THE EFFECTS OF ASHWAGANDHA ON DELAYED ONSET MUSCLE SORENESS

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Submitted to the Faculty of the Graduate College of the

Oklahoma State University in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

July, 2021

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DELAYED ONSET MUSCLE SORENESS

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ACKNOWLEDGEMENTS

"Life moves pretty fast. If you don't stop and look around once in a while, you could miss it"- Ferris Bueller

Life sure has moved fast for me, especially when trying to finish a doctorate in 3 years. Throwing in a global pandemic really sped things up a bit and slowed things down at the same time and allowed me the chance to look around at all of the people responsible for helping me achieve my goals of pursuing my doctorate in such an incredible field. I'm extremely thankful for not only my time at Oklahoma State University, but for the connections, experiences, and relationships formed along the way.

I personally want to thank my advisor and now colleague, Dr. Bert Jacobson. I want to thank you for taking a chance on me and providing the funds, advice, and opportunities to nurture my passion of education and well-being. I want to thank you for always having an open door and allowing me to come "bug you" whenever I needed to. I

also want to thank my committee members, Dr. Jam Khojasteh, Dr. Michael Trevino, and Dr. Jay Dawes. You all have had an incredible impact on my journey throughout my dissertation, as well as in the classroom. Being able to set up Zoom meetings or walk down the hallway to ask for input has been such a blessing.

I want to thank my parents Garry and Rhonda Diehl for your unwavering support throughout this last decade of school. Your constant encouragement and motivation has allowed me to pursue my dreams and build a life few get to experience. Words cannot express my gratitude fully and I love you so much for all that you've done for me. I also want to thank my Fiancé Kersten. You have been my rock throughout all of my endeavors

and have pushed me even through the hardships. I also want to thank my sister Katelyn and brother-in-law Ruwan. You both have helped me so much throughout my education and have been such a wonderful support system. Katelyn, not many sisters are willing to help their little brother by getting their brain zapped all for the good of science. Thank you!

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Date of Degree: JULY, 2021

Title of Study: THE EFFECTS OF ASHWAGANDHA ON DELAYED ONSET MUSCLE SORENESS

Major Field: HEALTH AND HUMAN PERFORMANCE

Abstract: Delayed onset muscle soreness (DOMS), is a phenomenon that typically occurs within 8-48 hours, and can peak within 24-72 hours after a bout of intense resistance training[1]. To combat this pain, analgesics such as Nonsteroidal Anti-inflammatory Drugs (NSAIDs) are commonly used. Many negative risks and side effects are associated with NSAID use including but not limited to: gastrointestinal (GI) distress, ulcers, hypertension, acute renal failure, and other cardiovascular related incidences [25]. Ashwagandha is an Indian herbal supplement believed to have anti-inflammatory and analgesic properties. The purpose of this study was to assess the effects of Ashwagandha on DOMS following a bout of intense exercise. Fifteen (n=15) college aged individuals (18-23 years of age), volunteered to participate in this study and were randomly placed in either an experimental condition (n=8), (750mg dose of Ashwagandha), or a placebo condition (n=7). The subjects completed an intense lower-body exercise protocol consisting of 5 sets of 20 weighted lunges at 40% body weight, along with 3 sets to failure of 75% body weight on a leg press machine. Testing measurements consisted of a Visual Analog Scale (VAS), pain pressure threshold, thigh circumference, peak power output, ground contact time, peak velocity, and vertical jump. Measurements were taken at pre-test, post resistance training bout, day 3, and day 6. VAS measurements were recorded days 1-5. Statistical analysis showed a statistically significant difference in peak power (W) output on Day 3 in the experimental group, (M= 1659.873, SD 614.104) compared to the control group (M=1401.214, SD= 306.669), $t(10.558)=1.051$, $p<0.016$. No other measurements showed statistically significant difference among the groups. This demonstrates Ashwagandha has the ability to improve, or maintain peak power output measures on the third day following an intense bout of lower body resistance training exercises.

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CHAPTER I

Introduction

1.1 Introduction

Soft tissue soreness is a sensation that occurs as a result of intense bouts of physical activity and can be felt immediately, hours after, or even days following activity. Muscle soreness is classified as a type I muscle strain and refers to the immediate soreness perceived by the athlete, patient, or subject immediately after participating in exercise [2]. Increases in mechanical stress trigger inflammatory responses within the muscle cell causing the production of reactive oxygen species, which in turn promote the transcription of nuclear factor kB (NF-kB) which leads to secondary muscle damage. This secondary muscle damage can lead to prolonged decreases in muscular strength, power, range of motion, and increased creatine kinase (CK). This in turn causes the immediate sensation of soreness or pain[3]. This pain is usually short lived and can alleviate almost immediately following the bout of exercise or activity. In contrast, delayed onset muscle soreness (DOMS) is the sensation of pain or soreness that is prolonged after exercise and can peak in intensity from 24-72 hours post exercise and usually subsides within 5-7 days post-exercise[4]. It is theorized that the primary sequence of muscle soreness begins with the disruption of sarcomeres, followed by the impaired excitation-contraction coupling, calcium signaling, and finally the activation of calcium-sensitive degradation pathways [5].

The mechanisms behind DOMS are not fully understood and there are many competing theories behind the phenomenon. Some of the claims behind the cause of DOMS revolve around: lactic acid accumulation[6] , muscle spasms[7, 8], microtrauma[9, 10], connective tissue damage[11], inflammation[12], and electrolyte and enzyme efflux[13].

The largest contributor to DOMS stems from eccentric muscle activity where the force that is developed, can be as great as twice that developed during isometric muscle contractions. This is counterintuitive because the total number of active cross bridges in a strongly bound state is approximately only 10% greater than during an isometric activity [14]. Eccentric muscle actions result from an external load or force that is greater than the force that the muscle exerts, thus causing the muscle to lengthen. This eccentric contraction is also known as negative work due to the fact that the movement is in the opposite direction of the force generated [15]. Since the force generated by the muscle is maximized by eccentric contractions, fatigue and the sensation of soreness is greater compared to other muscle contractions such as isokinetic and isotonic actions. When eccentric muscle contractions are performed, the muscle itself acts similar to a shock absorber. Locomotor muscles provide a spring like, shock absorbing function especially during descents or inclines. Lastayo et al. has even shown that during normal locomotion, muscles are collectively doing near equal amounts of positive and negative work. The energy that is absorbed during the muscle tendon stretch can be dissipated as heat, as well as the elastic strain energy can be stored and recovered if an immediate shortening concentric contraction immediately follows [15]. Once a muscle has been eccentrically contracted and fatigue has occurred, decreases in maximal force production has been seen as early as one hour after exercise. This sensation of fatigue can be felt as severe and similar to pain. Researchers have

suggested that there is not only a sensory condition[14] present, but also an affective component such as related unpleasantness of the soreness [16].

The earliest roots of today's medicine and pain management dates back to European medicine in the 17th century with the use of opium, laudanum, and the mixture of opium in sherry [17]. As technology advances, so does our ability to understand chemicals, herbs, and natural products at the cellular level. By the beginning of the 19th century, scientists were able to extract and dissect therapeutic drugs such as opium into different molecules causing the introduction into morphine [18]. Further isolation of ingredients led to the formation of non-opioid analgesics. For example, scientists were able to extract the glycoside of salicylic acid from white willow bark that has been shown to decrease pain. While analgesics such as morphine have been shown to be effective at treating severe bouts of pain, common soreness and pain relief from daily activities does not require such potent analgesics [19]. For instance, Cheshier, et al. discovered White Willow Bark has the capability of decreasing pain due to delayed onset muscle soreness [20]. The salicylic acid and salicylates are the ingredients found in the plants and herbs and have been used for thousands of years. The salicylic acid was first chemically synthesized in 1860, thus becoming the first form of Aspirin as we know it today. These aspirin like drugs are also known as non-steroidal anti-inflammatory drugs (NSAIDs), and are one of today's most commonly used forms of pain management [21]. While NSAIDs have been shown to be quite effective at pain management even in those with osteoarthritis of the knee, physicians still have difficulty prescribing the proper dose for each individual [22]. Once a patient has been dosed properly, NSAIDs show great effectiveness at mitigating soreness and pain but can come with a myriad of other negative effects. NSAIDs are one of the most successful drugs in modern medicine in

alleviating pain, fever, as well as decreasing inflammation. A retrospective study completed in 2015 evaluating 3,050 subjects with chronic pain, showed 97% of the population used NSAIDs for at least 21 consecutive days[23]. While the effectiveness has been shown, the American Geriatric Society has recommended that the chronic use of all NSAIDs, especially high dose aspirin should be avoided due to the increased risk of gastrointestinal (GI), bleeding[24]. While NSAIDs are by far the most common and most cost effective remedies for pain management from activities of daily living, they also come with a price. NSAIDs may cause GI ulcers, GI distress, serious cardiovascular events, hypertension, acute renal failure, and worsening of preexisting heart failure [25]. If those negative effects are not enough to persuade someone to search for an alternative pain management solution, research has also shown adverse effects after chronic NSAID use in the areas of: acute kidney injury (AKI), hypertension, dementia and cognitive decline, depression, decreased bone mineral density (BMD), urinary incontinence, psychiatric symptoms including psychosis, agitation, anxiety, paranoia, delirium, mania and hallucinations, increased cancer risk, stroke, and finally, other drug interactions [24].

The myriad of potential negative side effects from NSAIDs has caused extensive research to be done in the field of herbal supplements, nutritional supplements, and nutraceuticals. While western medicine has exponentially furthered the technology and success rate in modern medicine, traditional eastern medicine practices can provide an alternative to remedy pain in comparison to NSAIDs without the risk of negative effects. Like many other obscure herbal supplements, Ashwagandha has received very little attention with regards to formal research in the area of its anti-inflammatory properties and ability to reduce pain similar to aspirin.

1.2 Purpose:

Currently no research has been done on the effects of Ashwagandha on delayed onset muscle soreness (DOMS). Currently, research has shown positive effects of other nutritional supplements as a means for combating inflammation, soreness, and pain associated with exercise and specifically delayed onset muscle soreness. The known supplements that have extensive research to back their claims of prevention and treatment of DOMS are: saffron, turmeric, caffeine, ginger, cinnamon, black tea, pomegranate juice, chamomile, watermelon juice, cherry juice, and garlic [26]. Recently, the popularity and interest surrounding Ashwagandha has started to increase, but the research supporting the effectiveness of this supplement is rare. Therefore, the purpose of this study was to evaluate the effects of Ashwagandha on DOMS, athletic performance, and lower limb inflammation and sensitivity.

1.3 Specific Aims:

1. To investigate if Ashwagandha has any effect on the parameters of DOMS, (perceived pain, soreness in thigh/swelling), compared to placebo following an intense, exhaustive lower-body resistance training protocol.
2. To investigate if Ashwagandha could aid in the maintenance of vertical jump height, following an exhaustive lower body resistance training session.

1.4 Research Questions

1. Does Ashwagandha decrease DOMS compared to placebo following an exhaustive lower body resistance training session?
2. Does Ashwagandha improve or maintain vertical jump height (peak power output) compared to placebo following an exhaustive lower body resistance training session?

1.5 Hypotheses

- H: Ashwagandha (Withania Somnifera) will have an effect on delayed onset muscle soreness (DOMS)?
- H1: Ashwagandha will have a statistically significant effect on DOMS and thigh and calf circumference, and perceived pain.
- Ho: No statistical significant differences will be noted with regards to Ashwagandha and DOMS.
- Ho_2: No statistical significant difference will be noted in regards to Ashwagandha and thigh circumference.
- H_{o3}: No statistical significant difference will be noted in regards to Ashwagandha perceived pain.

1.6 Delimitations:

1. Participants were limited to 18-35 years of age.
2. The investigation required the recruitment of approximately 15 males and 15 females to complete the study.

3. All participants had to be clear from injury or surgery to lower body within 12 months of the study.
4. Subjects could not participate if they were diagnosed with any type of arthritis, stomach ulcers, diabetes, high blood pressure, psychoactive disorders, hypothyroidism, immunosuppression, autoimmune disorders, multiple sclerosis, and Lupus.
5. The subjects had to refrain from lower body exercise during the entirety of the study including: ham curls, leg extensions, lunges, leg press, squats, deadlifts, etc.
6. Subjects could not consume any anti-inflammatory drugs during the experiment.
7. Participants could not complete the study if an allergy to salicylates were present or if a known sensitivity to salicylates were present.
8. Recruitment method consisted of a convenience sample consisting of recruitment via online web meetings, classroom visits, word of mouth, and email correspondence.

1.7 Limitations:

1. Researchers did not require a food diary or log so there was no way to know for sure what the subjects consumed outside of the laboratory.
2. Researchers did not motivate the subjects during the testing or workout routine. Therefore, individual motivation could not be measured.

3. A sleep or daily activity log was not used, therefore, amount of sleep, possible stress levels, or outside activity that may promote inflammation was not accounted for.
4. Experiment took place during the Covid-19 Pandemic which could have caused increased stress, decreased motivation, and decreased willingness of subjects to volunteer.
5. Researchers could not control when the visual analog scale (VAS) was filled out along with supplemental adherence each morning.
6. Researchers could not control timing of participant involvement within the semester. Some participants' motivation could have been influenced due to midterm or finals week involvement.

1.8 Assumptions:

1. The researchers assume the participants were honest when completing health history, physical activity readiness questionnaire, and screening sheets.
2. The participants refrained from consuming NSAIDs during the entirety of the study.
3. The participants refrained from participating in any lower-body exercises during the entirety of the study.
4. The participants completed the VAS first thing in the morning upon waking up.
5. The participants consumed their Ashwagandha or placebo dose first thing in the morning upon waking up.

6. The fatiguing protocol used in the study was effective in eliciting DOMS.
7. The laboratory testing equipment was calibrated correctly and accurate readings were recorded.
8. The participants were truthful in their end-of-the-study questionnaire.
9. The statistical measurements and calculations were correct.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this literature review is to compare a safer and risk free alternative to aspirin and other NSAIDs. The history of Ashwagandha and its' efficacy in alleviating musculoskeletal swelling and pain are examined.

2.1 Aspirin (2- Acetoxybenzoic Acid) Dosage

As stated previously, aspirin (2-Acetoxybenzoic Acid) the most common NSAID, has a proven history of being efficient in mitigating soreness, inflammation, pain, swelling, and many other musculoskeletal disorders after a bout of physical activity or exercise with as little as 200mg [27]. In fact, aspirin is the most widely used drug worldwide and has been said to be one of the most important pharmacological achievements of the twentieth century. The earliest form of aspirin can be dated back 3500 years ago. Salicyline which is a glycoside can be found in the bark of willow trees

and has been used in the treatment of inflammation as far back as ancient Egypt. Those same salicylate acids were first synthesized with acetic anhydride into what we know now as Acetylsalicylic acid (aspirin), in 1897[28].

2.2 Aspirin Risks and Side Effects

While NSAIDs are one of the most popular and widely used medication to mitigate pain and soreness, many times, the adverse reactions outweigh the pain relief. NSAIDs make up over 7.5% of all sales in Europe and that does not even include over-the-counter (OTC) medicine. These numbers are also expected to rise as the cases of rheumatic disease incidences continue to climb [29]. The negative effects seen with NSAID use can even be noticed at the topical level. NSAIDs contain acidic properties. The effects of chronic use of NSAIDs have been reported to induce a wide spectrum of mucosal damage that can range from erosions to ulcers. Erosions can consist of damage to the mucosa itself while ulcers include the damage penetrating through the muscularis mucosa. These erosions are mainly found in the fundus and can develop and heal rapidly. Ulcers on the other hand can pose a much greater complication. Ulcers occur in the antrum, can have a more protracted development, and can be present much longer than erosions [30]. The mechanism of action of NSAIDs include the inhibition of the cyclooxygenase (COX) both 1 and 2. COX 1 and 2 are the key product in the biosynthesis of prostaglandins and NSAIDs have the ability to block both of these. The prostaglandins produced from COX 1 play an important role in the gut by stimulating the synthesis and secretion of mucus and bicarbonate. The production of these two, increase

the mucosal blood flow and promote epithelial proliferation. The negative effect of NSAIDs associated with this process is due to the fact that NSAIDs will inhibit these enzymes which in turn will create a gastric environment that is more susceptible to topic attack by endogenous and exogenous factors. Also, COX 1 can block the platelet production of thromboxane which in turn can increase bleeding within the GI system if an active bleeding site is present [29]. Not only can the upper GI tract be negatively affected, but the lower GI tract can be affected as well. While a decrease in hospitalizations due to upper GI distress has decreased in the last 10-20 years, an increasing amount of lower GI distress associated with NSAID use has been on the rise. Some of these complications included bleeding from colonic diverticula, and bleeding from the rectum. GI bleeding was the most common symptom noted during the study by Lanas et al [31].

2.3 Ashwagandha (Withania Somnifera)

2.3.1 History and Use: Ashwagandha

The traditional medicine system of India is known as Ayurveda. The goal and philosophy behind Ayurveda seeks to treat and integrate body, mind, and spirit using a comprehensive holistic approach especially by emphasizing diet, herbal remedies, exercise, meditation, breathing, and physical therapy. Ashwagandha, also known as Indian Cherry or Indian Ginseng is a traditional medicinal plant that has been used in south Asia for over a millennia[32]. While there are over 23 Withania species, only Withania Somnifera and Withania Coagulans are believed to have medicinal

properties. The roots from Ashwagandha have over 200 formulas in Ayurveda, Siddha, and Unami medicine. Currently in the US, Ashwagandha is sold as an herbal supplement in either a powder or capsule form. Ashwagandha is a safe home remedy that has been used as a tonic to treat a myriad of ailments. The geriatric population has benefited from using it to help control and mitigate hand and limb tremors [33]. It has also been prescribed for all kinds of muscle weaknesses and has been proposed to increase strength and promote vigor [33]. Ashwagandha has also been rumored to be an aphrodisiac and a rejuvenator. It has also been the drug of choice when battling rheumatic pain and inflammation of the joints and even in some paralytic conditions [33]. Ashwagandha, depending on how its prepared, has also shown prominence in treating insanity and hypertension along with helping heal skin lesions, boils, ulcers, and reducing pus formation, inflammation, and healing process promotion[33]. There are even claims that Ashwagandha can decrease cancer evidence in vitro[34]. While all of these claims and benefits of Ashwagandha use are an encouraging alternative, the use of Ashwagandha to promote exercise performance and to decrease inflammation decrease are the goal of this study. While some studies have shown prominence in animal-based research, additional research is needed within human subject research [35].

2.3.3 Ashwagandha Mechanism of Action:

The practitioners of Ayurveda have labeled Ashwagandha as an adaptogen thus, the mechanism of action of Ashwagandha directly on diseases is not the case, but rather the drug promotes homeostasis and physiological stability. Adaptogens are herbs that can improve the response to stress and help the body adapt by normalizing the physiologic processes in times of increased stress. An ideal adaptogen must reduce stress-induced

damage, be safe, must exhibit stimulating effects, must be innocuous, must not perturb any bodily function and must be devoid of any negative effects such as withdrawal symptoms. Adaptogens can exert their stress-protective method by helping to regulate homeostasis due to several different mechanisms of action that are associated with the hypothalamic pituitary adrenal axis (HPA) [36]. Regarding inflammation and immune regulation, researchers have shown evidence that Ashwagandha's mechanism of action is driven by the lymphocyte and natural killer (NK) cell activation. The anti-inflammatory properties of Ashwagandha can be attributable to the targeting of cysteine 179 of IKK beta leading to the inhibition of Nf-kB activity[32].

2.3.4 Ashwagandha and Delayed Onset Muscle Soreness:

Currently, the available research investigating Ashwagandha and its role in mitigating DOMS is extremely minimal. The majority of current research involves animal-based research, but the results are promising in that population. The goal of this study was to see how these results can translate to human subject-based research.

2.3.5 Ashwagandha and Arthritis:

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disorder and is an inflammatory disease that is marked by synovial hyperplasia with a local invasion of bone and cartilage leading to joint destruction [37]. To study arthritis and inflammation in rat models, the collagen-induced arthritis (CIA) model has been extensively studied and has been shown to induce inflammation in rodents. This model has been shown to replicate inflammation similar to the effects of RA by inducing synovial hyperplasia, mononuclear cell infiltration and cartilage degradation. Gupta et al., [32] utilized a

similar procedure to evaluate Ashwagandha on inflammation within rats. The researchers studied albino female rats aged 6-10 weeks of age within a 12:12 hour of light to dark schedule. Female rates were used based on previous findings that autoimmune arthritis is mediated by sex hormones causing a higher prevalence within female rats. Six rats were used in each groups and were divided into the following: normal control, arthritic control, Ashwagandha 600 mg/kg, Ashwagandha 800mg/kg, and methotrexate 3mg/kg. The doses were administered orally with a syringe at 10:00 am daily. Sterile water was given to the control group and untreated arthritic control rats. The experimental treatments were given from day 20 post collagen immunization and continued up to the 45th day. To assess the degree of arthritis, the researchers utilized a grading system as follows: Grade 0= no sign of arthritis, Grade 1=Redness and swelling in paw, Grade 2= Deformity in Paw, Grade 3= Ankylosis in paw, and Grade 4= Maximal swelling and deformity with ankylosis. To measure the amount of pain induced, the researchers utilized a similar grading scale as follows: 0= normal footprint, 1= partial footprint (no heel), 2= Fingers only, 3= absence of one footprint, 4= total absence of footprint. These measurements were tallied after the rat's hind paws were dipped in ink and allowed to walk on white paper for a distance of 60cm. After analysis, the researchers found that the administered dose of 600mg/kg of Ashwagandha to arthritic rats significantly decreased the severity of arthritis by suppressing the symptoms of arthritis and improving the functional recover of motor activity and radiological score [37].

While animal studies provide the necessary groundwork into human research, they don't explain fully the benefits of how Ashwagandha may affect inflammation, pain, or soreness in humans. Ramakanth et al. [38] designed a study evaluating the effects of

Ashwagandha on knee joint pain in humans. The researchers conducted study consisting of sixty patients with knee join pain and discomfort. The design of the study was constructed using a randomized double blind procedure. The patients were divided into one of three groups consisting of: Ashwagandha 250mg dose, Ashwagandha 125mg dose, and a placebo that were consumed twice daily. To assess pain and discomfort, a modified Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[39], a Knee Swelling Index (KSI), and finally a Visual Analog Scale (VAS). These measurements were taken at baseline, 4 week, 8 week, and 12 week. After analysis, the researchers found both significant decreases in pain and inflammation in both 250mg, and 125mg dose of Ashwagandha. The researchers concluded that while both groups showed significant inflammation and pain decreases, the 250mg group showed even greater decline in those variables and participants were free of any GI distress or disturbances [38].

To date, there is very little research evaluating Ashwagandha and its effects on inflammation and (chronic) pain. There is even less research looking at the effects of Ashwagandha on acute pain. Murthy et al.,[40] designed a study evaluating Ashwagandha on pain threshold force and pain tolerance. The study consisted of a placebo controlled, randomized double blind design The subjects were given a single oral dose of 1000mg Ashwagandha or placebo. Pain was assess using a Randal Selitto[41] score before and three hours a following drug/placebo administration. Pain threshold and pain tolerance force and time were also evaluated after drug administration. Following a 10-14 day washout period, the subjects repeated the same testing battery. At the conclusion of the study, analysis showed statistically significant difference in increased

pain threshold force and time as well as pain tolerance force in the experimental condition compared to the placebo group. The researchers concluded that a similar study evaluating the effects of Ashwagandha in populations suffering from chronic pain needs to be addressed [40].

Ashwagandha has also shown promise in promoting athletic performance. Ziegenfuss et al. [42]studied the effects of Ashwagandha on boy composition, muscular strength, power and endurance, 7.5km cycling time trial, and clinical blood chemistries. The researchers utilized a randomized double blind study with participants being randomly assigned to either a placebo condition or a 500mg dose of Ashwagandha. Measurements were taken at baseline and then at 12 weeks after training and supplementation. The supplements were consumed daily every morning. The training sessions consisted of upper and lower body resistance training two days per week with a 4 day split. The workouts consisted of the following exercises: bench press (pectoralis major), lat pulldown (latissimus dorsi), shoulder press (deltoid) , seated row (latissimus dorsi), shoulder shrug (trapezius) , dip (deltoid) , biceps curl (biceps), triceps pushdown, leg press (hamstring), squat (gluteus major), deadlift (erector spinae), lunge (hamstring), leg curl (hamstring, gluteus major), leg extension (quadriceps femoris), and calf raise (gastrocnemius). The participants completed initially three sets of 12-15 repetition maximum (RM) loads but finished the study performing 4-6 sets of eight RM loads. Rest periods between sets ranged from 1-3 minutes. Following the completion of the 12-week training period and supplementation period, the researchers found statistical significant differences between the experimental condition and placebo. Specifically, the researchers noted statistically significant increases in the 1-RM back squat and 1-RM bench press.

Following Dual-energy X-ray absorptiometry, the researchers also noted favorable body composition changes in the experimental condition compared to placebo. Further analysis showed improvements in average squat power, peak bench press power, 7.5 km time trial performance, and perceived recovery scores. While there were no statistically significant changes in blood chemistry, the researchers concluded that a 500mg dose of Ashwagandha can improve upper and lower-body strength, favorable distribution of body mass, and is well-tolerated with little to no adverse reactions.

Similar to Ziegenfuss et al, [42], recent literature has surfaced that corroborates the claims of increased athletic performance following Ashwagandha supplementation. Wankhede et al., [43] designed a randomized prospective double-blind, placebo-controlled clinical study that evaluated the effects of Ashwagandha with 57 young male subjects. The study consisted of an 8-week period of supplementation and training with the subjects divided into either a placebo controlled group or an experimental condition consuming 300mg of Ashwagandha twice daily. The subjects completed upper and lower-body resistance training exercises consisting of barbell squat, leg extension, seated leg curl, machine chest press, barbell chest press, seated machine row, one-arm dumbbell row, machine biceps curl, dumbbell biceps curl, cable triceps press-down, dumbbell shoulder press, and straight arm pull down. The subjects completed this routine 3 times per week with rep and set schemes ranging from 1-3 sets of 12-15 repetitions. Measurements were taken at baseline and after the 8-week training point. Measurements consisted of muscle strength, muscle size, body composition, serum testosterone level and muscle recovery. To test for muscle strength, the 1-RM test was utilized. Muscle recovery was evaluated by using serum creatine kinase levels as measures of muscle

injury. Following analysis, the researchers noted statistically significant increases in muscle strength in the experimental group compared to placebo group in the bench press and leg extension, greater muscle size increase in the arms and chest. The experimental group had significantly greater reduction of exercise-induced muscle damage compared to the placebo group with regards to the stabilized serum creatine kinase levels. The experimental group also revealed higher testosterone levels compared to the placebo group as well as a greater decrease in body fat percentage. While not directly related to delayed onset muscle soreness, these results lend themselves to the goal of decreasing inflammation with regards to lower serum creatine kinase levels. Elevated levels of serum creatine kinase are especially present after strenuous exercises and are extremely prominent in subjects experiencing DOMS [44].

There is a myriad of research supporting the idea that increases in upper and lower-body strength yields greater overall performance. Recent literature has shown the positive effects of Ashwagandha on muscle strength and power following supplementation along with a training regimen. Recent literature has also shown the positive effects of Ashwagandha on the cardiorespiratory system as well. Shenoy et al., [45] performed a research study evaluating the effects of Ashwagandha supplementation for 8 weeks in elite Indian Cyclists. Twenty male and twenty female elite level cyclists ranging from 18-27 years of age were randomly divided into an experimental group receiving 500mg of Ashwagandha daily or a placebo group that received starch capsules for eight weeks. Baseline measurements of a maximal aerobic capacity ($V0_2$ max), metabolic equivalent, respiratory exchange ratio (RER), and total time to exhaustion were taken before the eight-week training period. After eight weeks, the subjects completed the

same baseline testing battery. After analysis, the researchers noted statistically significant improvement in the experimental condition in all parameters. The placebo group did not show any statistically significant improvement in any of the measured parameters. The researchers concluded that a 500mg dose of Ashwagandha daily can have statistically significant improvements in regards to cardiorespiratory performance.

CHAPTER III

MATERIALS AND METHODS

3.1 Participants:

Fifteen subjects volunteered to participate in this study including eight males and seven females. In order to participate in the study, the subjects had to be considered recreationally healthy and be free from any signs or symptoms of disease, without any musculoskeletal injury, free from any type of arthritis, (rheumatoid, gout, osteoarthritis, etc), previous surgery within the last six months involving hip, knee, ankle or low back, be at least 18-35 years of age, and have no low back pain. During the study, the subjects could not participate in any lower body resistance training or exercise in order to minimize additional inflammation after the first exercise routine. Along with refraining from exercise, the subjects were asked to refrain from taking any NSAIDs, analgesics, corticosteroids, or any nutritional supplement that could influence inflammation. The first of four laboratory visits included completing a health history questionnaire, subject screening sheet, university approved IRB informed consent, instructions on how and when to consume the supplement, instructions on how to complete the daily VAS, as well as requirements and expectations for the study. The participants then completed a familiarization session that consisted of the four repeated counter-movement vertical

jump assessment as well as a VAS. The subjects were then randomly placed in either an experimental group (n=8), or placebo group (n=7).

Table 1

Column1	Experimental	Placebo
Age (years)	20.875	21.142857
Height (cm)	68.97637795	67.255343
Weight (kg)	83.8125	82.1
BMI (kg/m^2)	26.89944752	28.353072

3.2 Methodology:

A double-blind, randomized placebo-controlled study design was utilized to evaluate the effects of Ashwagandha on delayed onset muscle soreness (DOMS) as well as to determine the effects of Ashwagandha on athletic performance following a strenuous bout of lower-body resistance exercise. Subjects consumed 750mg of Ashwagandha or a placebo following baseline testing and an exhaustive bout of lower-body resistance training in order to elicit DOMS. The subjects' performance, inflammation markers, and subjective visual analog scale (VAS) data was tracked throughout the training study. The VAS consists of a 10 centimeter line with points spaced 1 millimeter a part from each other. This allows the subject to score or rate their perceived pain from one extreme to the other. At the far left of the VAS an extreme score of 1mm would denote no pain whatsoever while the 100mm mark would denote extreme debilitating pain. The use of VAS has been used in scholarly research and has been validated when measuring phenomena such as anxiety, nausea, pain, fatigue, dyspnea and hunger [46]. Statistical analysis was completed to compare means between both the

experimental and placebo groups. This investigation was approved by the Oklahoma State University Institutional Review Board for human subject research.

3.3 Study design:

This study included four visits to the Applied Neuromuscular Physiology Laboratory and five days of supplementation. The first visit consisted of a familiarization period with the measurement devices, informed consent, VAS, screening sheet, and health history questionnaire, as well as instruction and information regarding the experimental supplement protocol. Subjects were instructed to contact the researchers immediately if any adverse reactions to the supplement occurred. During the second session, subjects completed baseline measurements were recorded consisting of: muscle soreness, mid-thigh circumference via medical tape measure, (Mabis DMI Healthcare Des Moines, IA), pain pressure threshold via (Wagner Force Ten Digital Force Gauge Greenwich, CT), along with basic anthropometric data via standard mass scale. The subjects' thigh circumference was measured approximately halfway between the iliac crest and the proximal border of the patella. Once, the halfway point was measured, the researchers marked that spot with a Sharpie, (Newell Brand, Atlanta, GA), to ensure measurements of the same spot occurred. This same spot would denote the location of where the digital force gauge was placed. To measure the sensitivity and perceived pressure, the researchers placed the point of the gauge on the subjects' mid-thigh, and instructed the participants to alert the researcher when the first sensation of pressure was felt. The researcher instructed the participants that the pressure should not be painful but to alert at the onset of discomfort. To measure baseline athletic performance, a testing battery of a four repeated counter-movement vertical jump was utilized to account for

jump height, ground-contact time, and peak power and peak velocity was recorded via Just Jump Sytem (Just Jump; Pro Biotics Inc, Huntsville, AL, USA) along with testing peak power and peak velocity via a tendo unit, (Tendo Sports, Trencin, Slovak Republic). After baseline measurements were recorded, the subjects performed a lower body resistance training session designed to induce DOMS. Following the resistance training session, the subjects then repeated the initial testing battery to measure the parameters of DOMS along with the same four counter movement jump test. To ensure minimal bias, the researcher was given the supplements in a brown paper bag to eliminate knowing which subjects received either the experimental or control pills. Subjects were then given their bag of supplements along with the list of instructions on how and when to consume the supplement. The morning after the first training session, participants were instructed to begin their supplement consumption and begin their VAS recording to measure their soreness. Subjects were required to repeat the same testing battery on day three (72 hours), and day six (144 hours). On the final day of the study, participants were asked whether they had any adverse reactions, if they thought they received the experimental or placebo condition, along with why they thought they might have had placebo or the experimental condition.

3.4 Fatiguing Protocol:

The fatiguing protocol that was utilized was similar to Cheshier et al. due to the ability to elicit DOMS quickly with fewer exercises[20]. The lower-body protocol consisted of a brief 3–5 minute warm-up on a stationary bicycle followed by 5 sets of

twenty repetitions of weighted lunges. A successful lunge consisted of walking forward and lowering the body until the knee bends to 90^{O} with the front thigh parallel to the floor. Forty percent of the subject's body weight was used to calculate the load carried in each arm (20% in each hand). The subject then performed 5 sets of twenty repetitions (10 each leg) holding a dumbbell in each hand, with one minute of rest between each set. The subject then performed 3 set to failure on a leg press machine with a 75% of total body weight load. The subjects completed 3 sets to failure in conjunction with a metronome (Pro Metronome, Xanin Technology, Hangzhou, China), set at 60 beats per minute[47].

3.5 Treatment Conditions:

Subjects were randomly assigned to either the experimental condition, or the placebo (control) condition via computer generated list. The experimental condition consisted of a 750mg once daily dose of Ashwagandha (Withania Somnifera) taken preferably with a meal early in the morning. The supplement was purchased from a commercially available supplement site, which has a long standing of quality supplements that are independently tested. The subjects received their supplement in either a white or brown unmarked container that mitigated any bias or knowledge from the researcher. The placebo or control group were instructed to consume their capsules in a similar fashion to the experimental group. The placebo pills were gelatin capsules filled with powdered sugar. All participants were instructed to start their supplement consumption the morning after session two and to continue until session six. The subjects then brought their container of capsules back to the laboratory to test for compliancy. A

compliance of 80% was used to be included in the statistical analysis. All subjects were in compliance. This standard was used in a previous study [20].

3.6 Statistical Analysis:

Due to small sample size, independent samples t-tests were used to look at family variables including performance markers and inflammation or perceived pain markers. The performance maker variables included: Jump Height (cm), Peak Power (W), and Peak Velocity (m/s). These variables were measured and calculated at the three time points, Pre-DOMS (pre-test), Day 3 (mid-point), and Day 6 (post-test). The inflammation or perceived pain variables measured included VAS scores of the: hamstring, gluteus maximus, and quadriceps femoris muscles. These variables were measured and calculated at the time points Day 2, Day 3, and Day 4 which showed the greatest changes in perceived soreness. Analysis of data was completed using SPSS Statistical Software (Version 27.0, IBM Corporation, Chicago IL). A bonferoni correction factor was used to correct for the multiple t-tests. An alpha (α) level was set at .016 due to the multiple t-tests.

CHAPTER IV

RESULTS

4.1 Statistics

Eight healthy males (n=8), and seven healthy females (n=7) successfully completed the study. Eight participants were randomly placed in the experimental condition (Ashwagandha), and seven participants were randomly placed in the control (placebo) condition. The results of the independent t-test revealed a statistically significant difference in Peak Power (W) between the experimental group (M= 1659.873, SD 614.104) to the control group (M=1401.214, SD= 306.669), $t(10.558)=1.051$, $p<0.016$. Further analysis shows no other statistically significant results between the two groups. Both groups had a 100% compliance rate with no adverse reactions reported.

Figure 1

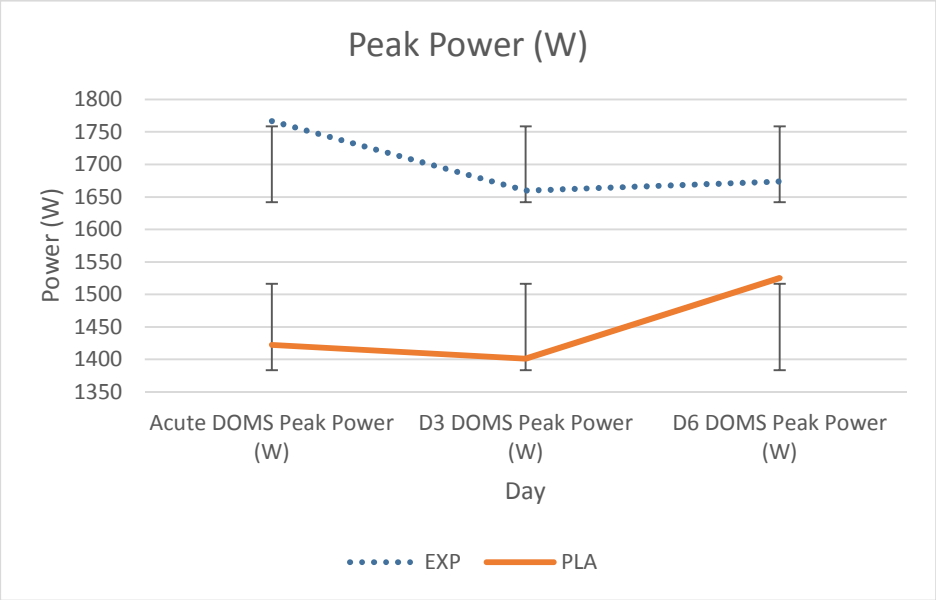


Table 2

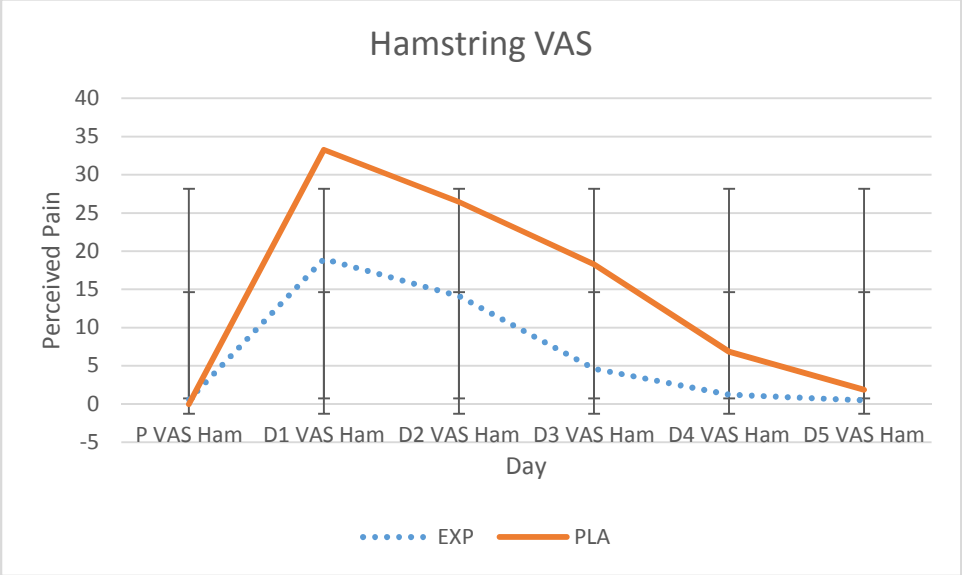
Descriptive Statistics

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance	Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
Age	15	4	19	23	21.00	.926	.857	1.615	1.121
Weight	15	63.5	57.0	120.5	83.013	22.7712	518.530	-1.490	1.121
Height	15	38.6	154.4	193.0	173.160	11.1652	124.663	-.633	1.121
BMI	15	20.822	18.215	39.037	27.57781	6.619499	43.818	-1.134	1.121
Valid N (listwise)	15								

4.2 Hamstring VAS:

While there was no significant difference noted, there was a trend of decreased perceived pain or soreness in the hamstring VAS scores within the experimental group.

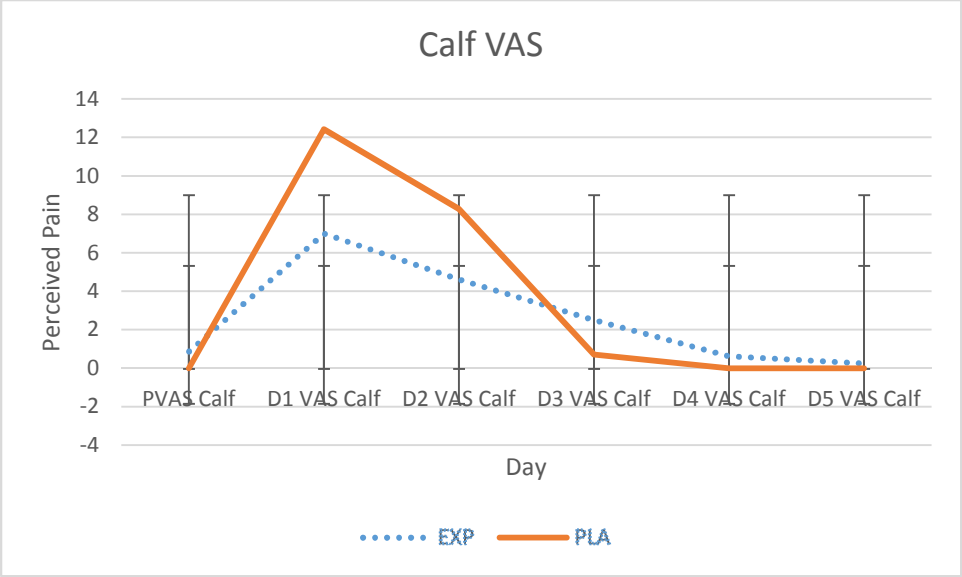
Figure 2



4.3 Calf VAS:

While there was no significant difference noted, there was a trend of decreased perceived pain or soreness in the calf (gastrocnemius) comparing the experimental to the conditional group.

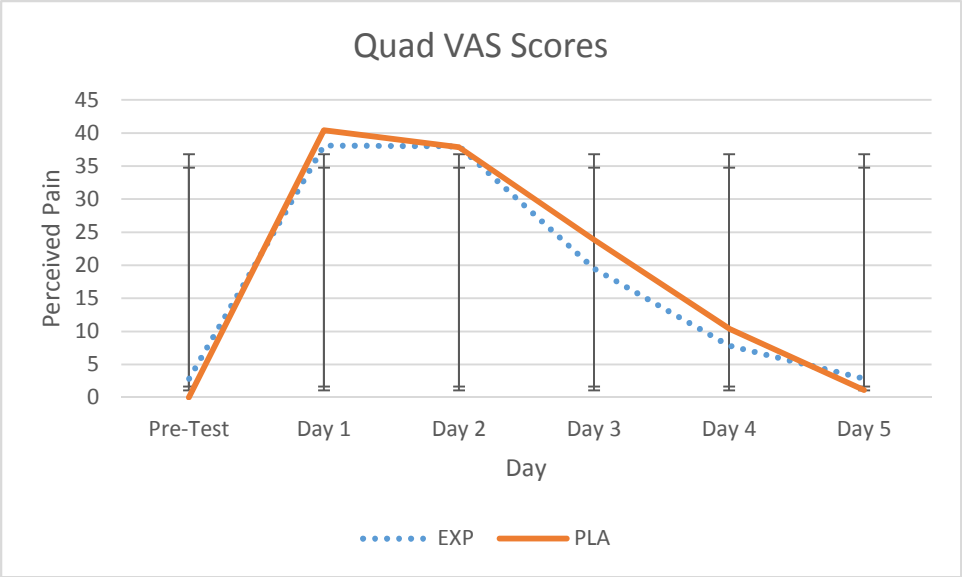
Figure 3


Calf VAS
14
12
10
8
6
4
2
0
-2
-4
Perceived Pain
PVAS Calf
D1 VAS Calf
D2 VAS Calf
D3 VAS Calf
D4 VAS Calf
D5 VAS Calf
Day
EXP
PLA

4.4 Quadriceps VAS Scores:

There were no statistically significant difference between the experimental group and the control group. The graph below demonstrates a minimal difference in day 2 to day 3 regarding perceived soreness or pain.

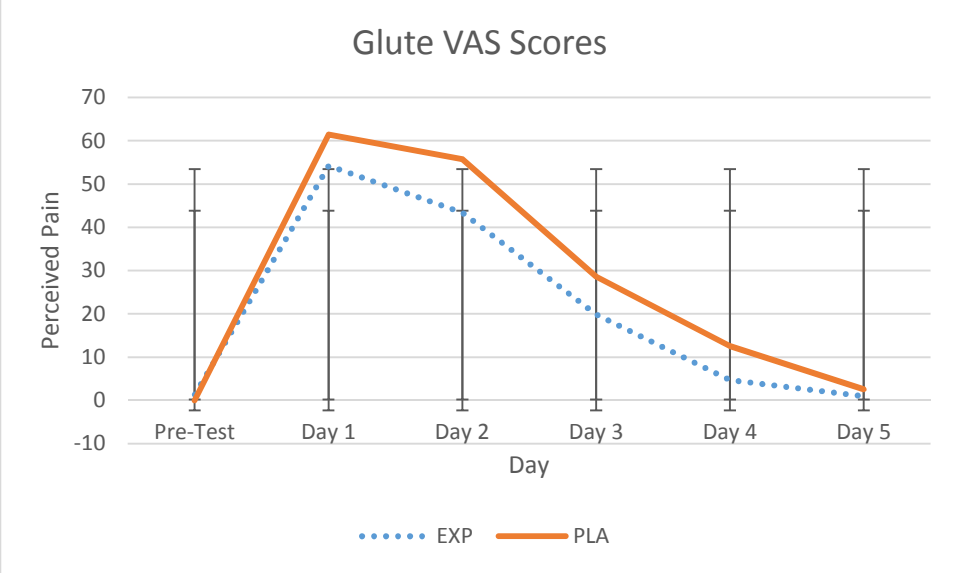
Figure 4



4.5 Gluteus Maximus VAS Scores:

There were no statistically significant difference between the experimental group and the control group. The graph below demonstrates a minimal difference in day 2 regarding perceived soreness or pain.

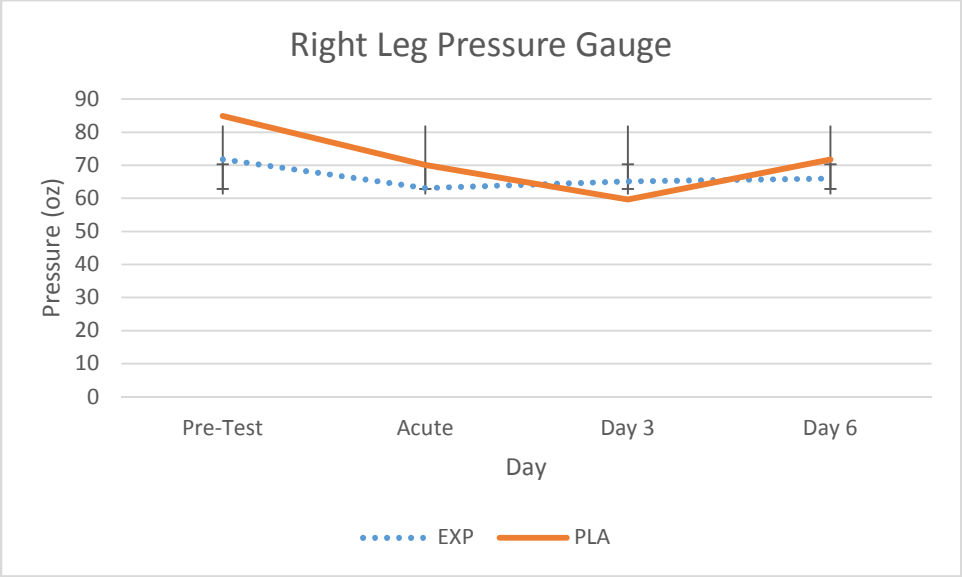
Figure 5



4.6 Right Leg Pressure Gauge Scores (oz):

There was no statistically significant difference noted between the experimental and placebo pressure gauge scores.

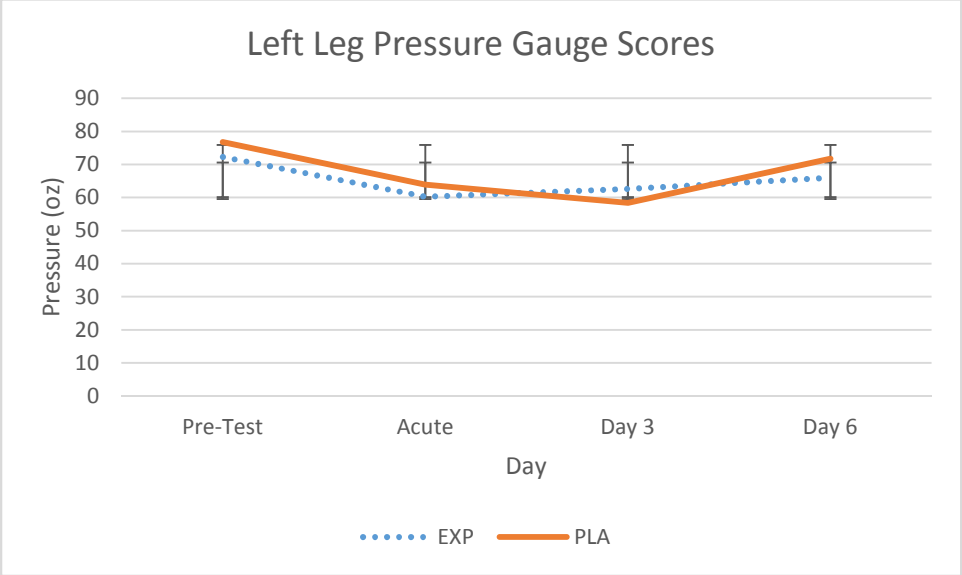
Figure 6



4.7 Left Leg Pressure Gauge Scores (oz):

There was no statistically significant difference noted between the experimental and placebo pressure gauge scores.

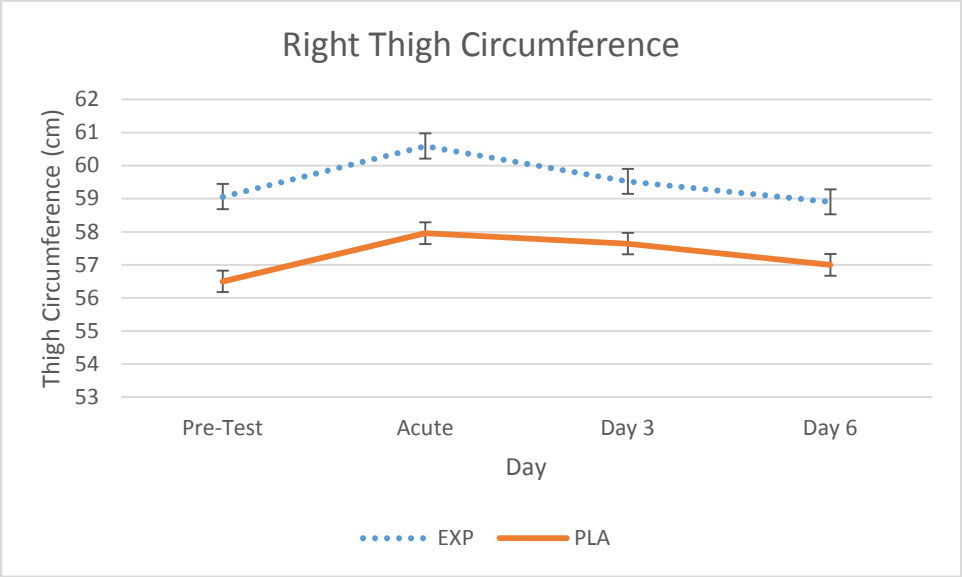
Figure 7



4.8 Right Thigh Circumference (cm):

There was no statistically significant difference between thigh circumference in the experimental compared to the placebo conditions in the right leg but there is a trend of a larger circumference in the experimental condition that was present at baseline and continued to post-testing.

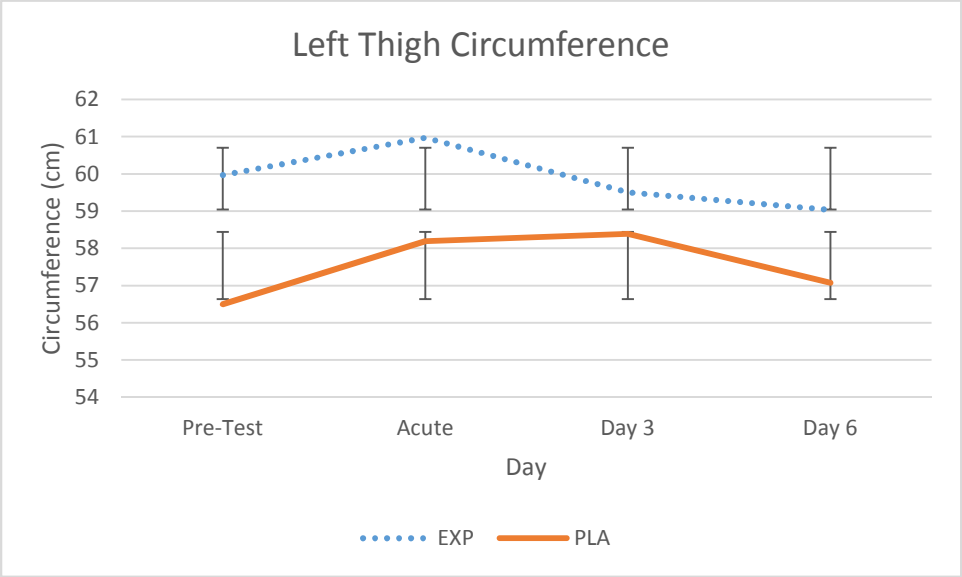
Figure 8



4.9 Left Thigh Circumference:

There was no statistically significant difference between thigh circumference in the experimental compared to the placebo conditions in the left leg but there is a trend of a larger circumference in the experimental condition that was present at baseline and continued to post-testing.

Figure 9



CHAPTER V

CONCLUSION

5.1 Discussion:

Currently, this is the first investigation of its kind to evaluate the effects of Ashwagandha on the parameters of DOMS as an alternative to NSAIDs. While none of the primary findings were found to be significant, the statistically significant difference in peak power (W) at Day 3 is encouraging especially to athletes or those interested in maintaining a certain level of performance. This knowledge can be especially useful to athletes that participate in multiple day long events such as track and field events or ultra-endurance events where power can drastically diminish [48]. In similar studies, the increase in ground contact time and jump height were drastically diminished after the fatiguing protocol demonstrating that our subjects were physically fatigued and DOMS ensued [49].

5.2 Pain Pressure Threshold:

The use of pain pressure threshold devices have been used to measure pain perception within a fatigued or sore muscle [50]. The pain pressure threshold gauge

allows the researchers to determine the sensitivity of the muscle before and after exercise to quantifiably determine the level of soreness based on the perceived pain from the subject. While not statistically significant, the experimental group showed a slightly higher pain pressure gauge scores on Day 3 which corresponds to the statistically significant increase in Peak Power (W) compared to the control group.

5.3 Athletic Performance:

Currently, to our knowledge, there is only one other study assessing Ashwagandha and its effects on athletic performance. While Ziegenfuss et al. [42] utilized an upper body muscular power analysis, the researchers chose to utilize a Jump Mat and Tendo unit to assess muscular power, vertical jump, and ground contact time. The researchers noted a statistically significant difference at Day 3 Peak Power (W) comparing the experimental group (M= 1659.873, S 614.104) to the control group (M=1401.214, SD= 306.669), t=1.007, p=0.012. No other athletic performance variables showed statistically significant differences among the groups.

5.4 Thigh Circumference:

To our knowledge, this is the first study to assess the effects of Ashwagandha on thigh circumference following a bout of intense lower-body resistance training. Other studies have utilized thigh circumferences to assess inflammation and recovery before and after intense bouts of exercise [51]. No statistically significant differences were noted

between the experimental and control group. The experimental group did in fact have larger thigh circumference scores throughout the study compared to the placebo group but these values were not statistically significant.

5.5 Visual Analog Scores:

To the best of our knowledge, currently, there are no other studies that have assessed perceived pain or soreness after the consumption of Ashwagandha using a VAS. While there were no statistically significant differences noted between the experimental or placebo groups, the calf and hamstring muscle VAS scores showed a trend of having lower values in the experimental group in days 1-3 post exercise. While not significant, this trend is encouraging for athletes that are lower-body resistance trained.

5.6 Conclusion:

The purpose of this study was to assess the effects of Ashwagandha on the parameters of DOMS as well as performance measures as an alternative to NSAIDs. Currently, to the best of our knowledge, no other studies have investigated Ashwagandha on the effects of DOMS and its effects on performance markers. Currently, the research is extremely limited in the realm of human performance. After 5 days of supplementation of a 750mg daily dose of Ashwagandha, a statistically significant difference in peak power was shown at the third day of supplementation between the experimental and placebo condition. This demonstrates the ability of Ashwagandha to maintain or increase peak

power after an intense bout of exercise. Whether this significant difference is due to diminished perceived pain, or the supplement providing an ergogenic effect, we cannot confirm. While no other statistically significant effects were noted between the two groups, the experimental condition showed decreased VAS scores in the hamstring, glute, and quadriceps muscles at the 3-day mark. While every study is without limitations, this study is no exception. Designing a study and recruiting subjects during the Covid-19 global pandemic caused decreased participation and the ability to design a study and recruit participants as efficiently compared to earlier times. This could be the root cause behind the small sample size. We can speculate that this may have caused subjects to may not feel as safe as they usually are which could cause decreased internal motivation, decreased desire during exercise, and increased stress factors that could play a major role in inflammation thus causing the claimed anti-inflammatory effects of Ashwagandha to become minimized. The limitations within this study are as followed: small sample size, subjects internal and external motivation, lack of food, supplement, and sleep log, possible non-compliance with VAS log times, and Covid-19 interference. For future studies, the researchers recommend: possible higher dosages, multiple doses during the day, increased length of supplementation beyond one week, higher sample size, food and sleep log, and larger subject age range.

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APPENDICES

APPENDIX A

Informed Consent:

Applied Exercise Science

The Effect of Ashwagandha on Delayed Onset Muscle Soreness

Background Information

You are invited to be in a research study of the effects of the supplement Ashwagandha in the alleviation of common muscle soreness following a workout. We ask that you read this form and ask any questions you may have before agreeing to be in the study. Your participation in this research is entirely voluntary. There is not penalty for refusal to participate, and you are free to withdraw your consent and participation in this project at anytime. Your decision whether or not to participate in this study will not affect your grade in any class.

This study is being conducted by: Cody Diehl under the direction of Dr. Bert Jacobson, both of School of Kinesiology, Applied Health and Recreation at OSU.

Procedures

If you agree to be in this study, we would ask you to do the following things:

1. Initially, you will report to the Neuromuscular Physiology lab (session 1) to sign the informed consent and fill out the health history questionnaire. You will become familiar with the procedures of the vertical jump and practice a few jumps to make sure you are comfortable with the procedures. In addition, your weight and height will be measured for a body mass index calculation.

2. On your scheduled day (session 2), you will report to Neuromuscular Physiology lab. First, you will rate your initial muscle soreness on the visual analog scale. Then your right and left thigh circumference will be measure at the belly of your quadriceps. Following thigh circumference, a pressure algometer will used to provide pressure to

3. Initially, you will report to the Neuromuscular Physiology lab (session 1) to sign the informed consent and fill out the health history questionnaire. You will become familiar with the procedures of the vertical jump and practice a few jumps to make sure you are comfortable with the procedures. In addition, your weight and height will be measured for a body mass index calculation.

4. On your scheduled day (session 2), you will report to Neuromuscular Physiology lab. First, you will rate your initial muscle soreness on the visual analog scale. Then your right and left thigh circumference will be measure at the belly of your quadriceps.

5. Following thigh circumference, a pressure algometer will used to provide pressure to induce tenderness or pain on your right and left thigh. Then you will be assessed on the vertical jump.

6. At the Colvin Recreation Center, you will first perform a warm-up on the cycle ergometer for approximately 5 minutes. You will then perform lower body exercise to induce skeletal muscle fatigue. Exercises will include:
    a. 5X10 lunges (60% body weight)
    b. 3X10 leg press (75% body weight)

7. Immediately following exercise you will go back to the Neuromuscular Physiology lab and sit for 10-15 minutes. Then you will have another measurement of thigh circumference. In addition, the amount of pressure required to induce pain/tenderness assessed again. Then you will perform one more vertical jump. Finally, you will be given an oral supplement (1 capsules, 750mg) Ashwagandha or a placebo with 1/3 of a protein bar.

8. You will then be asked to continue supplement/placebo dosing for the next 5 days with food in the morning, noon, and at night. In addition you will rate you soreness on a visual analog scale as soon as you wake for the next **5** days. During this time, you will be asked to refrain from participating in any lower body exercise to not interfere with the current study.

9. On day 3 (session 3), you will report back to the Neuromuscular Physiology lab where you will again perform the vertical jump test, have a measurement of your thigh circumference and the amount of pressure to induce pain/tenderness assessed again.

10. On day 6 (session 4), you will report back to the Neuromuscular Physiology lab at your scheduled time to return your visual analog scale and have a final measurement of thigh circumference, the amount of pressure to induce pain/tenderness assessed for the last time, and perform the final vertical jump test.

Participation in the study involves the following time commitment: The total time commitment in the Neuromuscular Physiology lab for session 1 is 30 minutes. Session 2 which takes place in the Neuromuscular Physiology Lab and Colvin Recreation Center, the total time commitment is about 60 minutes. Day 3 (session 3) and Day 6 (session 4) time commitment at the Neuromuscular Physiology lab for vertical jump test, thigh circumference, and pressure to induce pain/tenderness is about 15-20 minutes each.

Risks and Benefits of being in the Study

The study involves the following foreseeable risks: Loss of privacy. In order to assist with the offset of this risk, all data and biospecimens will be coded immediately and will never have your name attached. In case of injury or illness resulting from this study, emergency medical treatment will be available at University Health Service on the OSU Stillwater campus. No funds have been set aside by Oklahoma State University to compensate you in the event of illness or injury.

The benefits to participation are:

There are no direct benefits to you. More broadly, this study may help the researchers learn more about Ashwagandha and may help determine if Ashwagandha is effective in decreasing skeletal muscle soreness following exercise.

Reduce the Risk of COVID-19

Screening: Researchers and participants who show potential symptoms of COVID-19 (fever, cough, shortness of breath, etc.) will **NOT** participate in this study at this time. In addition, before any scheduled sessions both the researchers and participants must self-screen for COVID-19. If any participant is suspected of having or been exposed to COVID-19 during the duration of this study they will not be allowed to further participate in the study until they have tested negative for COVID-19.

Temperature Check: If the subjects' body temperature exceeds 100.4 degrees, they will be unable to participate.

Physical distancing: Whenever possible, we will maintain at least 6 feet of distance between persons while conducting the study.

Mask/Covering: Researchers and participants will be required to shield their mouth and nose with a cloth face cover or mask during the study, even when maintaining at least 6 feet of distance. Only during the fatiguing protocol will the participants not be required to wear their face covering. Tissues will be available to cover coughs and sneezes.

Gloves: When the researchers has direct contact with the participant (thigh circumference and using the pressure algometer to provide pressure to induce tenderness or pain) the research will wear latex free-gloves to further reduce the risk of COVID-19 exposure.

Handwashing: Researchers and participants will wash hands or use a hand sanitizer containing at least 60% alcohol before all testing procedures.

Disinfecting materials: When feasible, researchers will clean and disinfect surfaces between participants, using an EPA-registered disinfectant or a bleach solution (5 tablespoons of regular bleach per gallon of water) for hard materials and by laundering soft materials. Disinfected materials will be handled using gloves, paper towel, plastic wrap or storage bags to reduce the chance of re-contamination of materials.

Electronics: Alcohol-based wipes or sprays containing at least 70% alcohol will be used to disinfect shared touch screens, mice, keyboards, etc. Surfaces will be dried to avoid pooling of liquids.

Compensation

You will receive no payment for participation in this this study.

Voluntary Nature of the Study

Your participation in this research is voluntary. There is no penalty for refusal to participate, and you are free to withdraw your consent and participation in this project at any time. The alternative is to not participate. You can skip any questions that make you uncomfortable and can stop the interview/survey at any time.

Confidentiality

Only qualified research personnel and the Oklahoma State University Institutional Review Board (IRB) will have access to the database containing study information (P.I., and the advisor of the P.I). You information will be stored on the primary investigator's computer that is not accessible to anyone other than the primary investigator. The signed consent forms will be kept for 3 years per federal guidelines. Once your demographic data is obtained from the medical questionnaire (height, weight, gender, and age) and put onto an excel document, your medical history questionnaire will be shredded. All data collected (visual analog scale scores, height, weight, gender, and age) will be saved on the hard drive of the PI's work computer. All data collected will be reported as groupings (position) and will not be linked to participants. The researchers will not be able to remove your data from the dataset once your participation is complete. The

Seretean Wellness Center can be accessed by the public, but the room that contains all documents remains locked on a regular basis and can only be accessed by the PI.

The list of corresponding names and ID numbers will be stored in a locked desk in the PI's office. The data will be stored on a hard drive that will be locked in the Primary investigators office. The hard drive will only be accessible while the PI is at work, and will only be used while he or she is at work. To elaborate, your medical questionnaire will be destroyed immediately after your demographic data input has been recorded and saved onto the PI's office computer. For example, if subject M1 comes in on a Monday, shortly after he finishes his initial assessment procedures, meets all inclusion criteria, and all data is saved on the PI's computer (the same day), his health data will be shredded. In addition, all information that pertains to the individuals who do not meet inclusion criteria will also be shredded immediately.

It is important to note that the data collected during this research study may be shared with other researchers in the future. If this happens, no identifiable information will be shared. It is unlikely, but possible, that others responsible for research oversight may require us to share the information you give us from the study to ensure that the research was conducted safely and appropriately. We will only share your information if law or policy requires us to do so. Finally, confidentiality could be broken if materials from this study were subpoenaed by a court of law.

Contacts and Questions

The Institutional Review Board (IRB) for the protection of human research participants at Oklahoma State University has reviewed and approved this study. If you have questions about the research study itself, please contact Cody Diehl at (937)-902-7845, cody.diehl@okstate.edu or Dr. Bert Jacobson at (405) 744-933, bert.jacobson@okstate.edu. If you have questions about your rights as a research volunteer or would simply like to speak with someone other than the research team about concerns regarding this study, please contact the IRB at (405) 744-3377 or irb@okstate.edu. All reports or correspondence will be kept confidential.

Statement of Consent

I have read the above information. I have had the opportunity to ask questions and have my questions answered. I consent to participate in the study.

Signature:_____ Date: _____

Signature of Investigator:_____ Date: _____

APPENDIX B

Recruitment Form

Hello,

My name is Cody Diehl and I am a doctoral student in the Health and Human Performance Department at Oklahoma State University. We are conducting a research in the Applied Neuromuscular Physiology Laboratory, 192 Colvin Center, which investigates the effects of Ashwagandha on delayed onset muscle soreness. You may be eligible to participate in the study if you have been recreationally active, healthy and are between the ages of 18-35.

If you choose to participate in this study, your BMI, thigh circumference, muscle fatigue post-exercise, vertical jump, and overall muscle fatigue using a Visual Analog Scale (VAS) will all be measured. You will be placed in either a placebo or experimental group. The experimental group will consume 750mg of Ashwagandha daily supplied to you by me, Cody Diehl.

If you have any question about the study or if you need to contact me about participation, you may contact me at 937-902-7845, cody.diehl@okstate.edu

Thanks for your consideration,

Cody Diehl

APPENDIX C

Screening Sheet

Ashwagandha Screening Sheet

Age:_____

Sex:_____

Exclusion Criteria:

Please indicate yes or no to the following:

Allergic or sensitive to Salicylates such as Asprin:_____
_____

Diagnosed with Asthma, Diabetes, Gout, Gastritis:_____
_____

Diagnosed with Hemophilia, Stomach Ulcers or with Kidney/Liver issues, psychoactive disorders, Lupus, Immunosuppression, Autoimmune Disorders, and Multiple Sclerosis:_____
_____

Chronic Low Back Pain:_____
_____

Surgery within the last 12 months involving the knee, ankle, hip or back:_____
_____

Diagnosed of all types of Arthritis:_____
_____

Currently pregnant or breastfeeding:_____
_____

Taking any of the following medications: anticoagulants (blood thinners), beta blockers, diuretic (water pills), Methotrexate and Phynytoin (Dilantin):_____
_____

Health History

Name _____ Date_____

Work Phone _____ Home Phone (Cell) _____

E-mail address_____ Preferred method of contact: Call, email, or text

Person to contact in case of emergency_____

Emergency Contact Phone _____

Gender _____ Age _____(yrs) Height _____(ft)_____(in) Weight_____(lbs)

Does the above weight indicate: a gain____ a loss____ no change____ in the past year?

If a change, how many pounds?_____(lbs)

A. JOINT-MUSCLE STATUS (✓Check areas where you currently have problems)

Joint Areas	Muscle Areas
( ) Wrists	( ) Arms
( ) Elbows	( ) Shoulders
( ) Shoulders	( ) Chest
( ) Upper Spine & Neck	( ) Upper Back & Neck
( ) Lower Spine	( ) Abdominal Regions
( ) Hips	( ) Lower Back
( ) Knees	( ) Buttocks

( ) Ankles

( ) Feet

( ) Other_____

( ) Thighs

( ) Lower Leg

( ) Feet

( )

Other_____

B. HEALTH STATUS (✓Check if you currently have any of the following conditions)

( ) High Blood Pressure

( ) Heart Disease or Dysfunction

( ) Peripheral Circulatory Disorder

( ) Lung Disease or Dysfunction

( ) Arthritis or Gout

( ) Edema

( ) Epilepsy

( ) Multiply Sclerosis

( ) High Blood Cholesterol or Triglyceride Levels

( ) Allergic Reactions to Rubbing Alcohol

( ) Hemophilia

( ) Chronic Low Back Pain

( ) Asthma

( ) Acute Infection

( ) Diabetes or Blood Sugar Level Abnormality

( ) Anemia

( ) Hernias

( ) Thyroid Dysfunction

( ) Pancreas Dysfunction

( ) Liver Dysfunction

( ) Kidney Dysfunction

( ) Phenylketonuria (PKU)

( ) Loss of Consciousness

( ) Allergic or Sensitive to Saliculates

( ) Stomach Ulcers

( ) Chronic Knee Pain

( ) Gastritis

C. SURGICAL STATUS ((✓Check if you have had surgery involving the following in the past 12 months)

( ) Knee ( ) Back

( ) Ankle ( ) Hip

D. PHYSICAL EXAMINATION HISTORY

Approximate date of your last physical examination_____

Physical problems noted at that time_____

Has a physician ever made any recommendations relative to limiting your level of physical exertion? _____YES _____NO

If YES, what limitations were recommended?_____

_____

E. CURRENT MEDICATION (PRESCRIPTION OR OVER THE COUNTER) AND SUPPLEMENT USAGE
(List the drug name and the condition being managed)

MEDICATION	CONDITION
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Are you currently taking any of the follow medications? Please circle **YES** or **NO** for the following questions.

Anti-coagulants (blood thinners) YES NO

Beta Blockers YES NO

Diuretics (water pills) YES NO

Methotrexate YES NO

Phenytoin (Dilantin) YES NO

Prescription NSAID YES NO

Corticosteroids YES NO

F. **FEMALES ONLY**: Please circle **YES** or **NO** for the following questions.

Are you pregnant? **Yes No** Are you currently breastfeeding? **Yes No**

G. **PHYSICAL PERCEPTIONS** (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after *physical activity* (PA); or during *sedentary periods* (SED))

PA	SED		PA	SED	
( )	( )	Chest Pain	( )	( )	Nausea

( ) ( ) Heart Palpitations ( ) ( ) Light Headedness

( ) ( ) Unusually Rapid Breathing ( ) ( ) Loss of Consciousness

( ) ( ) Overheating ( ) ( ) Loss of Balance

( ) ( ) Muscle Cramping ( ) ( ) Loss of Coordination

( ) ( ) Muscle Pain ( ) ( ) Extreme Weakness

( ) ( ) Joint Pain ( ) ( ) Numbness

( ) ( ) Other_____( ) ( ) Mental Confusion

H. FAMILY HISTORY (✓Check if any of your blood relatives . . . parents, brothers, sisters, aunts, uncles, and/or grandparents . . . have or had any of the following)

( ) Heart Disease

( ) Heart Attacks or Strokes (prior to age 50)

( ) Elevated Blood Cholesterol or Triglyceride Levels

( ) High Blood Pressure

( ) Diabetes

( ) Sudden Death (other than accidental)

I. EXERCISE STATUS

Do you regularly engage in aerobic forms of exercise (i.e., jogging, cycling, walking, etc.)? YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you regularly lift weights? YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you regularly play recreational sports (i.e., basketball, racquetball, volleyball, etc.)? YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you perform any lower body resistance exercises? YES NO

Do you perform any of these lower body resistance exercises on a regular basis? (✓Check if you currently perform any of these lower body resistance exercises)

Leg Extension ( ) Leg Press ( )

Squats ( ) Lunges ( )

Hamstring Curls ( ) Dead Lift ( )

APPENDIX D

Visual Analog Scale

DIRECTIONS:

PLACE A MARK ON THE LINE THAT MOST REFLECTS YOUR MUSCLE DISCOMFORT IN YOUR QUADRICEP (QUADS), HANSTRING, CALF AND BUTTOCK EACH MORNING FOR EACH CATEGORY.

VISUAL ANALOG RATING SCALE

Each **morning (when you wake)** place a mark **X** on the line to indicate your discomfort.

Day 1

Calf None ----------

Extreme

Quads None ----------

Extreme

Hamstring None ----------

Extreme

Buttock None ----------

Extreme

Day 2

Calf None ----------

Extreme

Quads None ----------

Extreme

Hamstring None ----------

Extreme

Buttock None ----------

Extreme

Day 3

Calf None ----------

Extreme

Quads None ----------

Extreme

Hamstring None ----------

Extreme

Buttock None ----------

Extreme

Day 4

Calf None ----------

Extreme

Quads None ----------

Extreme

Hamstring None ----------

Extreme

Buttock None ----------

Extreme

Day 5

Calf None ----------

Extreme

Quads None ----------

Extreme

Hamstring None ----------

Extreme

Buttock None ----------

Extreme

APPENDIX E

Ashwagandha Study: Participant Instructions

Visual Analog Scale:

For the next 5 days (starting tomorrow) you will rate your muscle soreness when you first wake up in the morning on the Visual Analog Scale that you have received for quads, hams, calf and buttocks.

Supplementation: 1capsules 1X daily

For the next five days (starting tomorrow) you will continue to take your supplement 1 times a day (morning) with food. It does not have to be a full meal. Just make sure you eat something when you take your supplement.

Exercise:

For the next 6 days (until your last session with me) you are to refrain from any type of lower body exercise (resistance or aerobic) that could potentially interfere with your muscle fatigue and recovery. *Just take it easy for the next week.*

Medication:

Until you meet with a member of the research team one last time, you are to refrain from taking any of the following medications: Ibuprofen or any other anti-inflammatory medication (prescription, over the counter, or supplements) **AND** analgesic (pain) medications such as Tylenol or any other pain medication (prescription, over the counter, or supplements).

Additional Instructions:

Please refrain from massaging, icing, heating (such as heating pads) or using any type of topical cream such as Icy Hot or Biofreeze to treat the DOMS.

Adverse Events:

If you believe you are experiencing any adverse effects from consuming the supplement, do not take any more of the supplement and contact Cody or Dr. Jacobson immediately.

When do you need to report back to the Neuromuscular Lab?

Day 3 of Supplementation: Thigh Circumference, Pressure Gauge and Vertical Jump

Date _____ Time _____

You will report back to the lab for thigh circumference, pressure gauge and vertical jump. This should only take 10-15 minutes of your time. Make sure you wear shorts. It is important that you keep your scheduled time.

Day 6 Final VAS, Thigh Circumference, Pressure Gauge, and Vertical Jump Test:

Date_____ Time_____

You will report back to the lab to complete your final thigh circumference, pressure gauge, vertical jump test, final VAS, and answer a few question regarding the study. This should only take 10-20 minutes. Make sure you wear shorts. It is very important that you keep your scheduled time.

***** Make sure you bring your VAS scores from the last 5 days and your sandwich bags that the supplements came in to turn in******

VITA

Cody L. Diehl PhD(c) CSCS

Contact Information

Work Address:
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Advisor:
Dr. Bert Jacobson Email: bert.jacobson@okstate.edu
Oklahoma State University Office
Phone: (405)-744-9337
Head- School of Kinesiology
Applied Health and Recreation
Regents Professor
Seretean Endowed Professor

Education and Training

Oklahoma State University, Stillwater, Oklahoma……2018-Expected Defense (Spring/Summer 2021)
Doctor of Philosophy: Health and Human Performance
Advisor: Dr. Bert Jacobson

Oklahoma State University, Stillwater, Oklahoma……..Expected Spring 2021
School of Educational Studies Graduate Certificate in Statistical Methods and Analyses

Western Michigan University, Kalamazoo, Michigan……..2016-2018
Masters of Science: Exercise Science
Advisor: Dr. Timothy Michael

University of Dayton, Dayton, Ohio……2011-2016
Bachelors of Science: Exercise Science