysis of Creatine Kinase (CK) and C-reactive Protein (CRP) each time (Drobnic et al., 2014; Mertens-Talcott et al., 2008). Baird et al. (2012) reported normal levels of CK at 35-175 U/L and Pearson and colleagues (2003) reported normal levels of CRP at < 1.0 mg/dL. The volunteer’s number was recorded on the serum gel tube. The tube was collected by the investigator and taken to the refrigerated centrifuge (Thermo Fisher Scientific). After centrifuged, the blood sample was stored in a freezer at -20 degrees. The blood samples were then taken to the Mercy Hospital laboratory for analysis.

**Perceived Muscle Soreness**

Ratings of perceived muscle soreness were assessed with the use of a Visual Analogue Scale (VAS). The scale had a 10cm horizontal line, anchored by the words no
pain on the left end and very severe pain on the right end (Gould, 2001). In order to complete this assessment, volunteers were asked to step up (concentric muscular contraction) and step down (eccentric muscular contraction) onto a 40cm box. Upon the completion of the activity, volunteers rated the soreness they experienced on the quadriceps, hamstring, and gastrocnemius muscles while completing the task (McLeay et al., 2012). Volunteers rated their perceived muscle soreness at baseline, after, 24 and 48 hours after downhill running.

**Joint Flexibility Assessment—Range of Motion (ROM)**

Range of motion (ROM) was measured with a goniometer. Supine passive knee flexion and passive straight leg raise test were performed. During the knee flexion test the volunteer was placed on the supine position. The flexion, extension, abduction, adduction, and rotation at the hip joint will be set at 0 degrees. The investigator placed one hand on the volunteer’s ankle, while the other hand was placed on volunteer’s anterior thigh of the dominant leg. The thigh was moved to 90 degrees of hip flexion, followed by knee maximum flexion. The end of maximum knee flexion was reached once the researcher felt resistance or the volunteer attempts to overcome the resistance by displaying an additional hip flexion. The goniometer was aligned with the longitudinal axis of the tibia and the longitudinal axis of the femur and the angle was measured (Markovic, 2015).

During the hip flexion test, the volunteer was also placed on the supine position and the flexion, extension, abduction, adduction, and rotation at the hip joint is also 0 degrees. A strap was placed over the non-dominant leg in order to secure the leg to the
The investigator slowly lifted the volunteer’s dominant leg into hip flexion while the knee is knee fully extended. Once the investigator felt tension in the posterior thigh, flexion of the knee and lumbar column, along with posterior pelvic tilt, the motion was stopped. The goniometer was then placed in the longitudinal axis of the femur and in a parallel line to the examining table so the joint angle could be measured (Markovic, 2015). The investigator took two measurements and used the average. Volunteers ROM was measured at baseline, after, 24 and 48 hours after downhill running.

**Vertical Jump Displacement Test**

Volunteers’ vertical jump displacement was measured with the use of the Vertical Jump Trainer equipment (VERTEC). The volunteer performed 3 countermovement jumps (CMJ) at baseline, after, and 24 and 48 hours after downhill running. Each volunteer was given 2 preliminary trials to learn the technique.

The first step on this test was to measure volunteers’ vertical reach. Volunteers then dropped down to a self-selected level before jumping maximally (Magnúsdóttir, Borglisson, & Karlsson, 2014). Unrestricted countermovement and free arm swing was allowed preceding the jump. The difference between vertical reach and jump height was used to represent volunteer’s vertical jump performance. Volunteers had an opportunity to be familiarized with the test procedure prior to data collection. Each volunteer performed 3 trials and the average score was recorded and used for statistical analyses (Kargarfard et al., 2015).
Agility Test

In order to complete the agility test, volunteers began with both feet behind the start at point 1. At their own discretion, each volunteer sprinted forward 9.14m (10 yards) to point 2. Volunteers then shuffled to the left 4.57m (5 yards) and touched the cone on point 3 with their left hand. Next, volunteers shuffled to the right 9.14m and touched the cone on point 4 with their right hand. Afterwards volunteers shuffled to the left 4.57m and touch cone on point 2 with their left hand. Volunteers finished by running backwards, passing the finish line at point 1. The t-test is outlined in Figure 1.

![Figure 1. t-test](image)

Volunteers performed a familiarizing trial as they walked through the course. A clock with electronic sensor was placed at point 1. The clock started when volunteers crossed point 1 and again stopped when volunteers crossed the sensor plane again. The test was repeated if volunteers failed to touch the cone, crossed their feet when shuffling, and/or did not face forward at all times. Three trials were performed and the average time was used for statistical analyses (Pauole et al., 2000). Volunteers performed the t-test at baseline, after, and 24 and 48 hours after downhill running.
Potential Risk

The participation in this study was associated with DOMS, which has been shown to subside linearly within 8–10 days (Clarkson et al., 1992). Furthermore, volunteers could have experienced hematoma and pain from blood draw (Pettross, 2011). Tiffin University Health and Wellness Center was prepared to provide medical assistance in the case unusual situations arose throughout the study. The registered nurse Fran Ford confirmed her participation. All volunteers were eligible for treatment in the Health and Wellness Center as all of them are enrolled at the university.

Statistical Analyses

Independent samples t-test was used to evaluate descriptive statistics between the experimental and control groups (age, height, weight, relative strength, and VO\textsubscript{2max}) of the experimental and control groups. To examine the differences across four time periods (baseline, after the DOMS inducing protocol, 24 and 48 after downhill running between the intervention group [Açai supplementation] and control [placebo] group, and for the interaction between time and group), a mixed factor 2 group × 4 time repeated measures ANOVA was used for each of the outcome measures. In the presence of a statistically significant F ratio, Bonferroni correction was used for post hoc analyses. Statistical analyses were performed using SPSS software (Version 20). A 1-repetition maximum (1RM) was used as a covariant for volunteers’ fitness status. Statistical significance was tested at a 95% significant level ($p < 0.05$). Values were presented as mean ± standard deviation unless otherwise specified.
CHAPTER IV
THE EFFECTS OF AÇAI (EUTERPE OLERACEA MART) ON DELAYED ONSET MUSCLE SORENESS (DOMS) AND PERFORMANCE IN COLLEGIATE MALE ATHLETES AND NON-ATHLETES

Both physically inactive individuals, who engage in periodic physical activity (i.e., “weekend warriors”), and athletes, who engage in exercise of high intensity and/or high volume, can experience delayed onset muscle soreness (DOMS). DOMS is characterized by muscle pain and stiffness that may limit physical function for several days after exercise (Drobnic et al., 2014). DOMS usually commences 12–24 hours following eccentric or intense exercise (Drobnic et al., 2014). It peaks in 2–3 days, and subsides linearly within 8–10 days (Clarkson et al., 1992).

In addition, DOMS may cause decreased range of motion, agility, and maximum muscular power, which limits performance (Baird et al., 2012; Bradley et al., 2007; Brown et al., 1999; Kargarfard et al., 2015; Page, 1995; Tokmakidis et al., 2003). Athletes who attempt to return to sport without proper recovery have the potential to increase the risk of injury such as muscle strain (Cheung et al., 2003; Rabin et al., 2014).

There are a variety of ways to reduce muscle soreness and enhance muscle recovery after intense exercise including whole-body vibration, cold-water immersion, massage therapy, foam roller, massage, proprioceptive neuromuscular facilitation (PNF), acupuncture, anti-inflammatory drugs (NSAID), antioxidants, and phytochemical supplementation (Declan et al., 2003; Hayter et al., 2016; Hyun-Gyu & Myoung-Kwon, 2015; Kargarfard et al., 2015; Timon et al., 2016). Dietary phytochemical
supplementation appears to be helpful to decrease the symptoms of DOMS following eccentric exercise such as downhill running due to its antioxidant and anti-inflammatory properties (Panza et al., 2015).

Açai is an exotic fruit that grows in a palm tree (Arecaceae) in the Amazon flood plain region, which has gained international attention due to its pharmacological properties (Bonomo et al., 2014). The nutritional and phytochemical composition of Açai has been found to contain potent anti-inflammatory and antioxidant properties (Honzel, Carter, Redman, Schauss, & Jensen, 2008; Jensen et al., 2011; Jensen et al., 2008; Schauss et al., 2006). The extracts and juices of the fruit Açai are believed to have antiproliferative, anti-inflammatory, antioxidant, and cardio-protective properties (Hogan et al., 2010; Kang et al., 2011; Paz et al., 2015; Xie et al., 2012; Yamaguchi et al., 2015).

The phytochemical composition of the fruit Açai (Euterpe Oleracea Mart) has shown positive effect on range of motion and decreased pain in individuals 44–84 years of age who demonstrated mild to moderate joint pain (Jensen et al., 2011). In addition, Açai supplementation increased plasma antioxidant capacity and decreased serum lipid profile, but did not affect sprint performance in junior hurdlers (Sadowska-Krepa et al., 2015). Furthermore, Açai supplement increased time to exhaustion, enhanced cardiorespiratory responses, and decreased metabolic stress and perceived exertion in aeronautical pentathlons, runners and sprinters. Individuals in the aeronautical field, work for the Brazilian Air Force (Carvalho-Peixoto et al., 2015).
The aim of this research was to investigate the effects of Açai on muscle soreness and physical performance on collegiate athletes and non-athletes after a bout of eccentric exercise. This study tested two hypotheses:

1. Açai supplementation reduce muscular soreness after downhill running;
2. Açai supplementation will result in higher scores on range of motion vertical jump displacement and agility running compared to the placebo group.

Methods

Tiffin University and Kent State University Institutional Review Board (IRB) approved all procedures.

Recruitment and Screening

The investigation was conducted as a double blind, counterbalanced/stratified, and placebo-control trial. Data collection started following the Kent State University and Tiffin University Institutional Review Board (IRB) approval. All the data were collected at Tiffin University Exercise Science Laboratory, Tiffin, OH. Volunteers between 17–25 years of age, collegiate athletes, and non-athletes were recruited. All volunteers who met the above criteria were pre-screened in person. The pre-screening included the American Heart Association / American College of Sports Medicine (AHA/ACSM) exercise pre-participation questionnaire (Pescatello, Arena, Riebe, & Thompson, 2014) and a health history questionnaire. Volunteers identified as low risk (presented one or less cardiovascular risk factor), and no history of muscle disorder, chronic inflammation, allergies of food from the palm trees family, and lower-extremity injury in the past 3 months qualified for the investigation.
During the first visit, once the pre-screening was completed, the qualified volunteer read and completed the informed consent, and baseline data were collected. Volunteer’s performed a VO\textsubscript{2max} test and 1 repetition maximum (1RM) leg press. In addition to the above measurements, volunteers’ age, anthropometric measurements (height via a stadiometer and body mass with a Health-o-Meter 402KL Physician Balance Beam Scale), resting heart rate, and blood pressure were measured and results were recorded.

The VO\textsubscript{2max} test was used to counterbalance the Açai extract supplement group and placebo group. For example, the first volunteer in the fair category, according to the normative data provided by the American College of Sports Medicine (Pescatello et al., 2014) was placed in one group (supplement), whereas the second volunteer to score in the fair category was be placed on the opposite group (placebo). The same procedure was repeated with volunteers who scored good, excellent, or superior. Volunteers who did not fall into the fair or above categories were dismissed from the investigation.

The VO\textsubscript{2max} standardized maximal treadmill protocol included a fixed grade at 3% and an increase in speed by 1km/h each minute until maximum sustained effort. The treadmill speed was initially set at 6km/h (Drobnic et al., 2014). Expired air was sampled using the PARVO Medics TrueOne 2400 Metabolic Cart. The test was completed on a motorized treadmill (MT200 Gait Trainer Treadmill) from Spirit Medical Systems Group.

Volunteers were instructed to maintain their usual diet and to abstain from physical activity 48 hours prior to downhill running and throughout the study.
Volunteers were also asked to abstain from the use of supplements and/or sports drinks containing antioxidants and anthocyanin, a class of flavonoids, rich foods such as red, purple, or blue fruits, juices, and tea throughout the investigation. Volunteers reported their nutrient intake during each visit (Carvalho-Peixoto et al., 2015).

A 1-repetition maximum (1RM) leg press test was performed during baseline. This test was performed in order to ensure that volunteers’ fitness status would not be a variable, which could influence the results of this investigation. In order to determine if the 1RM between groups were statistically significant, volunteers’ relative muscular strength was calculated by dividing volunteers’ 1RM leg press by their body weight (relative strength = leg press 1RM / body weight).

**Demographic Measurements**

Volunteers’ age, blood pressure, heart rate, height, weight, VO_2max, and relative strength were measured.

**Eccentric Exercise Protocol**

During the second visit, volunteers performed a 30-minute run on a calibrated motorized treadmill (MT200 Gait Trainer Treadmill) from Spirit Medical Systems Group. The 30-minute run protocol consisted of a 5-minute warm-up at grade of 0% at a speed chosen by the volunteer (Drobnic et al., 2014). After the 5-minute warm-up, treadmill speed was increased until 80% of predicted heart rate was achieved. The predicted heart rate of each volunteer was calculated with the use of the Karvonen Formula (Powers & Howley, 2015). Volunteers’ heart rate was measured with the use of a polar FT7 heart rate monitor. Once the volunteer maintained the new pace for 5
minutes, the treadmill grade was adjusted to 10% decline (Miller et al., 2004). After the 15-minute downhill running, volunteers also completed a 5-minute cool down at a self-selected pace and 5 minute seated passive recovery period. A pilot study performed in the Exercise Science lab at Tiffin University demonstrated that 15 minutes of downhill running produced DOMS. According to Eston and colleagues (1995), even a relatively short period of downhill running will result in muscle damage.

**Intervention**

During the first visit upon the completion of the baseline data collection, volunteers received 5 gelatin capsules, which were given to them by an independent researcher. The gelatin capsules either had the Açai supplement or the placebo (gelatin capsules filled with Domino sugar). The ConsumerLab report for the supplement, the Vitamin Shoppe Açai extract used in this study, revealed that the supplement contain Açai Berry 5:1 Extract (*Euterpe oleracea*). In addition, other ingredients found in the Açai extract include vegetable cellulose, dicalcium phosphate, magnesium stearate, and silica. No yeast, wheat, sugar, salt, soy, diary, citrus, fish, animal derivatives, preservatives, artificial colors or flavors added were found in the Açai extract.

Volunteers took 1 gelatin capsule, 1000mg Açai extract capsule or placebo, in front of the independent individual prior to leaving the laboratory. Volunteers were then instructed to take one gelatin capsule 20 minutes before dinner on day 1 and day 2, and 20 minutes before breakfast on day 2 and day 3. Volunteers initiated the supplementation 48 hours prior to the downhill running protocol and continued for 48 hours after it (Drobnic et al., 2014). Volunteers returned the empty bag to the researcher during the
second visit on day 3, the third visit on day 4, and the fourth visit on day 5 as a confirmation that the supplement or placebo gelatin capsules were taken.

**Outcome Measures**

Agility, vertical jump displacement, knee and hip flexion, and muscle soreness were measured at baseline, immediately after downhill running, 24 and 48 hours after the exercise bout. The *t*-test was used for the agility test (Pauole et al., 2000). In order to complete the agility test, volunteers began with both feet behind the start at point 1. At their own discretion, each volunteer sprinted forward 9.14m (10 yards) to point 2. Volunteers then shuffled to the left 4.57m (5 yards) and touched the cone on point 3 with their left hand. Next, individuals shuffled to the right 9.14m and touched the cone on point 4 with their right hand and then repeated the shuffle to the left. Volunteers completed the test running backwards, passing the finish line at point 1.

Volunteers performed a familiarizing trial as they walked through the course. A clock with electronic sensor was placed at point 1. The clock started when volunteers crossed point 1 and stopped when volunteers crossed the sensor plane again. The test was repeated if volunteers failed to touch the cone, crossed their feet when shuffling, and/or did not face forward at all times. Three trials were performed and the average time was used for statistical analyses (Pauole et al., 2000).

A vertical jump trainer (VERTEC) was used in the assessment of vertical jump displacement. The first step on this test was to measure volunteers’ vertical reach. Volunteers then dropped down to a self-selected level before jumping maximally (Magnúsdóttir et al., 2014). Unrestricted countermovement and free arm swing was
allowed preceding the jump. The difference between vertical reach and jump height was used to represent volunteer’s vertical jump performance. Volunteers had an opportunity to be familiarized with the test procedure prior to data collection. Volunteers performed three trials and the average score was used for statistical analyses (Kargarfard et al., 2015).

Supine passive knee flexion and passive straight leg raise test were used to test volunteers’ knee and hip range of motion (ROM) using a goniometer. Two measurements of knee flexion and hip flexion were averaged (Markovic, 2015). Ratings of perceived muscle soreness were assessed using a Visual Analogue Scale (VAS; Gould, 2001). Volunteers rated the soreness they experienced on the quadriceps, hamstring, and gastrocnemius muscles while stepping up (concentric muscular contraction) and down (eccentric muscular contraction) onto a 40cm box (McLeay et al., 2012).

**Statistical Analysis**

All data were analyzed using Statistical Package for Social Sciences (SPSS) software (Version 20). Independent samples *t*-test was used to evaluate descriptive statistics (age, height, weight, VO\(_{2\max}\), 1RM, and resting heart rate) between the experimental and control groups. To examine the differences across four time periods (baseline, immediately after, 24 and 48 after the DOMS inducing protocol [downhill running] between the experimental group [Açai supplementation] and control [placebo] group), a mixed factor 2 groups × 4 time repeated measures ANOVA was used for each of the outcome measures. In the presence of a statistically significant F ratio, Bonferroni
correction was used for post hoc analyses. Statistical significance was set at a 95% significant level ($p < 0.05$). Data are represented as mean ± standard deviation (SD).

**Results**

Twenty-eight volunteers were recruited for the study (see Figure 2). One volunteer did not meet the VO$_{2\text{max}}$ criteria because he scored ‘poor’ on the VO$_{2\text{max}}$ test and 7 volunteers were not willing to participate in 4 blood draws. Therefore, all 8 volunteers were disqualified from the investigation, leaving 20 volunteers eligible to complete it. There were no significant differences between the placebo ($n = 10$) group and the Açai ($n = 10$) group in age, height, weight, relative strength, and VO$_{2\text{max}}$ (Refer to Table 6). The mean and standard deviation of all variables are presented on Appendix H.

![Figure 2. Recruitment of volunteers](image-url)
Table 6

Descriptive Statistics of Volunteers

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<th>Placebo</th>
<th>P-Value</th>
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<tr>
<td>Age (years)</td>
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<td>Relative Strength (kg)</td>
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<td>.805</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82±13.5</td>
<td>78.6±14.2</td>
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</tr>
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</table>

Values are represented as mean ± standard deviation. \(N=10\) for the placebo group, \(N=10\) for the Açai supplement group.

Muscle Soreness Data

There was no significant group by time interaction \((p = .704, F = .149)\) in gastrocnemius muscle soreness (see Figure 3). In addition, there were no significant differences in gastrocnemius muscle soreness between the supplement (MSGS) and the placebo (MSGP) groups \((p = .441, F = .621)\). Furthermore, there was no main effect of time \((p = .082, F = 3.38)\).

There were no significant group by time interactions \((p = .974, F = .001)\) in hamstring muscle soreness. In addition, there were no significant differences in hamstring muscle soreness between the supplement (MSHS) and the placebo (MSHP) groups \((p = .198, F = 1.78)\). However, there was main significant effect of time \((p = .031, F = 5.5)\).
Figure 3. Gastrocnemius muscle soreness. Changes in muscle soreness before, after, and 24 and 48 hours after downhill running and total average muscle soreness adding all 4-time periods. Dark bars supplement group; lighter bars placebo group. Bars represents mean ± SE.

Post Hoc Bonferroni analyses (Pairwise Comparisons), which estimated marginal means (the mean difference is significant at .05) revealed significant difference between before and 24 hours after ($p = .013$) and between 24 and 48 hours after ($p = .018$) downhill running. Furthermore, there was no significant difference between before and after ($p = .800$), between before and 48 hours after ($p = .661$), between after and 24 hours after ($p = .83$), and between after the 48 hours after ($p = 1.00$) downhill running. Specifically, there was an increase in hamstring muscle soreness (see Figure 4) in both the Açai supplement group and the placebo group 24 hours after the downhill protocol. In addition, both the Açai supplement group and the placebo group show the same effect, meaning the same pattern of pain, throughout the 4-time period.
There were significant group by time interactions ($p = .023, F = 6.3$) in quadriceps muscle soreness (see Figure 5). In addition, there was a significant difference between muscle soreness quadriceps supplement (MSQS) and the muscle soreness quadriceps placebo (MSQP) groups ($p = .011, F = 8.2$). Furthermore, there was main effect of time ($p = .002, F = 14$). Post Hoc Bonferroni analyses (Pairwise Comparisons), which estimated marginal means (the mean difference is significant at .05), revealed significant differences between before and 24 hours after ($p = .002$), between before and 48 hours after ($p = .007$), and between 24 and 48 hours after ($p = .043$) downhill running. Furthermore, there were no significant differences between before and after ($p = .149$), between after and 24 hours after ($p = .445$), and between after the 48 hours after ($p =$
1.00) downhill running. Specifically, the protocol resulted in increased quadriceps muscle soreness on both the Açai supplement group and the placebo group 24 and 48 hours after the downhill protocol. However, the soreness reported by the placebo group was significantly higher compared to the Açai supplement group.

**Figure 5.** Quadriceps muscle soreness. Changes in muscle soreness before, after, and 24 and 48 hours after downhill running and total average muscle soreness adding all 4-time periods. Dark bars supplement group; lighter bars placebo group. + = significantly different than before; * = significantly different than placebo, $p < 0.05$. Bars represents mean ± SE.

**Range of Motion Data**

There was no significant group by time interactions ($p = .519$, $F = .433$) in knee flexion (see Figure 6). In addition, there were no significant differences in knee flexion between supplement (KFS) and the Placebo (KFP) groups ($p = .423$, $F = .673$).

Furthermore, there was no main effect of time ($p = .101$, $F = 2.9$).
Figure 6. Knee ROM. Changes in knee range of motion before, after, and 24 and 48 hours after downhill running and total average ROM adding all 4-time periods. Dark bars supplement group; lighter bars placebo group. Bars represents mean ± SE.

There was no significant group by time interaction ($p = .404, F = .731$) in hip flexion (see Figure 7). In addition, there were no significant differences between hip flexion supplement (HFS) and the hip flexion placebo (HFP) groups ($p = .090, F = 3.2$). However, there was main significant effect of time ($p = .003 F = 11.9$). Post Hoc Bonferroni analyses (Pairwise Comparisons), which estimated marginal means (the mean difference is significant at .05) revealed significant difference between before and 24 hours after ($p = .006$) downhill running and between before and 48 hours after ($p = .044$) downhill running. Furthermore, there was no significant difference between before and after ($p = 1.00$), between after and 24 hours after ($p = .088$), between after the 48 hours after ($p = .215$), and between 24 and 48 hours after ($p = 1.00$) downhill running. This suggests that the protocol resulted in decreased hip flexion on both the Acai supplement
group and the placebo group after, and 24 and 48 hours after the downhill protocol compared to baseline. In addition, both the Açai supplement group and the placebo group show the same effect, meaning the same ROM pattern, throughout the 4-time period.

**Figure 7.** Hip ROM. Changes in hip range of motion before, after, and 24 and 48 hours after downhill running and total average ROM adding all 4-time periods. Dark bars supplement group; lighter bars placebo group. + = significantly different than baseline. Bars represents mean ± SE.

**Agility Test Data**

There was no significant group by time interactions \( (p = .306, F = 1.1) \) in the agility test (see Figure 8). In addition, there was no significant difference between Açai supplement agility (AgilityS) and the agility placebo (AgilityP) groups \( (p = .528, F = .413) \). However, there was main significant effect of time \( (p = .019 F = 6.6) \). Post Hoc Bonferroni analyses (Pairwise Comparisons), which estimated marginal means (the mean difference is significant at .05), revealed significant difference between before and 48
hours after ($p = .045$) downhill running. Furthermore, there was no significant difference between before and after ($p = .068$), between before and 24 hours after ($p = .323$), between after and 24 hours after ($p = 1.00$), between after the 48 hours after ($p = 1.00$), and between 24 and 48 hours after ($p = 1.00$) downhill running. Both the Açai supplement group and the placebo group were more agile 48 hours after the downhill protocol compared to baseline.

![Agility T-test](image)

*Figure 8.* Agility t-test. Changes in agility before, after, and 24 and 48 hours after downhill running and total agility scores adding all 4-time periods. Dark bars supplement group; lighter bars placebo group. $+$ = significantly different than baseline. Bars represents mean ± SE.

**Vertical Displacement Data**

There was no significant group by time interactions ($p = 1.37, F = 2.4$) in vertical displacement (see Figure 9). In addition, there were no significant differences between
vertical displacement supplement (VDS) and the vertical displacement placebo (VDP) groups \((p = .492, F = .493)\). Furthermore, there was no main effect of time \((p = .362, F = .877)\).

![Vertical Displacement Test](diagram)

**Figure 9.** Vertical Displacement test. Changes in vertical displacement before, after, and 24 and 48 hours after downhill running and total vertical displacement adding all 4-time periods. Dark bars supplement group; lighter bars placebo group. Bars represents mean ± SE.

**Discussion**

The aim of this study was to evaluate the effects of Açai on muscle soreness and physical performance on collegiate athletes and non-athletes. Both the intervention and control groups were represented equally as there were no significant differences between groups’ neither VO\(_{2\text{max}}\) nor relative strength.

Açai supplementation significantly decreased perceived muscle soreness in the quadriceps muscle compared to the placebo. However, the same effect was not seen in
the gastrocnemius and hamstring muscles. According to Brunett et al. (2010), the quadriceps muscles face a heavy eccentric load during downhill running as they brace forward momentum on the downward grade. Muscle soreness in the quadriceps muscle was not seen in female athletes while stepping up and back down a 40cm box after 300 eccentric quadriceps contractions (McLeay et al., 2012). Although muscle soreness did not reach statistical significance in both the gastrocnemius muscle and the hamstring muscle between groups, volunteers in the Acai group reported slightly less pain in all 4-time period as compared to the volunteers in the placebo group. Drobnic and colleagues (2014) saw similar results as volunteers in the curcumin group reported less pain than the volunteers in the placebo group after downhill running. Curcumin as Acai have antioxidant and anti-inflammatory properties that has been evident in innumerable experiments. Furthermore, Curcumin has shown to reduce inflammation and the negative side effects associated with eccentric-induced muscle damage (Davis et al., 2007).

There was no statistical significance between the Acai and placebo groups in the agility t-test. However, there was a significant decrease in time between before and 48 hours downhill running in both groups, which can be attributed to learning effect (Munro & Herrington, 2011). Although the results for the agility test did not reach statistical significance between groups, volunteers in the Acai group reported slightly faster times in all 4-time period as compared to the volunteers in the placebo. Other studies showed similar results in which agility did not reach statistical significance at 24 and 48 hours after the intervention (Cockburn, Bell, & Stevenson, 2013; Kargarfard et al., 2015).
However, the study by Kargarfard and colleagues (2015) showed that agility was compromised by the presence of DOMS after 72 hours (Kargarfard et al., 2015).

Non-significant results were also found in vertical displacement scores. However, volunteers in the Açai group scored slightly better over all 4-time period as compared to the volunteers in the placebo group. Previous research has shown that DOMS decreases maximal power during tasks such as vertical jump after eccentric exercise on both the supplement group and the placebo group (Kargarfard et al., 2015; Tokmakidis et al., 2003).

Although hip and knee ROM showed no significant interaction between group and time, hip flexion decreased 24 and 48 hours after downhill running in both groups. This finding is similar to the finding of Thompson and colleagues (2003) in which both the Vitamin C supplement group and the placebo groups showed decreased ROM on leg flexors and extensors.

Limitations and Future Directions

There are several limitations to this study including a small sample size, a short duration of supplementation, and a 48-hour maximum assessment period. A sample size power analysis with hamstring muscle soreness data suggests that sample size should increase to 30 to produce a power of 0.80 and alpha of 0.05. The sample size was determined by the G*Power 3.1.9.2 power analysis software.

A previous study by Jensen and colleagues (2011) documented decreased pain levels and increased range of motion 2–4 weeks after the beginning of Açai supplementation ($N = 14$). In addition, a study by Kargarfard and colleagues (2015)
showed a significant increase in vertical displacement 72 hours after the intervention ($N = 15$). Furthermore, based on the results for quadriceps muscle soreness, future studies should examine knee ROM while the volunteers lie in a prone position, as both ends of the quadriceps muscle would be extended, which will create greater stress on the muscle as it is stretched. Although learning effect in performance tests such as the $t$-test has been confirmed by previous study (Munro & Herrington, 2011), the $t$-test is considered reliable for use (Munro & Herrington, 2011; Pauole et al., 2000). In order to prevent learning effect three different types of agility test could have been used. The volunteers would be asked to perform all three agility tests during baseline; however a different test would be used after, and 24 and 48 hours after downhill running.

Lastly, future studies should add an additional assessment at 72 hours after intervention. According to previous study, vertical displacement (Kargafard et al., 2015) and muscle soreness (Kanda et al., 2013) peaked at 72 hours after intervention.
CHAPTER V

THE EFFECTS OF AÇAI (EUTERPE OLERACEA MART) ON CREATINE KINASE AND C-REACTIVE PROTEIN IN COLLEGIATE MALE ATHLETES AND NON-ATHLETES FOLLOWING DOWNHILL RUNNING

The sensation of soreness following unaccustomed eccentric muscle action or high intensity/high volume physical activity and the acute inflammatory response was first proposed during the late 1970s (L. Smith, 1991). The high tension created on the muscle tissue by eccentric exercise results in structural damage. In turn, this structural damage leads to the disruption of Ca++ homeostasis in the muscle fibers that were injured. These injured muscle fibers will then go through necrosis, which peaks 2 days after exercise. Furthermore, the accumulation of chemical substances such as bradykinin, serotonin, and histamine following muscle tissue damage elicit action potentials in free nerve endings of group-IV sensory neurons in the arterioles, capillaries, and at the musculotendinous junctions (Armstrong, 1984). The stimulation of these nociceptors sensory neurons, which are sensitive to chemicals released as a result of muscle tissue damage and inflammatory products such as CRP, lead to the sensation of DOMS (Armstrong, 1984; Norris, 2011).

C-reactive protein (CRP) is a highly conserved plasma protein, which participates in the systemic response to inflammation. Therefore, the concentration of CRP in the plasma increases after tissue injury (Black, Kushner, & Samols, 2004). CRP increases during the first few days after high-intensity or high volume exercise (Dufaux et al., 1984; Strachan et al., 1984).
Creatine kinase (CK) is an enzyme that has been used as a marker for contractile tissue damage (Deyhle et al., 2016; Köhne et al., 2016; Magrini et al., 2017). This enzyme is found in the cytosol as well as in the mitochondria of tissues in which energy demands are high (Baird et al., 2012). According to Baird and colleagues, skeletal muscle has high levels of CK. Eccentric muscle contractions in activities such as downhill running initiate cell damage and muscle cell disruption (Brown et al., 1999). This cellular disturbance can cause CK to leak from the muscle cells to the blood serum, which allows one to use the measurement of serum CK activity as an indicator of muscle tissue damage (Brancaccio, Maffulli, & Limongelli, 2007; Totsuka, Nakaji, Suzuki, Sugawara, & Sato, 2002). According to Gagliano and colleagues (2009), serum CK levels vary from 35 to 175 U/L in the general population.

Delayed onset muscle soreness (DOMS) and associated inflammation usually commences 12-24 hours following eccentric or intense exercise (Drobnic et al., 2014). It peaks in 2–3 days, and subsides linearly within 8–10 days (Clarkson et al., 1992). Even though the etiology and the cellular mechanisms of DOMS are not known, potential contributors include skeletal muscle damage and enzyme efflux and inflammation (Armstrong, 1984; Cheung et al., 2003; Peake et al., 2005). The skeletal muscle damage theory, which was first proposed by Hough in 1902, focused on the disruption of the contractile component of the muscle tissue following eccentric exercise. This theory was supported with the measurement of blood enzymes such as CK, which diffuses into the interstitial fluid following the disruption of the z-lines and damage to the sarcomere (Cleak & Eston, 1992). According to Cheung and colleagues (2003), researchers have
agreed that no single theory can explain the onset of DOMS. Furthermore, in order to
explain DOMS, some researchers have proposed a unique sequence of events, which
include: (a) the high tensile forces created by eccentric exercise damage muscle and
connective tissue; (b) this damage if following by an acute inflammatory response

Forms of treatment for DOMS include, but it is not limited to pharmaceuticals,
herbal remedies, acupuncture, stretching, ice, massage, and nutritional supplements
(Connolly et al., 2003). Although phytochemicals are not created equally, dietary
phytochemical supplementation appears to be helpful to decrease the symptoms of
DOMS following eccentric exercise such as downhill running (Panza et al., 2015).
Dietary phytochemical supplementation has positive results in decreasing the symptoms
of DOMS due to the antioxidant and anti-inflammatory properties of the phytochemicals
in the supplements (Panza et al., 2015).

The polyphenols and flavonoids (antioxidants and anti-inflammatory) present in
Açai are both important exogenous sources in the fight against free radicals and oxidative
stress caused by exercise (Paz et al., 2015). The three major anthocyanins, group of
polyphenols, found in Açai are cyanidin 3-rutoside, cyanidin 3-digllycoside, and cyanidin
3-glucoside (Jensen at al., 2008). The major flavonoids found in Açai include 2S,
3S-dihyrokaempferol, 2R,3R dihyrokaempferol, 3-O-β-D-glucoside, isovitexin, velutin,
and trimethoxyflavone (Kang et al., 2011). Açai increases the plasma antioxidant
capacity of 2.3 fold for Açai juice and 3 fold for Açai pulp (Mertens-Talcott et al., 2008).
In addition, Sadowska-Krepa and colleagues (2015) were able to demonstrate that Açai significantly improved blood antioxidant capacity and lipid profile.

The aim of this research is to investigate the effects of Açai on markers of muscle damage and inflammation on collegiate athletes and non-athletes after a bout of eccentric exercise. This study tested two hypotheses:

1. Açai supplementation will reduce CK, a marker of muscle damage, after downhill running;
2. Açai supplementation will reduce, C-reactive protein, a marker of inflammation after downhill running.

**Methods**

Tiffin University and Kent State University Institutional Review Board (IRB) approved all procedures.

**Recruitment and Screening**

On Day 1, the pre-screening and the baseline data were collected at Tiffin University Exercise Science Laboratory, Tiffin, OH. For details on pre-screening, investigation format, and volunteers’ information, see Chapter 4. The nurse practitioner, Frances Ford, who works at the Tiffin University Health and Wellness Center, supervised the phlebotomists who performed the blood draws. Each volunteer visited the Tiffin University Exercise Science Laboratory 2 times and the Health and Wellness Center 4 times in 3 days.

During the first visit, pre-screening and informed consent were completed along with baseline data collection. Volunteer’s age, resting heart rate, blood pressure, and
anthropometric measurements were recorded along the results of a VO$_2$max test and 1 repetition maximum (1RM) leg press. For details on the VO$_2$max test protocol and the 1RM leg press test, see Chapter 4.

Volunteers were instructed to maintain their diet throughout the study. In addition, volunteers were instructed to abstain from physical activity 48 hours prior to downhill running and throughout the investigation. Supplements and/or sports drinks containing antioxidants and anthocyanin, a class of flavonoids, rich food such as red, purple or blue fruits, juices, and tea were to be avoided throughout the investigation. Volunteers reported their nutrient intake during each visit (Carvalho-Peixoto et al., 2015).

**Demographic Measurements**

Volunteers’ demographic measurements were obtained during the first visit on day 1. For details on the demographic measurements, see Chapter 4.

**Eccentric Exercise Protocol**

Volunteers performed eccentric exercise during the second visit on day 3. For details on the 30-minute downhill running protocol performed by the volunteers during this investigation, see Chapter 4.

**Intervention**

During the first visit, an independent individual provided the volunteers with 5 gelatin capsules. The gelatin capsules either had the Açai supplement or the placebo, which were gelatin capsules filled with Domino sugar. For details on the supplement, how much was taken by volunteers throughout the investigation, and when supplement was taken, see Chapter 4.
**Outcome Measures**

Blood levels of creatine kinase and high sensitivity C-reactive Protein were measured at baseline, after downhill running, and 24 and 48 hours after the exercise bout. During the second visit, on day 3, samples were taken twice via a venipuncture from the antecubital space on the volunteers’ preferred arm. During the third and fourth visits, days 4 and 5, volunteers’ went back to the Health and Wellness Center so their blood could be drawn for the 24 and 48 hours period.

The investigator used the assay kits Vacuette manufactured by greiner bio-one. Mercy Laboratories, located in Tiffin, OH provided these assay kits to the investigator. According to Mercy Laboratories procedures, due to the large amount of quality control, samples were analyzed singularly. However, the analyses of samples with abnormal levels were duplicated. The assay dimeric enzyme method was used in the analysis of CK, while the assay amino turbidimetric latex particles for agglutination was used for the analysis of high sensitivity CRP (Vezdos-Lilje, 2015). CK values were obtained on a Roche-Hitachi cobas c 501 analyzer. Samples obtained from volunteers were compared to those determined using the CKL reagent on a COBA INTEGRA 800 analyzer. Furthermore, CRP levels were obtained on a Roche-Hitachi cobas c 501 analyzer. Samples obtained from volunteers were compared to those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (Vezdos-Lilje, 2017). According to Mercy laboratories chemistry coordinator, Mr. Vezdos-Lilje (2017), the coefficient of variation of the CK essay was 0.4, whereas the coefficient of variation of the hsCRP was 0.5.
Statistical Analysis

Data analyses were completed with the use of the SPSS software (Version 20). Independent samples t-test were used to examine volunteer demographics and a mixed factor 2x4 ANOVA was performed to examine changes in CRP and CK. The Post hoc analyses Bonferroni was used in the presence of significance. For details, see Chapter 4.

Results

Twenty volunteers completed the investigation. For details, see Figure 2 in Chapter 4. There were no significant differences between the placebo \((n = 10)\) group and the Açai supplement \((n = 10)\) group in any of the demographic measurements (Ch. 4, Table 6).

Creatine Kinase (CK) Data

There was no significant group by time interactions \((p = .867, F = .029)\) in creatine kinase levels. In addition, there were no significant differences between creatine kinase placebo (CKP) and the creatine kinase supplement (CKS) group \((p = .751, F = .105)\). However, there was a significant main effect of time \((p = .022, F = 6.6)\). Post Hoc Bonferroni analyses (Pairwise Comparisons), which estimated marginal means (the mean difference is significant at .05), revealed significant difference between before and after downhill running \((p = .000)\), before and 24 hours after \((p = .001)\) downhill running, between after and 24 hours after \((p = .011)\), and between 24 and 48 hours after \((p = .000)\) downhill running in the level of CK in both the supplement and placebo groups.

Furthermore, there was no significant difference between before and 48 hours \((p = .392)\) and between after and 48 hours after \((p = 1.00)\) downhill running on CK levels on both
the groups. CK levels increased on both the Açai supplement group and the placebo group after and 24 hours after the downhill protocol compared to baseline. In addition, both the Açai supplement group and the placebo group showed the same effect, meaning the same increase in decrease pattern, throughout the 4-time period. Both groups showed an increase in CK level after and 24 hours after downhill running and a decrease in CK level between 24 and 48 hours after the bout of exercise. Furthermore, the placebo group reported higher levels of CK compared to the Açai supplement group throughout the 4-time period (see Figure 10).

Figure 10. CK levels. Changes in CK levels before, after, and 24 and 48 hours after downhill running and total average CK level adding all 4-time period. Dark bars supplement group; lighter bars placebo group. + = significantly different from baseline. Bars represents mean ± SE.
C-reactive Protein (CRP) Data

There were no significant group by time interactions ($p = 1.00, F = .000$) in CRP levels (see Figure 11). In addition, there were no significant differences in CRP levels between C-reactive protein placebo (CRPP) and the C-reactive protein supplement (CRPS) groups ($p = .177, F = 1.9$). Furthermore, there was no main effect of time ($p = .504, F = .792$).

![CRP Levels](image)

*Figure 11.* CRP levels. Changes in CRP level before, after, and 24 and 48 hours after downhill running and total average CRP level adding all 4-time period. Dark bars supplement group; lighter bars placebo group. Bars represents mean ± SE.

Discussion

There were no statistical differences in CK levels between the Açai group and the placebo group. However, there was a significant difference in the main effect of time. CK levels were significantly higher after and 24 hours after the downhill running protocol in the placebo group. According to Hamill and colleagues (1991), CK response
peaks at 24 hours after downhill running; this was also seen in this investigation. CK levels dropped slightly at 48 hours and even though it was still higher than before and after the intervention. However, this difference was not significant. Although CK levels did not reach statistical significance between groups, volunteers in the Açai group had lower levels of CK in all 4-time period as compared to the volunteers in the placebo group. In other studies downhill running causes muscle damage of the same magnitude as plyometric or maximal eccentric exercises, which was shown with an increase of plasma creatine kinase (Chen, Nosaka, & Wu, 2008; Hamill et al., 1991).

The findings on this study have been similar to the findings from Drobnic et al. (2014). CK levels peaked 24 hours after a bout of 45-minute downhill running. In addition, CK levels tended to increase less in the supplement group compared to the placebo group, even though the difference in neither study was statistically significant. In another study, CK levels were not significantly different between the Ginger supplement group and the placebo group (Matsumura, Zavorsky, & Simoliga, 2015). Ginger is important in Chinese herbal medicine and it is used as an anti-inflammatory (Ali, Blunden, Tanira, & Nemmar, 2008). As Açai, Ginger blocks the production of interleukins and tumor necrosis factor alpha, which are anti-inflammatory actions that may assist in the reduction of inflammation caused by exercise-induced muscle damage (Cooke, Rybalka, Williams, Cribb, & Hayes, 2009; Xie et al., 2012).

According to Gagliano and colleagues (2009), the general population shows baseline serum CK levels between 35-175 IU/L. Otherwise, Mercy Laboratories expected values for CK levels is based on the findings from Klein and colleagues (2001).
According to Klein et al., healthy men show baseline serum CK levels between 39–308 IU/L. If compared to CK values proposed by Gagliano and colleagues, the results of this investigation indicated that both the placebo and the Açai supplement groups displayed CK levels above normal at baseline. Conversely, if compared to CK values proposed by Klein and colleagues, the results of this investigation indicated that both the placebo (207.1 IU/L) and the Açai supplement (202.8 IU/L) groups displayed normal CK levels at baseline.

Volunteers in the Açai group showed slightly lower CRP levels in all 4-time period as compared to the volunteers in the placebo group. CRP level showed very small change within the volunteers in the Açai group, while it peaked in the placebo group 24 hours after downhill. These results match findings from Strachan and colleagues (Strachan et al., 1984).

In contrast, the findings of this investigation differ from several other studies. Although in this study, CRP returned to baseline by 48 hours, Taylor and colleagues (1997) reported a significant increase in CRP level 24 hours after 21km of canoeing, 97 km of cycling, and 42km of running. Weight and colleagues (1991) also reported significant increase in CRP levels after and 24 hours after a 42km marathon race.

**Limitations and Future Directions**

This study has several limitations including a small sample size, limited biomarker analysis, and low intensity/duration during downhill running. A power analysis of data for CRP suggests that a sample size of 60 would provide a power of 0.80 and an alpha of 0.05. Several additional biomarkers could be examined including
inflammatory cytokines IL-6 and TNF-α (Xie et al., 2012). Furthermore, Açai pulp has a unique flavone called velutin that can reduce levels of tumor necrosis factor - α (TNF-α) and Interleukin-6 (IL-6; Xie et al., 2012).

It is possible that increasing the duration and intensity of downhill running could yield different results. Strachan and colleagues (1984) reported that levels of CK and CRP increase with increased in exercise length and/or intensity.
CHAPTER VI

SUMMARY

This study suggests that 2000mg of Açai Berry 5:1 Extract (Euterpe oleracea Mart) may be beneficial to attenuate perceived DOMS-related muscle pain in the quadriceps muscle. While the results on the hamstring and gastrocnemius muscle soreness along with the performance tests were not statistically significant, volunteers in the Açai group recorded less muscle soreness and scored better in all of the performance tests including agility, muscular power, and flexibility before, after, and 24 and 48 hours after downhill running compared to volunteers in the placebo group.

Although the results for CK and CRP were also not statistically significant between the Açai group and the placebo group, volunteers on the Açai group showed lower CK and CRP levels before, after, and 24 and 48 hours after downhill running compared to the placebo group. Furthermore, both groups showed the same effect throughout the 4-time period on both the CK and CRP levels.

Future research should build upon this investigation by increasing the sample size, increase length of supplementation and evaluation, and add the analysis of IL-6 and TNF-α blood biomarkers for inflammation levels. In addition, it is possible that 15 minutes downhill running at 80% of maximum heart rate was not enough to elicit significant changes in blood biomarkers and performance. Therefore, future studies should review the optimal intensity and duration of downhill running.
APPENDICES
APPENDIX A

LETTER OF INFORMED CONSENT
Appendix A

Letter of Informed Consent

Title: THE EFFECTS OF AÇAI (EUTERPE OLERACEA MART) ON DELAYED MUSCLE SORENNESS (DOMS) ON COLLEGIATE MALE ATHLETES

Name of Researcher: Ana Paula Fantini
Department of Arts & Sciences
Tiffin University
Phone # 419-448-3373
fantiniap@tiffin.edu

I am a faculty member at Tiffin University in the Department of Arts & Sciences. As part of the fulfillment of the requirements for the degree of Doctor of Philosophy in Exercise Physiology at Kent State University, I am conducting this research under the supervision of Dr. Angela Ridgel and Dr. Ellen Glickman. I am inviting you to take part in this research. Your participation is voluntary. As an athlete, you might gain benefits from this study as your performance might be affected positively. You may withdraw from the study at any time. The research is described below. This description informs you about the purpose, benefits, potential risks or discomfort that you may experience along with your responsibilities as a participant and my responsibilities as a researcher. I encourage you to discuss with me any questions you might about this research prior to signing this form.

Purpose
This research is being conducted to test the effectiveness of Açai (Euterpe Oleracea Mart) extract on delayed onset muscle soreness following downhill running. The researcher will examine if the fruit’s extract leads to decrease in muscle damage and increase in performance. Participants’ muscle soreness, knee and hip joints’ range of motion, vertical jump displacement, agility, and blood C-reactive Protein and Protein Kinase will be assessed. The research will last 4 consecutive days and it will be conducted Exercise Science Laboratory at Tiffin University.

Benefits & Potential Risks
The results obtained in the preparticipation period will determine if you should be able to exercise safely without consulting your physician or other appropriate health care provider. Participants’ exercise capacity and state of cardiovascular health will be determined through the VO2max test. Furthermore, the results will show if Açai’s extract attenuates the effects of DOMS and improve performance.

Participants’ medical history and AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire will be completed prior to participation to assure that you have no medical conditions that would make your participation in this
Research dangerous for you. In addition, Tiffin University Health and Wellness Center will provide medical assistance in the case unusual situations arise. The participation in this study is associated with delayed onset muscle soreness, which will subside linearly within 8-10 days. Furthermore, the participant might experience hematoma and pain from blood draw. Your participation is completely voluntary. You may withdraw from this research at any time.

**Responsibilities of the Participant**

Participants’ have the responsibility of promptly report previous experiences of heart-related symptoms such as shortness of breath with low-level activity, pain, pressure, tightness, and/or heaviness in the chest, neck, jaw, back, and/or arms. These and other unusual feelings with effort during exercise are very important to be noted by the participant as it may affect the safety of participants’ exercise test. Therefore, participants are responsible for fully disclosing their medical history including medication taken, allergies, etc., as well as symptoms that may occur during the test. Furthermore, participants must follow the instructions given to them by the researcher throughout the study.

**Responsibilities of the Researcher**

All the information in this research will be kept strictly confidential and anonymous. There will not be any identifying information on any of the forms if not necessary. To further protect participants’ identities, documents with identification, including this letter of informed consent will be sealed in an envelope and stored separately. Additionally, the results of this study will be presented as a group and no individual participants will be identified.

This research has been reviewed and approved by the Institutional Review Board (IRB). If you have any questions, please contact Professor Ana Paula Fantini by phone at 865-456-9797 or e-mail at fantiniap@tiffin.edu.

**By signing this letter of informed consent, you are indicating that you fully understand the above information and agree to participate in this research.**

Participant’s Signature: __________________________ Date: _____/_____/_____

Researcher’s Signature: __________________________ Date: _____/_____/_____
APPENDIX B

PRE-PARTICIPATION HEALTH SCREENING
Appendix B

Pre-Participation Health Screening

On the next page you will find the American Heart Association (AHA)/American College of Sports Medicine (ACSM) Health/Fitness Facility Preparticipation Screening Questionnaire. Individuals with multiple Cardiovascular Disease (CVD) risk factors (see below) should be encouraged to consult with their physician prior to initiating a vigorous intensity exercise program as part of good medical care and should progress gradually with their exercise program of any exercise intensity. Please complete the form on the next page as accurately as you can.

Atherosclerotic Cardiovascular Disease (CVD) Risk Factors and Defining Criteria
<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Defining Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Men $\geq 45$ yr; women $\geq 55$ yr (12)</td>
</tr>
<tr>
<td>Family history</td>
<td>Myocardial infarction, coronary revascularization, or sudden death before 55 yr in father or other male first-degree relative or before 65 yr in mother or other female first-degree relative</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>Current cigarette smoker or those who quit within the previous 6 mo or exposure to environmental tobacco smoke</td>
</tr>
<tr>
<td>Sedentary lifestyle</td>
<td>Not participating in at least 30 min of moderate intensity, physical activity ($40%-&lt;60%$ VO$_{2\text{R}}$) on at least 3 d of the week for at least 3 mo (22,30)</td>
</tr>
<tr>
<td>Obesity</td>
<td>Body mass index $\geq 30$ kg $\cdot$ m$^{-2}$ or waist girth $&gt;102$ cm (40 in) for men and $&gt;88$ cm (35 in) for women (10)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Systolic blood pressure $\geq 140$ mm Hg and/or diastolic $\geq 90$ mm Hg, confirmed by measurements on at least two separate occasions, or on antihypertensive medication (9)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Low-density lipoprotein (LDL) cholesterol $\geq 130$ mg $\cdot$ dL$^{-1}$ (3.37 mmol $\cdot$ L$^{-1}$) or high-density lipoprotein (HDL) cholesterol $&lt;40$ mg $\cdot$ dL$^{-1}$ (1.04 mmol $\cdot$ L$^{-1}$) or on lipid-lowering medication. If total serum cholesterol is all that is available, use $\geq 200$ mg $\cdot$ dL$^{-1}$ (5.18 mmol $\cdot$ L$^{-1}$) (21)</td>
</tr>
<tr>
<td>Prediabetes*</td>
<td>Impaired fasting glucose (IFG) = fasting plasma glucose $\geq 100$ mg $\cdot$ dL$^{-1}$ (5.55 mmol $\cdot$ L$^{-1}$) and $\leq 125$ mg $\cdot$ dL$^{-1}$ (6.94 mmol $\cdot$ L$^{-1}$) or impaired glucose tolerance (IGT) = 2 h values in oral glucose tolerance test (OGTT) $\geq 140$ mg $\cdot$ dL$^{-1}$ (7.77 mmol $\cdot$ L$^{-1}$) and $\leq 199$ mg $\cdot$ dL$^{-1}$ (11.04 mmol $\cdot$ L$^{-1}$) confirmed by measurements on at least two separate occasions (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative Risk Factors</th>
<th>Defining Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-density lipoprotein (HDL) cholesterol</td>
<td>$\geq 60$ mg $\cdot$ dL$^{-1}$ (1.55 mmol $\cdot$ L$^{-1}$)</td>
</tr>
</tbody>
</table>

*If the presence or absence of a CVD risk factor is not disclosed or is not available, that CVD risk factor should be counted as a risk factor except for prediabetes. If the prediabetes criteria are missing or unknown, prediabetes should be counted as a risk factor for those $\geq 45$ yr, especially for those with a body mass index (BMI) $\geq 25$ kg $\cdot$ m$^{-2}$, and those $<45$ yr with a BMI $\geq 25$ kg $\cdot$ m$^{-2}$ and additional CVD risk factors for prediabetes. The number of positive risk factors is then summed.

*High HDL is considered a negative risk factor. For individuals having high HDL $\geq 60$ mg $\cdot$ dL$^{-1}$ (1.55 mmol $\cdot$ L$^{-1}$), for these individuals one positive risk factor is subtracted from the sum of positive risk factors.
Assess your health status by marking all true statements

History
You have had:

___ a heart attack
___ heart surgery
___ cardiac catheterization
___ coronary angioplasty (PTCA)
___ pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ heart valve disease
___ heart failure
___ heart transplantation
___ congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Symptoms
___ You experience chest discomfort with exertion
___ You experience unreasonable breathlessness
___ You experience dizziness, fainting, or blackouts
___ You experience ankle swelling
___ You experience unpleasant awareness of a forceful or rapid heart rate
___ You take heart medications

Other health issues
___ You have diabetes
___ You have asthma or other lung disease
___ You have burning or cramping sensation in your lower legs when walking short distance
___ You have musculoskeletal problems that limit your physical activity
___ You have concerns about the safety of exercise
___ You take prescription medications
___ You are pregnant

Cardiovascular risk factors
___ You are a man ≥45 yr
___ You are a woman ≥55 yr
___ You smoke or quit smoking within the previous 6 mo
___ Your blood pressure is ≥140/90 mm Hg
___ You do not know your blood pressure
___ You take blood pressure medication
___ Your blood cholesterol level is ≥200 mg • dL⁻¹
___ You do not know your cholesterol level
___ You have a close blood relative who had a heart attack or heart surgery before age 65 (father or brother) or age 65 (mother or sister)
___ You are physically inactive (i.e., you get <30 min of physical activity on at least 3 d per week)
___ You have a body mass index ≥30 kg • m⁻²
___ You have prediabetes
___ You do not know if you have prediabetes

___ None of the above

If you marked two or more of the statements in this section you should consult your physician or other appropriate health care as part of good medical care and progress gradually with your exercise program. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guide program or almost any facility that meets your exercise program needs.

*Professionally qualified exercise staff refers to appropriately trained individuals who possess academic training, practical and clinical knowledge, skills, and abilities commensurate with the credentials defined in Appendix D.
Logic model for classification of risk
Cardiovascular (CV) & Cardiovascular disease (CVD)

Review Health/Medical History for: Known Disease, Signs/Symptoms, CVD Risk Factors

Known CV, Pulmonary, Metabolic Disease? (see Table 2.3)

Cardiovascular: Cardiac, peripheral vascular, or cerebrovascular disease
Pulmonary: COPD, asthma, interstitial lung disease, or cystic fibrosis
Metabolic: Diabetes mellitus (Types 1 and 2) or renal disease

Pain, discomfort in the chest, neck, jaw, arms, or other areas that may result from ischemia
Shortness of breath at rest or with mild exertion
Dizziness or syncope
Orthopnea or paroxysmal nocturnal dyspnea
Ankle edema
Palpitations or tachycardia
Intermittent claudication
Known heart murmur
Unusual fatigue or shortness of breath with usual activities

Major Signs or Symptoms Suggestive of CV, Pulmonary, Metabolic Disease?

Yes

Number of CVD Risk Factors

Age
Family History
Current Cigarette Smoking
Sedentary Lifestyle
Obesity
Hypertension
Dyslipidemia
Prediabetes

<=2

High Risk

Moderate Risk

Low Risk

<2

ACSM’s Guidelines for Exercise Testing and Prescription (9th Ed.).

Participant’s Risk Category: ________________

___________________________ ________________________________
Printed Name Signature Date
APPENDIX C

HEALTH HISTORY QUESTIONNAIRE
Appendix C

Health History Questionnaire

It is important to have an accurate assessment of your health status prior to the beginning of your participation to assure that you have no medical conditions that would make the test dangerous for you. This medical history form is confidential and only the researchers in this study will review it.

Participant’s #: __________________________________________________________

Date of Birth: ______/_____/______  Age: ____________________ in years

Height: ___________________________  Weight: ___________________________

Resting Heart Rate: _______________  Blood pressure: __________/_________

Ethnic group: ______ White  ______ African American  ______ Hispanic

_________ Asian  ______ Pacific islands  ______ American Indian ______ Other _________________________

Chronic Inflammation
Do you have a medical history of chronic inflammation?  ______ Yes  ______ No

Muscle Disorders
Do you have a medical history of muscle disorders?  ______ Yes  ______ No

Medication
Have you had immunosuppressant within the last two days?  ______ Yes  ______ No
Have you had anti-inflammatory within the last two days?  ______ Yes  ______ No

Allergies
Do you have any known allergies of food from the palm trees family such as dates or coconuts?  ______ Yes  ______ No

Have you had any lower extremity injury in the past 3 months?  ______ Yes  ______ No
Explain _____________________________________________________________

Do you feel that there are any other reason (s) not mentioned above for you not to participate in this research?  ______ Yes  ______ No
Explain _____________________________________________________________

________________________________________________________
Printed Name  Signature  ______/_____/______
APPENDIX D

PARTICIPANTS’ INSTRUCTIONS
Appendix D

Participants’ Instructions

1. Participants should maintain their usual diet and to abstain from the use of supplements and/or sports drinks containing antioxidants and anthocyanin rich food such as red, purple or blue fruits, juices, and tea throughout the study.

2. Participants will consume 500mg of Acai extract or matching placebo, twice a day, 20 minutes before breakfast and 20 minutes before dinner. Participants will initiate the supplementation 48 hours prior to the test and continue for 48 hours after the downhill running.

3. Participants should be rested and hydrated consuming 500ml of water starting 30 minutes before downhill running. Participants will also be allowed to drink water throughout the tests.

4. Participants must abstain from physical activity 48 hours prior to the beginning of the exercise protocol and throughout the study.

5. Participants should wear clothing that permit freedom of movement and include running shoes throughout the study.
Appendix E

Timeline of Events

**Day 1** – Baseline Data Collection
1. Letter of informed consent, Health History Questionnaire, Pre-participation Health Screening, anthropometric measurements, and discussion of participants’ instructions – Time 14 minutes
2. Muscle Soreness Assessment – Time 2 minutes
3. Joint Flexibility Assessment – Time 10 minutes
4. Vertical Jump Displacement Test – Time 2 minutes
5. Agility Test – Time 2 minutes
6. 1RM – Leg press – Time 5 minutes
7. VO$_{2\text{max}}$ Test – Times 20 minutes

Total Visit Time: 55 minutes approximately

**Day 2** – Take Placebo at Breakfast and Dinner
1. Meet researcher in the morning to consume Açai or placebo supplementation

Total Visit Time: 2 minutes approximately

**Day 3** – Downhill Running Protocol and Data Collection
1. Blood Sample – 5 minutes
2. Downhill running protocol – 30 minutes
3. Muscle Soreness Assessment – Time 2 minutes
4. Joint Flexibility Assessment – Time 10 minutes
5. Vertical Jump Displacement Test – Time 2 minutes
6. Agility Test – Time 2 minutes
7. Blood Sample – Time 5 minutes
8. Supplementation/Placebo - 2 minutes

Total Visit Time: 58 minutes approximately

**Day 4** – Perform 24 hour follow-up Data Collection
1. Muscle Soreness Assessment – Time 2 minutes
2. Joint Flexibility Assessment – Time 10 minutes
3. Vertical Jump Displacement Test – Time 2 minutes
4. Agility Test – Time 2 minutes
5. Blood Sample – Time 5 minutes
6. Supplementation/Placebo - 2 minutes

Total Visit Time: 23 minutes approximately

**Day 5** - Perform 48 hour follow-up Data Collection
1. Muscle Soreness Assessment – Time 2 minutes
2. Joint Flexibility Assessment – Time 10 minutes
3. Vertical Jump Displacement Test – Time 2 minutes
4. Agility Test – Time 2 minutes
5. Supplementation/Placebo - 2 minutes
6. Blood Sample – Time 5 minutes

Total Visit Time: 23 minutes
APPENDIX F

DATA COLLECTION SHEET
Appendix F

Data Collection Sheet

Volunteer’s #: _____________________________ Date: ____/___/____

**VO2max Test**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Score</th>
<th>Classification</th>
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<tbody>
<tr>
<td>VO2max Assessment</td>
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**Blood Sample**

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<thead>
<tr>
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<th>Baseline</th>
<th>After Downhill Running</th>
<th>24 hours after Downhill Running</th>
<th>48 hours After Downhill Running</th>
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<tbody>
<tr>
<td>C-reactive Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Creatine Kinase</td>
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**Perceived Muscle Soreness**

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<th>24 hours</th>
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<td>Perceived Muscle Soreness</td>
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<td>Score:</td>
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*Joint Flexibility Assessment – Range of Motion*
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<th>Baseline</th>
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<th>24 hours</th>
<th>48 hours</th>
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</thead>
<tbody>
<tr>
<td>Knee Joint</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
</tr>
<tr>
<td></td>
<td>2nd Trial:</td>
<td>2nd Trial:</td>
<td>2nd Trial:</td>
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<tr>
<td></td>
<td>Average:</td>
<td>Average:</td>
<td>Average:</td>
<td>Average:</td>
</tr>
<tr>
<td>Ankle Joint</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Average:</td>
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**Vertical Jump Displacement Test**

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<th>48 hours</th>
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</thead>
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<td>Vertical Jump Displacement</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
</tr>
<tr>
<td></td>
<td>2nd Trial:</td>
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<tr>
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<td>Best Score:</td>
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**Agility Test – T-Test**

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<th>48 hours</th>
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<tr>
<td>Agility Test</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
<td>1st Trial:</td>
</tr>
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<td>2nd Trial:</td>
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<td>Average:</td>
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**IRM Test**
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<th>Classification</th>
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<td>1RM Assessment</td>
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APPENDIX G

VISUAL ANALOGUE SCALE (VAS)
Appendix G

Visual Analogue Scale (VAS)

Pain Assessment (VAS)

Volunteer’s #: _____________________________ Date: _____/_____/_____

Place a vertical mark on the line below to indicate your level of pain today upon the completion of the activity.

No Pain | Very Severe Pain

Gastrocnemius VAS

No Pain | Very Severe Pain

Quadriceps VAS

No Pain | Very Severe Pain

Hamstring VAS
APPENDIX H

MEAN ± STANDARD DEVIATION OF ALL VARIABLES
### Appendix H

**Mean ± Standard Deviation of all Variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>After Downhill Running</th>
<th>24 hours after Downhill Running</th>
<th>48 hours after Downhill Running</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (Açaí)</td>
<td>202.8±63</td>
<td>234.5±75</td>
<td>333.9±115</td>
<td>249.5±92</td>
</tr>
<tr>
<td>CK (Placebo)</td>
<td>207±97.8</td>
<td>244.5±113</td>
<td>362.2±124.6</td>
<td>259±91</td>
</tr>
<tr>
<td>CRP (Açaí)</td>
<td>0.46±0.3</td>
<td>0.46±0.3</td>
<td>0.47±0.2</td>
<td>0.43±0.2</td>
</tr>
<tr>
<td>CRP (Placebo)</td>
<td>0.75±0.5</td>
<td>0.8±0.6</td>
<td>0.9±1</td>
<td>0.68±0.6</td>
</tr>
<tr>
<td>Hip ROM (Açaí)</td>
<td>78±4.9</td>
<td>76±7.3</td>
<td>72±5.4</td>
<td>73±4.3</td>
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<tr>
<td>Hip ROM (Placebo)</td>
<td>69.7±12</td>
<td>69.2±10</td>
<td>66±10</td>
<td>67±11</td>
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<tr>
<td>Knee ROM (Açaí)</td>
<td>148.5±6</td>
<td>148.7±6</td>
<td>148±5</td>
<td>149±5</td>
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<tr>
<td>Knee ROM (Placebo)</td>
<td>146.8±4</td>
<td>146±4</td>
<td>147.5±4.6</td>
<td>148±4.8</td>
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<td>Agility (Açaí)</td>
<td>8.2±0.4</td>
<td>7.8±0.5</td>
<td>7.9±0.4</td>
<td>7.9±0.6</td>
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<td>Agility (Placebo)</td>
<td>7.8±0.6</td>
<td>7.8±0.6</td>
<td>7.8±0.6</td>
<td>7.7±0.5</td>
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<tr>
<td>Vertical Jump. Disp. (Açaí)</td>
<td>58.3±7.3</td>
<td>58.5±7.3</td>
<td>57.5±6.1</td>
<td>57.5±7.2</td>
</tr>
<tr>
<td>Vertical Jump. Disp. (Placebo)</td>
<td>54.7±10.6</td>
<td>55.2±11.2</td>
<td>55.5±11.1</td>
<td>54.9±10.9</td>
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<tr>
<td>MS Quadriceps (Açaí)</td>
<td>0.08±0.07</td>
<td>0.96±1.3</td>
<td>1.25±1.4</td>
<td>0.43±0.4</td>
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<tr>
<td>MS Quadriceps (Placebo)</td>
<td>0.54±0.5</td>
<td>2.1±2.4</td>
<td>3.6±2.5</td>
<td>2.3±1.7</td>
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<tr>
<td>MS Hamstring (Açaí)</td>
<td>0.28±0.48</td>
<td>0.32±0.47</td>
<td>1.75±2.21</td>
<td>0.6±0.83</td>
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<tr>
<td>MS Hamstring (Placebo)</td>
<td>0.61±0.55</td>
<td>1.29±1.35</td>
<td>2.14±1.73</td>
<td>1.3±1.15</td>
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<tr>
<td>MS Gastrocnemius (Açaí)</td>
<td>0.11±0.16</td>
<td>0.8±1.5</td>
<td>1.3±2.3</td>
<td>1.47±2.4</td>
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<td>MS Gastrocnemius (Placebo)</td>
<td>0.6±0.98</td>
<td>1.4±2.2</td>
<td>1.89±2.26</td>
<td>1.03±1.28</td>
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</table>
REFERENCES
REFERENCES


Jensen, G., Ager, D., Redman, K., Mitzner, M., Benson, K., & Shauss, A. (2011). Pain reduction and improvement in range of motion after daily consumption of acai
(Euterpe oleracea Mart) pulp-fortified polyphenolic-rich fruit and berry juice blend. *Journal of Medical Food, 14*, 702-711.


