1. **Project Title: Efficacy of a controlled short-term trial of Cannabidiol (CBD) ingestion on reducing symptomatic response and facilitating recovery after induced muscle injury**

2. **Investigators:**

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3. **Abstract:**

Current research has shown evidence that phytocannabinoids may have a promising therapeutic potential in a variety of physical and psychological ailments, and cannabidiol (CBD) is of particular interest due to its positive safety profile, non-intoxicating effects and widespread capabilities in a number of musculoskeletal diseases. Three primary reasons people consume CBD on a global basis, in addition to the fact that it is non-intoxicating, are for symptomatic (pain) relief, anxiety reduction, and improved sleep quality. Very little is known about CBD and how it functions in the body from both an efficacy and mechanistic perspective, especially in humans. There is a large consumer base for this product that will be expanding exponentially in the next few years. Most of the evidence available is anecdotal from the personal testimony of consumers. We aim to determine the efficacy of a controlled short-term trial of CBD ingestion for reducing symptomatic response and facilitating recovery following induced muscle injury. We will assess, in serial fashion, symptomatic response, functional limitations and recovery of the quadriceps muscle following induced injury in which selected doses of CBD oil will be delivered using a sublingual route of administration during a 15-day pre-injury consumption and post-injury recovery phase. A double-blind, randomized, two-arm study design will be used and participants will be randomly assigned to either an active dose (n=15) or vehicle control group (n=15). Our clinical outcomes include measures of muscular pain and disability along with measures of pain-related fear and anxiety. Our laboratory-based study design is desirable and advantageous because it is a controlled method of tracking individuals using an experimental model of injury that is translatable to clinical populations. Another advantage of this study design is that it will address, in parallel fashion, two of the primary reasons people are consuming CBD – symptomatic relief and anxiety reduction. This exploratory study will provide preliminary data needed to support the hypotheses of a planned larger scale application.

4. Background:

Overview and Rationale for Study

Cannabidiol (CBD) is one of more than 100 cannabinoids found in the *Cannabis Sativa L.* or hemp plant. Hemp-derived CBD does not contain any psychoactive ingredients unlike other cannabis products such as ∆9-tetrahydrocannabinol (THC). CBD is manufactured and distributed in a variety of ways including isolate, and broad/full spectrum formulations. CBD isolates contain pure CBD (99.9%) and are tasteless and odorless, while broad and full spectrum products contain hemp-extracts that contain CBD (~4%), and very low levels of THC (broad spectrum 0%, full spectrum <0.3%), along with all naturally occurring cannabinoids, terpenes and essential oils from the plant extract. As a constituent, CBD is the same regardless of where it originates; the main difference is in the presence and concentrations of other compounds in the end-product. CBD does not cause intoxication or make you feel euphoric or "high". As a result, many physically-active Americans, including prominent professional athletes, have reported pain relieving effects of CBD that can reduce or eliminate the use of nonsteroidal anti-inflammatory drugs (NSAIDs) for activity-related pain with minimal to no side effects.[1] Long-term use of over the counter (OTC) medications, including NSAIDs, can pose a significant health risk, coupled with the rise in dependence on opioid medications has resulted in the deaths of tens of thousands of Americans annually and over 300,000 deaths during the past decade.[2,3] Consequently, many consumers of CBD-related products view them as alternatives to NSAIDs for treating such ailments as musculoskeletal and neuropathic pain, anxiety and insomnia, with little to no knowledge or evidence as to best practices regarding dose or method of administration. The sale of CBD and CBD related products is poorly regulated even as the hemp industry is rapidly growing. As of 2016, consumer CBD related sales totaled $262.2 million, and is estimated to reach $1.8-billion by 2022.[4] A 2019 CBD market report predicts that the industry will reach $22-billion in the near future.[5] The widespread use of CBD has gotten far ahead of the research to support the health-related claims that are being made by manufacturers. Therefore, scientific exploration and validation is necessary.

Hemp-derived CBD is a phytocannabinoid, and is chemically similar to components of the endogenous endocannabinoid system (ECS) that play an important role in the homeostasis of bodily functions including the regulation of tissue inflammation and pain.[6,7] Cannabidiol can account for up to 40% of the Hemp plant's extract, and does not violate the Controlled Substance Act if it contains less than 0.3% THC.[8] CBD can be taken into the body in multiple ways including inhalation (smoke or vapor), buccal (aerosol spray into the cheek), oral (swallowed as liquid or gelatin capsules) and sublingual (drops) routes of administration.

The World Health Organization (WHO) Expert Committee on Drug Dependence recommended not scheduling CBD within the International Drug Control Conventions. WHO cited the fact that there are no case reports of CBD abuse or dependence; no public health problems have been associated with CBD use. CBD has been found to be generally well-tolerated with a favorable safety profile; and that there is no evidence that CBD is likely to be abused by users.[9] Furthermore, the U.S. Health and Human Services Department (HHS) conducted a scientific review on CBD and concluded that it does not present a significant risk to public health. The U.S. Health and Human Services Department found that there is no evidence for classic drug withdrawal, no evidence that CBD causes physical or psychic dependence and no potential for abuse under the controlled substances act.[10] CBD products sourced from hemp, such as oils and tinctures, can be purchased on-line or in major drugstores, convenience stores and retail shops for human consumption

CBD's documented health and medical benefits open an important therapeutic window for scientific discovery. Current research has shown evidence that phytocannabinoids may have a

promising therapeutic potential in a variety of physical and psychological ailments, and CBD is of particular interest due to its positive safety profile and widespread capabilities in a number of neurological conditions and musculoskeletal diseases.[11] Broadly stated, CBD, irrespective of dosage and route of administration, has demonstrated anxiolytic, anti-inflammatory, and analgesic benefits primarily in animal models.[11] Currently the underlying mechanisms of action for its biological and therapeutic effects have not been elucidated and clinical research in humans regarding its efficacy is lacking.

Cell and Animal Studies

In vitro and in vivo studies have shown that, unlike THC, CBD has a low affinity for a variety of cannabinoid receptors in the brain, such as CB_1, CB_2, and several G-protein coupled receptors.[12-15] The lack of affinity between CBD and cannabinoid receptors is thought to obviate any potential therapeutic effects of CBD using this receptor-mediated pathway.[8,12] More recent evidence indicates that CBD works synergistically with the endocannabinoid system (ECS) in modulating pain, inflammation and anxiety.[10,16] Elmes et al.[16] postulates that CBD acts as an endocannabinoid (EC) reuptake inhibitor regulating concentrations of EC proteins, such as anandamide, in the central and peripheral nervous system. The elevated concentrations of these EC molecules is thought to potentiate their intracellular signaling activity in the nervous system, thus exerting anti-inflammatory, analgesic and anxiolytic effects in the body.

Another neuro-modulatory pathway of interest for CBD activity is the serotonin 5-HT neurotransmitter-receptor system known to mediate inhibitory neurotransmission for acute and chronic inflammatory pain.[17] Specifically, CBD has been shown to act as a partial agonist for the $5\text{-}HT_{1A}$ receptor.[18,19] In addition, CBD is an allosteric modulator of μ- and δ-opioid receptors with a high affinity for pain inhibitory neurotransmitters such as enkephalins and β-endorphins.[20] Cannabidiol has also been found to be an agonist for transient receptor potential (TRP) ion channels V_1 and A_1 located on primary sensory neurons projecting to the spinal cord and brain stem where they can modulate pain by interacting with GABA-related inhibitory neurotransmitter circuits.[10,17,21]

Human Clinical Trials

In clinical trials CBD is generally administered orally as either a capsule or dissolved in an oil solution or under the tongue (sublingual) using a titrated dropper. A wide range of oral doses have been reported in the literature, with most ranging from 100 to 800mg/day or 2-10mg of CBD per kg of body weight per day to approximately 25-50 mg/kg/d with only minor side effects and adverse events.

G.W. Pharmaceuticals (Salisbury, Wiltshire, UK) processes, manufactures, and markets the CBD drug Epidiolex® and has run numerous clinical trials that investigated the effects of CBD in the treatment of rare child seizure disorders related to epilepsy. Epidiolex® is a highly purified pharmaceutical grade CBD isolate which is qualitatively and quantitatively different than most other forms of hemp-derived CBD products consumed by individuals. Efficacy studies were performed in three randomized, double-blind, placebo-controlled clinical trials involving 516 patients with either Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS).[22-24] In these trials, Epidiolex®, taken along with other medications, was shown to be effective in reducing the frequency of seizures when compared with placebo. The results of these trials have set the initial standards for dose optimization with recommended doses of 2-10mg/kg/d with gradual incremental increases up to 25-50mg/kg/d for reducing the incidence and magnitude of seizures. The most common side effects that occurred in Epidiolex-treated patients in the clinical trials were sleepiness, sedation and lethargy; elevated liver enzymes; decreased appetite; diarrhea; rash; fatigue, malaise and weakness; insomnia, sleep disorder and poor-quality sleep; and infections.

Two published placebo-controlled, randomized clinical studies were conducted by the same research team at the University of Sao Paulo (Sao Paulo, Brazil). Both studies investigated the anxiolytic effects of CBD for the treatment of anxiety (social anxiety disorder). Bergamaschi et al.[25] compared the effects of a simulated public speaking test (SPST) on healthy controls (HC) and individuals with social anxiety disorder (SAD) who received a single dose of CBD (600mg) or placebo (0mg). The product was consumed orally in gelatin capsules (99.9% pure CBD powder dissolved in corn oil base) and provided by STI-Pharm, (Brentwood, UK) and THC-Pharm, (Frankfurt, Germany). The CBD group reported significantly less anxiety on self-reported symptom and mood scales than the placebo group, but not the HC group. Zuardi et al.[26] compared the effects of a single dose of CBD or placebo on a SPST using healthy individuals. The CBD dose-range was 100mg, 300mg or 900mg and subjects consumed the product orally in gelatin capsules (99.9% pure CBD powder dissolved in corn oil base at 100 and 200mg/mL) provided by Biosynthesis Pharma Group (BSPG-Pharm, Sandwich, UK). Their results were able to show that acute administration of CBD at the 300 mg (but not the 100mg and 900mg doses) was effective for reducing anxiety as self-reported on a mood scale in the post-speech phase.

In a placebo-controlled, randomized clinical trial conducted in the US (Mt. Sinai, NY), Hurd et al.[27] investigated the effects of CBD administration for the reduction of cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder. Participants ingested either 400mg or 800mg (between 5mg/kg and 10mg/kg of body weight) doses of CBD once daily for three consecutive days. The CBD was administered in oral solution (100 mg/mL; Epidiolex) and provided by G.W. Pharmaceuticals (Salisbury, Wiltshire, UK). Acute CBD administration significantly reduced both craving and anxiety induced by the presentation of salient drug use cues compared with neutral cues. CBD also demonstrated prolonged effects measures one week after short term (3-d) CBD exposure. In addition, CBD reduced the drug cue-induced physiological measures of HR and cortisol levels. There were no reported serious side effects of CBD exposure.

Overall, CBD administered in various forms demonstrated a favorable safety profile in these controlled clinical trials. There is also preliminary evidence that CBD may be a useful treatment for several other medical conditions. Several current studies located on clinicaltrials.gov will begin or have begun recruiting subjects and collecting data in efficacy studies investigating CBD ingestion and its therapeutic effects for reducing chronic and musculoskeletal pain and anxiety-related disorders (social anxiety disorders).

Experimental Injury Model

The experimental injury model we will employ for this study is a controlled model of muscle micro-trauma and recovery. This particular experimental model was selected because it allows us to control and standardize the mechanism of injury and allows us to track symptomatic response and physical impairment in a more clinically relevant manner when compared with other experimental pain models that are controllable but of shorter duration (e.g. thermal or pressure stimuli). The experimental model requires the participant to complete a single bout of high intensity resistance exercise. The exercise bout is able to systematically optimize eccentric overload of the muscle coupled with fatigue to produce controlled strain-induced damage to the contractile elements of the muscle. The resultant muscle injury produces a local inflammatory response with associated soreness, stiffness, disability, and functional deficits.[28-31] Our previous findings from work in the shoulder, arm and low back generate pain and disability in subjects. We have also demonstrated an established link between this pre-clinical pain model and a post-operative clinical pain model suggesting robust validity.[32,33] Last, these models form the basis for trials funded by the NIH to study other interventions for pain.

We will be able to study important bio-behavioral factors in a concomitant manner that are specific to the central tenets of ingesting a hemp-extract that contains CBD and other minor cannabinoids. Biological factors to be studied include clinical signs of reduced symptomatology and functional limitations as well as quicker recovery to baseline that are indicative of anti-inflammatory and analgesic effects of CBD ingestion. Additional prior work by our group shows robust associations among pain intensity and measures of negative affect. Thus, we will also be able to study pain-related fear-avoidant and anxiety behaviors that are involved in the participants' perception of the threat or anticipation of a painful experience related to intense exercise and injury before, during and after undergoing our experimental injury model.

5. Specific Aims of Study:

Our overall objective is to generate preliminary data in humans on the effects of ingesting a hemp-extract investigational product that contains CBD for reducing pain-related anxiety and symptoms related to induced muscle injury. We aim to determine the efficacy of a controlled short-term trial of hemp-derived CBD ingestion for reducing symptomatic response and facilitating recovery following induced muscle injury. We will assess, in serial fashion, symptomatic response (both physical and psychological factors), functional limitations and recovery of the quadriceps muscle following induced injury in which selected doses of a hemp-derived CBD oil will be administered orally during a 15-day pre-injury consumption and post-injury recovery phase. We will evaluate these responses with an experimental repeated-measure design spanning pre-treatment, baseline measurement, injury induction, short-term sublingual administration of a hemp-derived CBD extract (or placebo) and recovery.

Aim 1: To determine time-related patterns of symptomatic and functional recovery in response to sublingual administration of either a low-dose or a high-dose CBD extract after muscle injury. Based on the purported anti-inflammatory, analgesic, and anxiolytic effects of CBD ingestion, we **hypothesize** that short-term sublingual administration of a hemp-derived CBD extract will limit the symptomatic response and functional limitations after induced muscle injury allowing for an expedient recovery to baseline conditions.

Aim 2: To determine the impact of sublingual administration of a CBD extract on psychological factors known to influence pain intensity and duration. The investigation of psychological constructs will include evaluating the effects of sublingual administration of a CBD extract on pain-related anxiety, catastrophizing and fear pertaining to participants' views and perceptions toward engaging in strenuous physical exercise, sustaining a musculoskeletal injury and prolonged symptomatic and functional recovery. We **hypothesize** that the anxiolytic effects of sublingual administration of a CBD extract will diminish the threat and perceived physical stress of exercise-induced pain, physical harm, and prolonged symptomatic and functional recovery.

This exploratory study will provide preliminary data needed to support the hypotheses of a planned larger scale application. Preliminary data will help in better designing a future study aimed at identifying an efficacious dose range of hemp-derived CBD extract, as well as determining the cellular and molecular mechanisms that contribute to symptom resolution and recovery. Our findings may also be helpful for making future improvements for treating a broader range of inflammatory conditions that afflict the musculoskeletal system.

6. Research Plan:

Study Location & Design

The project will involve one study center located on the campus of the University of Florida. Data collection for the study will take place in the Sports Medicine & Human Performance Research Laboratory (187 Florida Gymnasium, 1864 Stadium Road, Gainesville, FL 32611). The study protocol will be submitted to the UF Gainesville Health Science Center Institutional Review Board (IRB-01) located in the Gainesville Health Science Center (Ruth K and Shepard Broad Building, 1300 Center Drive, Room 130, Gainesville, FL 32610).

The design will be a double-blind, randomized, vehicle-controlled, three-arm trial. Participants will be randomly assigned to either an active CBD dose group (n=15) or vehicle-control (placebo) group (n=15).. The effects of the active CBD dose groups will be compared to the vehicle-control. Subjects will be required to complete a 15-day study trial spanning a pre-supplement baseline visit, a controlled daily dosage regimen, pre-exercise baseline testing, eccentric exercise to induce muscle damage, and planned follow-up post-exercise data collection time points.

Study Population

Inclusion Criteria: a) male and female adults between the ages of 18-35 years and b) English speaking, and c) both female and male subjects must be currently practicing acceptable methods of birth control, such as abstinence, and methods of contraception (barriers, oral, patch or other prophylactic methods).

Exclusion Criteria: (a) use of cannabis products on a regular basis OR positive urine test for cannabis, (b) history of seizure disorder (self or family), traumatic brain injury, liver, kidney, or cardiovascular disease, (c) current medical condition that would prevent the participant from performing strenuous resistance exercise, (d) weight lifting for the lower extremities (legs) more than twice a week, (e) currently experiencing pain in the hips, leg, or knee region, (f) pregnancy, lactating or positive urine pregnancy test, (g) known allergy to CBD, coconut/sesame oil, or tree nuts (coconut).

If the participant self-reports that they are currently using tobacco products or a nutritional/dietary supplement, or if they take prescription anti-depressant/anxiety medications, the participant may continue to use the product(s) but they must be on a stable dose at least 3 months prior to randomization as well as during the 15-day study trial period. Also, if the participant is currently using OTC anti-inflammatory medication (e.g., Advil, Aleve, Aspirin) on a regular basis, we will ask that they stop taking the medication at least 7 days before randomization and refrain from using the product during their participation in the study trial.

Investigational Product & Dosing Schedule

Participants will be provided a 30mL bottle with a syringe dropper on day 1 with instructions to ingest the solution orally using a sublingual route of administration (0.5 cc ~1/2 dropper) twice per day (BID) 12 hours apart (morning and evening) at the prescribed daily dosages: Vehicle-control (0mg/30mL hemp extract = no hemp extract), and active Dose (2000mg/30mL hemp extract = 67mg/day). The sublingual route of administration allows the product to be absorbed directly into venous circulation, and thus bypasses the liver and eliminates the consequences of first pass metabolism; ultimately increasing its bio-availability. The bioavailability of CBD administered orally (including sublingual) is estimated to be 13-19% and its half-life estimated to be around 18-32 hours.[35,36]

SunFlora (St. Petersburg, FL) has been selected as the supplier for the investigational test article. SunFlora is a third-party tested hemp-derived product manufacturing company.

SunFlora is dedicated to producing high quality hemp-derived products formulated to be safely used by the public and are manufactured in accordance with the Food and Drug Administration (FDA) Current Good Manufacturing Practices (cGMP) for Dietary Supplements, 21 CFR Part 111. Their hemp derived products are assessed multiple times at production process control points throughout the manufacturing process. Dietary ingredients used in the manufacturing process are accompanied by certificates of analyses (CoAs) confirming their identity and specifications. All finished products are tested to ensure they meet specifications prior to being released for distribution. The label and all labeling satisfy FDA requirements found under 21 CFR 101. Medium chain triglyceride-based mixing agent blended with coconut oil will be used as the vehicle-control. The addition of the hemp extract to the mixing agent will not change the physical properties of the liquid, and thus will be indistinguishable from the vehicle-control.

Experimental Procedures and Timeline

Recruitment: Participants will respond to advertisements posted around UF campus and on CANVAS sites. Candidates who respond to the advertisements will be contacted by phone for a pre-screen interview to determine if he/she meets the entry criteria. (refer to phone screen for detailed information). Any information collected during the screen will be discarded. If the candidate meets the eligibility criteria, he/she will be sent the informed consent document electronically to read over and familiarize themselves with the protocol. Participants will be instructed not to sign the consent document until they arrive for their 1st visit. Participants will be required to report to the Sports Medicine & Human Performance Research Laboratory for six scheduled visits.

Physical Discomforts and Intervention Risks:

Exercise Protocol: Participants will likely experience some level of discomfort in their upper thigh (quadriceps muscle) following the exercise protocol. The discomfort will be in the form of localized muscle soreness, stiffness, and limited motion. These symptoms have been shown to last short-term and should be significantly diminished within 4-5 days. Based on our previous experience, the chance of this occurring is rare, occurring in less than 1% of the population (less than 1 out of 100). As with any type of resistance exercise there is a slight risk of repetitive strain injury. We have used this exercise protocol in our previous studies without any incidence of significant injury or any other related adverse reaction. All muscle resistance exercise sessions will be investigator supervised to minimize the probability of muscle injury. Furthermore, in the unlikely event that an injury may occur, a National Athletic Trainers' Association Board-certified athletic trainer (ATC) or licensed physical therapist (LPT) will be available to direct immediate treatment, if required. All study personnel are first aid and CPR trained and certified.

Mechanical Pressure: pressure is administered to the surface of the skin at an approximate rate of 30kPa/sec via a hand-held algometer fitted with a 10mm diameter rubber tip. Participants will be instructed to signal pain by pressing a button (kill switch) when the pressure sensation becomes unpleasant. The algometer will be immediately removed from the surface of the skin upon signal from the participant. The risk of bruising or other skin trauma as a result of this procedure is minimal. By design, participants stop the procedure at the onset of discomfort (unpleasantness) but will be informed that they also can elect to discontinue the procedure at any time. In a separate assessment, punctate pressure stimuli will be delivered to the surface of the skin using a nylon monofilament. The procedure involves 10 pressure applications at a rate of one application per second. The risk associated with this procedure is minimal. Some participants may experience mild skin irritation and/or redness at the immediate punctate site

that will resolve within 1-3 minutes following the procedure. Participants may stop the procedure at any time at their request.

Cold Stimulation: Cold is delivered by having the subjects immerse their hand in cold water. The process can produce temporary discomfort but will usually subside within seconds of withdrawal of the hand. No other risks are known currently. Participants may stop the procedure by removing their hand at any time.

CBD use: In previous clinical trials using hemp-derived products administered orally (liquid or capsule) and swallowed, a small percentage of patients experienced tiredness, change in appetite and gastro-intestinal discomfort following ingestion. In addition, published reports have indicated that CBD ingestion may lead to elevated blood levels of serotonin which could pose a very mild risk of serotonin syndrome or toxicity. We will be using daily doses of CBD that are considerably lower than the daily doses used in the clinical trials. Therefore, the chance of our participants experiencing these side effects is minimal.

Safety Monitoring and Stopping Criteria:

Safety monitoring will occur during the initial baseline visit as well as throughout the participant's duration in the study, including a 1-week follow-up contact after study termination. As noted below, the baseline assessment conducted during the first visit will include a brief physical exam (including vital signs) and a thorough medical and psychiatric history, including a review of past medical history, concomitant medications, inclusion/exclusion criteria, and potential contraindications. Safety monitoring will include active and passive surveillance procedures using pre-existing safety monitoring guidelines. Participants will be assessed actively at each laboratory visit by study personnel using the side effects/adverse events checklist. Each participant will be read each side effect/adverse event from the checklist and instructed to self-report as yes (go) or no (no go). If a participant experiences any of the side effects/adverse events from the checklist provided at the baseline visit, study personnel will then contact the study physician (Dr. Cook) for consultation and guidance. The side effects/adverse events will be triaged as mild, moderate or severe, and a determination will be made by the study physician as to whether the side effect/adverse event is due to the investigational test article or some other circumstance (e.g. food poisoning). If the reaction or event is determined to be due to the investigational test article and graded moderate to severe by the study physician, the participant will then be instructed to stop participation and advised to seek medical care by their primary physician or hospital. (Refer to stopping criteria below). In the event that emergency care needs to be provided the participant is instructed to immediately call 911 or seek care at a medical health facility (e.g. hospital). The study physician (Dr. Cook) would also be contacted for guidance as well. Any serious adverse reaction or event will be followed until full resolution. All adverse reactions and events will be reported to the UF IRB within 24-48-hrs and documented in the participant's file (redcap).

Stopping criteria:

1. The PI/physician decides that the participant should be withdrawn for **safety reasons** (presence of active lesions in the oral cavity/sublingual region or suffering side effects or adverse events associated with the use of the investigational product).
    a. List of side effects and adverse reactions are listed below*
2. Participant is unwilling to continue in the study.
3. Lack of compliance with protocol.
4. Investigator or study sponsor stops the study for any reason.
5. Participant becomes pregnant.
6. Participant is enrolled in error or lost to follow-up (lost to follow-up will be defined as a subject failing to attend planned study visits or failure to respond to contacts by study personnel after documented 2 attempts).

*Potential Side Effects and/or Adverse Events (AEs):

1. Feelings of anxiety (nervousness, restlessness or being tense, feelings of danger, panic, or dread), paranoia, or negative mood (depression or suicidal thoughts), or paranoia.
2. Poor quality of sleep (persistent insomnia).
3. Feelings of extreme fatigue, sleepiness or drowsiness.
4. Feelings of nausea (upset stomach, vomiting or gastro-intestinal (GI) distress (e.g. diarrhea).
5. Change in or loss of appetite.
6. Feelings of light-headedness or dizziness.
7. Visual disturbances such as blurry/double vision or dilated pupils.
8. Hypotension, hypertension, tachycardia, heart palpitations, syncope or dyspnea.
9. Development of a skin rash with redness and itching.
10. Loss of muscle coordination (clumsiness)
11. Muscle spasms

Visit 1 (Consenting, screening, randomization and test product distribution, Day 1)

Participants will be consented. Consenting will be performed by one of the co-investigators in a private room. The following procedures will be performed: urine sample for pregnancy (females) and toxicology screen (presence of cannabinoids), a brief physical exam (including vital signs: measurements for height, weight, pulse, blood pressure and body temperature), obtaining a thorough medical and psychiatric history, including a review of past medical history, concomitant medications, inclusion/exclusion criteria and potential contraindications, assessment of the oral cavity and sublingual area to rule out any active lesions or abnormalities (bleeding, sores, ulcers, abscesses, etc.). If any lesions are found to be present, the research assistant will document the findings and follow up with a consultation phone call with the study physician to determine next steps. The study participant may choose to be excluded from participation or wait until the condition fully resolves. If the participant continues with the study protocol, follow up oral assessments will be repeated at visits 2 through 6.

Results of urine screening for pregnancy and toxicology (presence of cannabinoids) will be available and assessed during the first visit. If the urine test (pregnancy or cannabinoids) comes back positive, the participant will be excluded from the study. The participant will complete several psychological questionnaires (FPQ-9, PCS, PASS-20, PSQI, & LEFS), followed by tests for muscle soreness and quantitative sensory testing (QST). Each participant will be provided a detailed safety monitoring checklist of side effects and/or adverse reactions that they may potentially experience from ingesting the investigational product. Participants will then be instructed on how to perform their own passive surveillance for identifying these side effects/adverse events and what to do if they experience any of them between study visits while they are ingesting the investigational test article (Days 1 through 10). Participants will be instructed to maintain current activity levels and not to initiate a resistance-training or weight-loss program for the duration of the study. Participants will then be randomly assigned to an experimental group and instructed on how to ingest the investigational test article (or placebo) and dosing procedures, which will be dispensed in a quantity to last for the next 15 days. Each participant will be provided a daily log chart to record their daily CBD doses for compliance and monitoring purposes. A pre-exercise visit will be scheduled. Each subject will receive $10 for completion of the test session.

During the passive surveillance period (Days 1 through 10) participants will be instructed to contact the PI (Borsa) if they experience any of the side effects/adverse events from the checklist provided at the baseline visit. Dr. Borsa/Bishop will then contact the study physician (Dr. Cook) for consultation. The side effects/adverse reactions will be triaged as mild, moderate or severe, and a determination will be made as to whether the side effect/adverse reaction is due to the investigational product or some other circumstance (e.g. food poisoning). If the event is determined to be due to the investigational test article and graded moderate to severe by the study physician, the participant will then be instructed to stop participation and seek medical care by their primary physician or hospital. Any participant that reports a serious adverse event will be followed to full resolution.

Visit 2 (Pre-exercise measurements and exercise protocol, Day 11)

Participants will be assessed actively (including vital signs) at each subsequent visit by study personnel using the side effects/adverse events checklist. Each participant will be read each side effect/adverse reaction from the checklist and instructed to self-report as yes (go) or no (no go). For participants who continue in the study, the following procedures will be performed: completion of psychological questionnaires (FPQ-9, PCS, PASS-20, PSQI, & LEFS), followed by tests of muscle soreness, quantitative sensory testing (QST), the timed stair climbing test (TSCT) and isometric strength measures to evaluate leg function. Participants will then undergo a standardized single bout of eccentric resistance exercise to the dominant quadriceps muscle. An isokinetic dynamometer (Biodex System 4 Pro, Biodex Medical Systems, Inc., Shirley, NY) will be used for the eccentric exercise protocol. Each participant will then perform five sets of twenty-five repetitions. Exercise will be performed using concentric (meaning shortening of muscles during contraction) and eccentric (lengthening of the muscle while still developing tension). The angular velocity will be set at 60°/sec for concentric and 90°/sec for eccentric actions. Participants will be given a three-minute rest period between sets. Each subject will receive $15 for completing the test session.

Visit 3, 4, & 5 (Follow-up measurements @ 24, 48, & 72-hr post-exercise, Day 12, 13, & 14)

The following procedures will be performed: completion of psychological questionnaires (PCS & LEFS), followed by tests of muscle soreness and leg function testing (TSCT and isometric strength measures). Participants will receive $5 for completing visits 3, 4 and 5.

Visit 6 (Follow-up measurements @ 96-hr post-exercise, Day 15)

The following procedures will be performed: completion of psychological questionnaires (FPQ-9, PCS, PASS-20, PSQI, & LEFS), followed by tests of muscle soreness, QST, and leg function testing (TSCT and isometric strength measures). At the end of the session each participant will be asked if they thought they were taking the active CBD product or placebo. Participants will receive $10 for completing visit 6.

Study personnel will perform a follow-up phone call at 1 week after completion from the study for continued safety monitoring. Study personnel will refer to the checklist to determine if the participant has experienced or is experiencing any side effects or adverse events using the safety monitoring guidelines. If the participant reports experiencing any of the side effects/adverse events from the checklist the study physician will be consulted immediately for guidance and the participant's condition will be followed until full resolution.

Primary Outcome Measures: Self-report **ratings of muscle soreness** will be used to determine efficacy for duration of soreness and peak intensity of soreness. A muscle soreness inventory will be used to self-report the level of soreness. The muscle soreness inventory consists of rating soreness on a visual analog scale (VAS). A 10cm line is drawn with 0 (no soreness) on the left pole and 10 (extreme soreness) on the right pole. The inventory asks participants to rate their level of soreness by placing a slash on the line that best represents

their current level of soreness and soreness at its worst, best and average over the past 24 hours. The highest worst soreness intensity recorded during the recovery time will be recorded as the peak intensity of soreness. Measures of anxiety and fear of pain/soreness will also be recorded using a similar VAS. **Disability** will be measured using the Lower Extremity Functional Scale (LEFS).[37] The LEFS is a self-report questionnaire containing 20 items concerning an individual's ability to perform everyday tasks. The scale can be used to evaluate the functional impairment of a patient post-exercise of one or both lower extremities. Each item includes a 0-4 point hierarchical grading scale with 0 being "extreme difficulty or unable to perform activity" and 4 being "No difficulty". Subjects will rate their level of function for each of the 20 items for a total of 80 points. Each subject's score will be divided by 80 and recorded as a percentage value. LEFS scores will be recorded regularly until recovery and the highest score during this period will be recorded as peak lower extremity disability.

Secondary Outcome Measures: Evoked Pain will be assessed using instrumented algometry. The mechanical pain threshold (MPT) technique will use a pressure algometer (Force Ten FDX, Wagner Instruments, Greenwich, CT). A pressure algometer is a manually operated force gauge calibrated in kilograms (kg) that administers focal pressure to an area of the body for the purpose of evoking pain or tenderness. The most sensitive point on the quadriceps muscle will be located via palpation and marked for testing. The examiner will apply pressure to the specified point at a rate of 1 kg/sec until the non-noxious pressure turns to pain. The subject will be instructed to indicate the change by saying "pain" at the point when the pressure turns to pain. Each measurement will be performed four times and the average of the four scores will be recorded in kg of force.

Quantitative Sensory Testing (QST): QST is a set of noninvasive tests used to assess pain perception. QST relies on the assessment of an individual's response to external stimuli applied using a stimulus modality (e.g.; pressure and/or temperature), and reflects the integrity of the corresponding sensory pathways to regulate pain transmission and processing by the central nervous system. We will be using QST methods to measure the participants' pain sensitivity and their ability modulate pain perception using endogenous inhibitory pathways.

The QST Protocol will include the following tests: temporal summation, pressure pain threshold and conditioned pain modulation.

Temporal Summation

1. A single punctate stimulus using a 6.65 level, 300g force Tactile Semmes-Weinstein monofilament (Fabrication Enterprises, White Plains, NY) will be delivered to the left inter-metacarpal space (contralateral side), followed by a VAS pain rating.
2. A series of 10 repeated punctate stimuli within a $1cm^2$ site @ 1-Hz will be delivered to the same space as the single stimulus, followed by peak pain rating (VAS).
3. Wind-up ratio will be calculated as peak pain (#2) ÷ single pain (#1).

Pressure Pain Threshold (PPT)

Pressure will be applied to the midpoint of the right (ipsilateral) trapezius muscle using a rubber-tipped algometer (Algomed Computerized Pressure Algometer, Medoc Advanced Medical Systems, USA, Durham, NC). The pressure will be increased at a constant rate of 30 kPA per second until the participant indicates the onset of pain (when the sensation of pressure turns to pain). At that point, the test stimulus will be immediately removed. The procedure will then be repeated for a second and third trial, slightly relocating the test applicator tip between each trial to avoid skin irritation. The amount of applied pressure will be recorded at the moment the subject indicates the onset of pain for each trial. The three values will be averaged and recorded as the pressure pain threshold (PPT).

Conditioned Pain Modulation (CPM)

The CPM assessment will include the application of two stimuli:
Test Stimulus (Pressure Pain). Pressure applied to the midpoint of the tibialis anterior (TA) muscle using the algometer and parameters previously described in the PPT test.
Conditioning Stimulus (Cold-pressor Pain). Participants will be instructed to immerse their hand up to the wrist into a cold-water bath maintained at a constant temperature of 12°C by a refrigerated water circulator, and constantly circulated to prevent warming around the hand.

The test stimulus (pressure pain) will be applied to the left TA muscle. Participants will then be instructed to immerse their right (contralateral) hand in the cold-water bath, with fingers splayed, to begin the conditioning stimulus, maintaining their hand in the water bath for as long as they can tolerate for a maximum of one minute. After thirty seconds of hand immersion, subjects will be asked to rate their pain intensity from the immersed hand using a verbal pain scale (0-100). With the participant's hand still immersed in the cold bath, the test stimulus will be repeated on the TA muscle. The subject will be instructed to remove their hand after one minute of immersion, and a final test stimulus will be immediately delivered at the same anatomical site (TA). If the subject chooses to remove their hand prior to 60 seconds of immersion, the final test stimulus (PPT) will be administered to the TA at that point.

Maximal Voluntary Isometric Contraction (MVIC) will be measured using the Biodex System 4 Pro (Biodex Medical Systems, Inc., Shirley, NY). MVIC is the maximum voluntary force produced during a static muscle contraction and is a test of leg muscle function. Subjects will be seated with their dominant leg placed in 60° of knee flexion. Each subject will perform three maximal voluntary isometric actions held for 5 seconds. The maximum of the three values will be recorded as peak torque in Newton-meters (N-m).

The Timed Stair Climbing Test (TSCT). To assess physical function and pain with movement, participants will complete a timed stair climb test at baseline and again at follow-up to evaluate functional recovery. Participants will be timed as they ascend (up) and descend (down) a flight of 10 stairs. After completing the trial, the participant will be asked to rate their level of perceived exertion (CR-10 Borg Scale) and level of perceived pain with movement (VAS).

Participants will complete a series of self-report questionnaires to identify their level of anxiety and fear-avoidant behavior patterns prior to completing the experimental injury protocol. The **Fear of Pain Questionnaire** (FPQ-9) is a 9-item, 5-point rating scale to quantify fear of specific situations that normally produce pain.[29] The **Pain Catastrophizing Scale** (PCS) is a 13-item, 5-point rating scale used to assess different thoughts that may be associated with experiencing pain.[38] The **Pain Anxiety Symptom Scale** (PASS-20) is a 20 item, 5-point rating scale that assesses 4 theoretically distinct components of pain-related anxiety including cognitive anxiety, fear of pain, escape/avoidance behavior, and physiological anxiety.[39] The **Pittsburgh Sleep Quality Index** (PSQI) is a self-report questionnaire that assesses sleep quality over a 1-month time interval. The measure consists of 19 individual items, creating 7 components that produce one global score, and takes 5–10 minutes to complete.

Participants will be informed during the consenting period, both in writing on the consent form and verbally, that participants will likely experience soreness and stiffness within the exercised muscles after completing the exercise protocol. We postulate that participants will likely experience some level of heightened fear and anxiety (pain-related or other) in the 10-day wash-in phase of the investigational test article before they undergo the experimental exercise protocol and recovery phase. We feel this will be quantifiable on the self-reported psychological questionnaires that the participants will complete before and after the experimental protocol.

Data Management & Analysis
All information and data collected will be stored in locked filing cabinets or in computers with security passwords and accessed only by members of the research team. This process facilitates

data entry and collection, allows for participants to complete self-report questionnaires, and offers optimal security for collecting research-related information. The proposed Data Management will be housed within the Human Performance Laboratory and a secure web-based platform approved by UF (e.g. Redcap) will be used.

Outcome measures will be analyzed using a two-way ANOVA with repeated measures (between-group; within-time) or distribution free test (e.g Friedmans) as indicated by data distribution. Statistical significance will be set at $p<0.05$. If significant interactions occur, the Tukey post-hoc test will be used to reveal where the differences occur. We will calculate effect sizes and estimates of precision for all outcomes. All data analyses will be performed using SPSS® for Windows 16.0 (SPSS, Inc., Chicago, IL).

A **Data and Safety Monitoring Plan** will consist of two components: (1) overseeing participant safety and data monitoring, and (2) performance of monitoring. Since this project is viewed as low potential risk, all key personnel will perform all data and safety monitoring on an as needed basis.

7. **Possible Benefits:**

There is no known direct benefit from participating in this study. Study participants have the option of being paid for their time performing research related duties or they may choose to receive extra credit for a course they are currently taking and that may or may not be considered a benefit. There are no other obvious benefits to the study participant.

The benefit to society is that completion of this investigation may increase scientific knowledge related to benefit of short-term sublingual administration of either a low-dose or high-dose CBD extract for relief of muscle pain and pain-related fear/anxiety symptoms. Another benefit from this study is that the data generate may be used to plan a larger clinical trial.

8. **Conflict of Interest:** No conflicts of interest exist for any study investigators or members.

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