A Pilot Study Testing Benefits of Ursolic Acid (UA) as a Countermeasure To Myopenia and Insulin Resistance in Chronic SCI

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1. Protocol Signature Page

PRINCIPAL INVESTIGATOR SIGNATURE

I have read this protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined herein, including all statements regarding confidentiality. I will make a reasonable effort to complete the study within the time designated. I will provide copies of the protocol and access to all information furnished by the Sponsor to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the Investigational Product and the study. I understand that the study may be terminated or enrollment suspended at any time by the Sponsor, with or without cause, or by me if it becomes necessary to protect the interests of the study patients. Any supplemental information that may be added to this document is also confidential and proprietary to Sponsor and must be kept in confidence in the same manner as the contents of this protocol.

I agree to conduct this study in full accordance with all applicable regulations and Good Clinical Practice (GCP).

_____ _____

Principal Investigator's Signature Date of Signature (DD/MM/YYYY)

Mark S. Nash, Ph.D. FACSM, FASIA
Principal Investigator's Name (Print)

2. Protocol Title

A Pilot Study Testing Benefits of Ursolic Acid (UA) as a Countermeasure To Myopenia and Insulin Resistance in Chronic SCI

3. Objectives

To address the need for an agent that maintains muscle mass and attenuates IR after SCI, we propose a 'first-in-humans with SCI' pilot trial that tests benefits of UA as a countermeasure to sarcopenia and IR.

Aim 1

- To explore effects of UA administration on upper extremity lean mass and peak strength in persons with paraplegia.

Aim 2

- To test effects of UA administration in sedentary individuals with tetraplegia on whole body resting insulin sensitivity, glucose/insulin/inflammatory responses to an oral glucose challenge (OGTT), and whole-body lean mass.

4. Study Endpoints

Primary Outcome Measure(s):

1. Change in Muscle Mass Using Dual X-ray Absorptiometry (DXA)
    a. Time Frame: Baseline, 12 weeks
2. Change in Peak Isokinetic Strength
    a. Time Frame: Baseline, 12 weeks
3. Changes in Fasting Insulin Resistance (IR)
    a. Time Frame: Baseline, 12 weeks
4. Changes in Glucose Disposal
    a. Time Frame: Baseline, 12 weeks

5. Background

The multi-year COVID-19 pandemic has resulted in a decline of overall physical activity, life-satisfaction, and health within the spinal cord injured (SCI) community.[1] **During the pandemic, people with SCI have experienced health decay to a far greater extent than the general population, primarily due to profound social distancing, fears of social engagement and infection, and restrictions in exercise engagement**.[2] Even without social isolation and exercise restrictions, trauma to the spinal cord is typically associated with physical and metabolic disorders that have wide-ranging effects on almost all body systems.[3] Our works,[4] and the works of other investigators[5] have reported a level-of-injury dependent myopenia – i.e., *clinically relevant muscle wasting* – which is commonly observed following spinal cord injury (SCI), and ultimately compromises post-SCI function, metabolism, body composition, and health. Adjustments to body composition that accompany SCI-related myopenia include a decline of metabolically active muscle mass accompanying the loss of actively contracting muscle, which reduces whole body caloric expenditure and establishes

an environment in which substantial gains in whole body fat mass are reported within one year of SCI. Other myopenia-related co-morbid pathologies contribute to cardioendocrine disease clusters, that include insulin resistance (IR).[4] This project addresses an overarching need for the community of spinal cord injured (SCI) individuals to maintain health and function throughout their lifespans. Left unresolved we can expect earlier functional decline and cardioendocrine morbidity in the SCI population. These risks also project onto the caregivers of those with SCI, who are also "living with an SCI" in the contemporary medical environment and are also struggling to recover from the COVID-19 pandemic.

Post-SCI Myopenia

Pathological changes in skeletal muscle have been extensively characterized in humans with SCI.[5] Rapid loss of sublesional muscle mass is explained by a 48% reduction of cross-sectional muscle area occurring as early as 6-weeks following injury. Atrophy ranging from 30-60% of total body lean mass has also been reported, the extent of loss depending on the muscle-specific phenotype and both duration and severity of impairment. (Figure 1.) This muscle atrophy is accompanied by an adaptive fiber-type transformation from a slow-oxidative (Type I) to fast-glycolytic (Type II) myosin heavy-chain phenotype, with both Type I and II fiber atrophy contributing to overall muscular wasting. This shift in muscle phenotype also has known linkage with insulin resistance and progression of cardioendocrine medical complications. Unfortunately, the maintenance or restoration of muscle mass and myosin phenotype following SCI has yet to be achieved by voluntary exercise or electrically-stimulated contractions of muscles located below the lesion level. Attempts to do so have also been dealt a significant blow by the multi-year COVID-19 pandemic, which has resulted in widespread social isolation for many people with SCI and exercise outreach programs. Outreach exercise programs using the Zoom platform have rushed in to fill this gap in needed physical activity. However, the unintended yet widely reported consequences of COVID estrangement have challenged the ability of persons with SCI to preserve levels of fitness needed to sustain essential daily functions, and also offset their prevalent cardiometabolic disease (CMD) risks. These health risks are accompanied by ongoing delays in post-pandemic social reintegration, as fears of respiratory infection still pervade the SCI community, and in all expectation, will do so well into the future.

Cardiometabolic Pathology and Insulin Resistance

The loss of muscle mass after SCI has been associated with hastened trajectories pf all-cause cardiovascular diseases (CVD), which have emerged within the past decade as significant sources of morbidity and mortality for persons with spinal cord injuries (SCI).[6] These trends forecast patterns of illness and death after SCI that increasingly resemble those of the non-disabled general population. Among these CVD risks is the increased hazard for cardiometabolic diseases (CMD), a clustering of interrelated prothrombotic and proinflammatory factors that confers health risks that are equivalent to either coronary artery disease or diabetes.

Notably, these risk equivalents may be experienced without actually having coronary disease or a clinical diagnosis of diabetes, making their occurrence an insidious health threat in need of focused early management.[7]

Ursolic Acid as a Countermeasure to Post-SCI Myopenia and Insulin Resistance

Ursolic Acid – naturally occurring triterpene compound – is an herbal-like isolate obtained from the leaves of various plants (rosemary, marjoram, lavender, thyme, and organum), fruits (apple fruit peel), and berries. UA modulates several signaling pathways associated with the development of chronic diseases. Among confirmed benefits of UA administration are anti-inflammatory, anti-oxidant, anti-obesity, anti-diabetic, cardioprotective, anti-skeletal muscle atrophy, and thermogenic effects. The mechanisms by which UA exerts these beneficial effects may involve regulation of insulin signaling in adipose tissue, as well as atrophy and metabolic signaling in skeletal muscles through the mTOR signaling pathway.[8]

Recent evidence suggests that the UA has beneficial effects on insulin resistance (IR) and related CMD health risks in persons without SCI, and also slows muscle wasting in instances of musculoskeletal disuse.[8] Given that 60 percent of the SCI population has a metabolic profile with IR, and that obligatory sarcopenia occurs within the first year following SCI, UA may represent a novel countermeasure to health risks associated with COVID-related social isolation.

Preclinical testing of UA following SCI

Potential benefits of UA for persons with SCI are supported by our pre-clinical work in which UA but not a control compound, maintained muscle mass and muscle function in mice that had undergone experimental spinal cord contusion injuries.

In a pre-clinical study,[9] we investigated UA as an agent affecting anabolic, metabolically-linked signaling pathways in muscle, and explored phenotypic outcomes in sublesional muscle following traumatic SCI. Our primary objectives were to examine following experimental SCI i. intracellular mTOR signaling, ii. The expression of related catabolic genes, and iii. The effects on these outcomes with UA treatment. We hypothesize that SCI would i. reduce growth pathways via mTOR signaling, ii. Increase the expression of catabolic genes, and iii. UA will attenuate these SCI-induced changes. In addition, we examined the effect of SCI on physical and functional measures, and whether UA-promoted signaling in muscle translated to improvements in these outcome measures. When examining effects of UA treatment on sublesional muscle mTOR signaling, catabolic genes, and functional deficits following severe SCI in mice we observed that UA treatment significantly attenuated SCI-induced decreases in activated forms of mTOR, and signaling intermediates PI3K, AKT, and S6K, and the upregulation of catabolic genes including FOXO1, MAFbx, MURF-1, and PSMD11. In addition, UA treatment improved SCI-induced deficits in body and sublesional muscle mass, as well as functional outcomes related to muscle function, motor coordination, and strength.

These findings provided first evidence that UA treatment may be a potential therapeutic strategy to improve muscle specific pathological consequences of SCI.

6. Inclusion and Exclusion Criteria

Inclusion Criteria

- 18-65 years of age
- Aim 1: Male and female individuals with paraplegia having chronic (≥ 1 year) complete and incomplete (AIS A/B/C) injuries from T2-T8
- Aim 2: Male and female individuals wi4.th chronic (≥ 1 year) complete and incomplete (AIS A/B/C) injuries from C4-C7

Exclusion Criteria

- Pregnant or planning to become pregnant
- Women who are breastfeeding

Number of Subjects

- Aim 1 will enroll up to 10 participants
- Aim 2 will enroll up to 10 participants

7. Study Recruitment Methods

Recruitment will include IRB-approved flyers posted throughout the Christine E. Lynn Rehabilitation Center for the Miami Project to Cure Paralysis; social media; word-of-mouth at the Christine E. Lynn Rehabilitation Center for the Miami Project to Cure Paralysis; online advertisement using IRB-approved language from flyers, telephone or email contact for those individuals who have previously consented for contact in other studies.

In addition, we will utilize the Miami Project to Cure Paralysis research registry of >3000 individuals with SCI who have expressed an interest in participating in research studies. The registry currently is composed mainly of individuals with traumatic SCI (89%) who are ≥18 years old. It includes men and women of different ages with SCI that differs by level, severity, duration, and cause. These individuals have completed and submitted a form that outlines their medical history. Their level of SCI and degree of completeness has also been evaluated. This registry often provides adequate subject enrollment for studies without the inherent expense and difficulty of coordinating recruitment at multiple sites.

8. Study Timelines

Participants that are eligible for Aim 1 will come to the research site (Miami Project) a total of up to 38 visits:

- One day for consenting and screening assessments; Screening assessments may be completed on 2 separate visits to accommodate scheduling conflicts. This will add an additional day to the total visits
- 3 times a week for 12 weeks for exercise intervention;
- one day for post-study assessments.

During this time, they will:

- Take an oral 400mg (8 capsules – 4 in the morning/200mg and 4 at night/200mg) supplement of UA every day for 12 weeks.
- Drug accountability will occur every month and study staff will dispense 2 bottle every month and collect all used or empty bottles
- Pregnancy tests will occur monthly for females of child-bearing potential
- Take part in an exercise program where you will perform strength-training exercises on one arm, 3 times a week, for 12 weeks.
- Complete pre and post intervention assessments (see section 8)

Participants that are eligible for Aim 2 will come to the research site (Miami Project) up to 4 visits:

- One day for consenting and screening assessments. On this visit they will receive the first 2 bottles of the UA.
- 2 monthly visits for drug accountability where study staff will dispense 2 bottles every month and collect used or empty bottles; monthly pregnancy tests for females of child-bearing potential
- One day for post study assessments

During this time, they will:

- Take an oral 400mg (8 capsules – 4 in the morning/200mg and 4 at night/200mg) supplement of UA every day for 12 weeks
- Complete pre and post intervention assessments (see section 8)

9. Product and Procedures

Ursolic Acid (UA)

UA will be sourced as Biotech Nutritions Ursolic Acid Gelatin Free Non-GMO capsules (i.e. form) ; 200mg (4 total capsules, 50mg each) taken twice daily by mouth with food and a non-alcohol-containing drink. UA is a non-Rx nutraceutical obtained without prescription. We will neither claim an FDA 'brand' for the product, nor propose a new or extended indication for its use.

Manufacturer: Biotech Nutritions
24875 Novi Road 865
Novi MI 48376
Ph: 888 662 7001

- Aim 1: will couple UA administration at doses that increase muscle mass with strengthening exercise performed in one arm only.
    - Subjects will receive 2 bottles of oral UA every month. Subjects will be asked to take 400 mg or 8 capsules (4 in the morning/200mg and 4 at night/ 200mg) daily for 12-weeks
    - Adherence to the UA intervention will be done once a month by study staff and will entail:
        - collection of used bottle(s)
        - counting remaining capsules, if any
        - calculation of compliance: [(days x 8) – capsules remaining
        - and dispensing a new bottle(s).

**Education regarding the importance of compliance to the dosing requirements will be part of the monthly drug accountability.

*** Research Studies Subjects will be offered participation in other PM&R and Miami Project to Cure Paralysis studies for which they may be eligible. The possibility of dual enrollment or partial overlap of participation in more than one study is conceivable. This could result in duplication of required assessments subjects would need to complete. However, to simplify participation, reduce the number of visits to our research lab, and mitigate testing redundancy, we may apply test results completed for one study to another in those cases.

- During this period, one upper limb (only), randomized by selection, will undergo exercise conditioning three times weekly using a strengthening program we have established to increase muscle mass and strength in people with SCI. The other arm will remain untrained as a control receiving the same whole body dose of UA.
- Lean mass will be examined before and after 12 weeks of treatment using dual-ray absorptiometry (DXA) and non-contrast CT scan of both arms, with the area of interest identified from the glenohumeral joint distally to the ulnar styloid.
- Peak isokinetic strength of both arms at 90 degrees of flexion will be tested in elbow flexion and extension on a Biodex dynamometer.
- Maximal inspiratory pressure will be measured with the Pro2 Fit device.

The co-primary outcomes will be lean mass and peak strength.

- Aim 2 subjects will:
    - Subjects will receive 2 bottles of oral UA every month
    - Subjects will be asked to take 400 mg or 8 capsules (4 in the morning/200mg and 4 at night/ 200mg) daily for 12-weeks.
        - Adherence to the UA intervention will be done once a month by study staff and will entail:
            - collection of used bottle
            - counting remaining capsules, if any
            - calculation of compliance: [(days x 8) – capsules remaining]
            - and dispensing a new bottles.

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- Whole body lean mass will be determined by DXA and CT scan before and after 12 weeks of treatment.
- Before and after the 12-week UA intervention an oral glucose tolerance test (OGTT: 75 g glucose as Trutol™) will be administrated following an overnight fast.
    - Bloods taken before glucose ingestion will be analyzed for glucose, insulin, and inflammatory biomarkers (TNF-alpha, IL-6, and CRP).
    - Bloods will also be taken at 30-minute intervals for two hours.
- Maximal inspiratory pressure will be measured with the Pro2 Fit device.

Co-primary outcomes will be the fasting IR determined by the HOMA v2 model and the rates of glucose disposal defined by the area under the glucose challenge curve (AUC).

Dual X-ray Absorptiometry (DXA)*

The regional composition for Fat Free Mass (FFM), Fat Mass (FM), % FM, and Lean Mass (LM), in regions of interest including the trunk, arms, android, and gynoid regions will be measured by DXA as described by Gorgey et al., Spinal Cord 2018. Body composition will be measured by whole body scans using a GE

Lunar Prodigy Advance scanner (GE Lunar Inc., Madison, WI,USA). Transfer to the DXA scanner will be performed using either a ceiling lift or assisted self-transfer with or without sliding board. Participants will be instructed to take off any metal, void their bladder, and dress in light clothes prior to transfer to the DXA table. Using the NHANES scanning method, both knees will be bound together using a large Velcro strap. Above the knee joints and each leg is placed in neutral position with the great toe facing upwards. Because of the difficulty in keeping the arms close to the body in large individuals or in cervical SCI, arms will be strapped near the body in the mid-prone position to ensure the total body is within the scanning field. The standard mode scan will be used with a radiation dose of 0.4 μGy. Scans are performed and analyzed by a certified DXA operator using current Lunar software. As part of quality assurance, the DXA scanner will be calibrated using a quality control phantom (L: 197.7, W: 60.1) according to the manufacturer guidelines for both short-term and long-term precision studies.

Non-contrast CT images (Structure)*

Computed tomography (CT) is a radiographic method that produces thin cross-sectional high-resolution images that may better differentiate and measure volumes of fat and lean tissue when compared to DXA. For this pilot study, chest, abdominal and upper extremity/arm scans will be performed at UM/Sylvester Department of Radiology; these will be obtained pre- and post-intervention using a 128-slice multi-dimensional whole-body scanner before and after the intervention. Before the scan, subjects will have their upper extremities ranged. Images for upper extremities will be attained from the glenohumeral joint to the hand. All images will be analyzed at a separate workstation using TeraRecon iNtuition imaging software.

Images are automatically segmented into adipose tissue, skeletal muscle, and bone/background based on location and their attenuation properties, with adipose having the lowest attenuation and bone/background having the highest attenuation. A CT attenuation range of -120 to -40 Hounsfield units (HU) will be used to encompass abdominopelvic adipose tissue, -190 to -30 HU will be used for intra muscular fat (IMF), and -29 to +150 HU will be used for skeletal muscle (needed for IMF quantification). Cross-sectional area (CSA; cm^2) will be automatically calculated by the software by summing the pixels of the tissue and multiplying it by the pixel surface area for each adipose tissue depot, while volume (cm^3) computed by multiplying the CSA by the image slice thickness and inter-slice space. Visual distinction will be used to determine visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), muscle and bone based on the anatomical locations of the tissues and CSA and volumes will be summed together from consecutive slices. Total VAT mass and total SAT mass (in grams) will be the product of the total volume and fat density (0.9196 g/cm^3). To account for muscle size, IMF CSA will be normalized to muscle CSA. The volume of IMF will be calculated as described above. All CT scans will be reviewed by a Radiologist. Subjects will be informed of any incidental abnormalities detected in the CT scans performed for this study and they will be referred to an appropriate provider. In

order to do a single scan of the upper limbs and abdominopelvic cavity, the chest will be included in the scan. It will also be reviewed for incidental findings.

*Total effective dose of radiation for DXA and CT scans (total pre and post) is expected to be around 22 mSv (millisievert) or around 7 years worth of background radiation.

Blood Analyte Testing

Subjects refraining from alcohol and caffeine ingestion starting 24 hours before testing will be studied in the postabsorptive state after a 10-hour overnight fast. Under antiseptic conditions, a 21-gauge Teflon catheter (Jelco) with multi-sample Luer connector will be inserted in a superficial arm vein by a State of Florida Certified Phlebotomist. The catheter is kept patent with sterile physiological (0.9%) saline. Blood samples obtained at rest and during the OGTT (taken at 30-minute intervals for two hours) will be collected in NaF (glycolysis), clot lysis activator (serum), and citrated Vacutainer tubes, and then centrifuged at 8,000g for 30 minutes to isolate the serum. These isolates will be tested on a Roche Mira-Cobas Automated Chemistry Analyzer (Roche Diagnostic Systems, Basel, Switzerland), as we have described.

Oral Glucose Tolerance Test (OGTT)

Baseline blood sample will be drawn (see to blood analyte for procedure details) after an overnight fast. Subjects will them be given Trutol glucose solution that contains seventy five (75) grams of glucose, similar in taste to a very sweet soda (usually orange flavor). Subjects will be instructed to complete ingestion within five (5) minutes.

After drinking the Trutol, blood will be drawn at exactly 30 minute intervals for two hours. Blood collection process is detailed in the Blood Analyte Testing section.

Subjects must refrain from eating/drinking anything other than water for the duration of the test, approximately 2.5 hours.

Insulin Resistance

Insulin resistance will be computed using the Homeostatic Model of Mathews (HOMA; computer version v.2: [https://www.dtu.ox.ac.uk/homacalculator/download.php]) Criterion scores for normal, glucose intolerant, and insulin resistant were published by Nash, et al., Arch Phys Med Rehabil, 2016.

Strength

Peak isokinetic forces generated during elbow flexion and elbow extension will be tested on a BiodexR dynamometer. Peak and average isokinetic forces will be tested through full range of flexion and extension.

Maximal inspiratory pressure (MIP) is a surrogate measure of the strength of inspiratory muscles, primarily the diaphragm. Using the PRO2Fit device, each participant will be asked to perform up to 6 MIP maneuvers, with a goal of matching the highest two according to device or ATS guidelines. The participant will be seated for the test and will be instructed to exhale slowly and completely, seal lips firmly around the new mouthpiece, and then "pull in hard, like you are trying to suck up a thick milkshake." Participants will rest for about one minute and then repeat the maneuvers.
The PrO2 is a mobile connected device that measures inspiratory muscle performance and capacity. The device has a removable mouthpiece that will be thoroughly hand washed (with soap and water) after each participant visit. In addition, we will use a single-use bacterial/viral filter (BVF) to provide additional protection for participants. Vitalograph's BVFs provide an efficient hygiene solution giving better than 99.99% cross-contamination efficiency. The outer housing will be cleaned with alcohol or sani-wipes after each visit.

Pregnancy

Urine pregnancy tests will be performed on all female subjects of childbearing potential. The first test will be done at the first visit after the consent is signed and then every month for the duration of the study.

10. Data and Specimen Banking

- Specimens – General – Whole blood will be assayed by the site laboratory (Diabetes Research Institute Biomarker and Immunoassay Laboratory and will be disposed of once analyzed. No residual samples will be banked.
- Electronic Data – All electronic files will be stored on password-protected computers at each center. Computer security is provided by data encryption, firewall protection, and backup on the Medical/Academic Center Servers. Only the PI and appropriately indicated study staff will have access to the data.
- Data for the trial will be managed using a secure data repository system database (box or redcap), and through paper and pencil. Regulatory trial management may use the Complion INC e-regulatory system platform. All data collected will be entered into a secured data collection system. All participating centers in this multi-site project will have access to the coded deidentified data collected as a part of the study.

11. Data Management

Randomization

- For Aim 1, the dominant or non-dominant arm assigned to resistance exercise (versus non-exercise) will be randomized in equal numbers using the 'RAND' function in Microsoft Excel.
- No randomization is required for Aim 2.

Data analysis

- Will be performed using a repeated measures design corrected for multiple comparisons (Bonferroni) with co-primary outcomes for Aim 1 of *mass* and *strength*, and Aim 2 for co-primary outcomes of *IR* and *glucose disposal*. Other outcomes will be considered exploratory.
- Non-parametric techniques will be used, or data transformed, if the data fail a test of normality or homogeneity of variance. $\alpha = 0.05$ for all testing will represent the threshold value used to judge whether a test statistic is significant.

12. Confidentiality and Subject's Data Safety

- Physical/paper records will be stored in a locked room at the Miami Project (Christine E. Lynn Rehabilitation Center, 1611 NW 12th Ave, 2nd Floor) in a lockable file cabinet only accessible to study personnel in the. A security badge is needed to open the office door where data are stored. Access to Miami Project (Lynn Center 2nd Floor) is via a university-issued hallway proximity card after passing a security guard located in the front lobby.

- There are two types of electronic records, 1) source data and 2) pdf versions of all paper and electronic data.
    - Source electronic data will be stored in the original collection location. In addition, a copy of the electronic file may be stored on the Miami Project Server in a working group with restricted access. The Miami Project server is behind UM firewalls and is backed up electronically daily, with tapes of older copies archived off site in a fire/hurricane secure facility.
    - All electronic and paper source data will also be stored electronically as pdf's in an electronic version of the participant's folder. Paper source data will be scanned, and electronic source data will be saved as a pdf.
- Data Storage – Duration
    - Physical records will be kept for a minimum of 3 years after enrollment is closed and destroyed by shredding services provided to our center. Electronic records will be kept for a minimum of 5 years after study closure.
    - Source data will be maintained for 3 years after study closure – as mandated by UM IRB guidelines - and will then destroyed by a secure method, typically shredding using a university provider. All disposal of source data, hard copy records, and electronic records will meet or exceed all standards such as HIPAA, FDA Security Regulations
- Data – Access
    - During execution of the study access to identifiable information or links to identifiable information will be restricted to study personnel. However, coded information will be accessible to study personnel and may be provided to non-study personnel on a limited basis to gain assistance with data entry, management, analyses, and interpretation.
- Provisions to Protect the Privacy Interests of Subjects
    - To protect subject privacy, participant consent forms will be stored in a file that is separate from their source data. Records/source data (physical and electronic) will be identified by a numeric ID. Numerical code will be linked to participant names and contact info in a password protected electronic file solely accessible by the PI and authorized study personnel. Linkage codes will be maintained until data analyses are completed. Once the data have been analyzed and published, the linkage codes will be destroyed.

13. Provisions to Monitor the Data to Ensure the Safety of Subjects

- Participants will be screened and approved for study participation by a trained staff member at each center, ensuring compliance with study inclusion/exclusion criteria.
- Testing will be performed at a medical-academic center that is located near an emergency room.
- Security personnel who staff the Lynn Rehabilitation Cen are “first responders” and will follow standing policies and procedures for emergencies in the Center.
- All study personnel at each center hold current CPR certificates.
- An Independent Medical Monitor will receive reports of AEs and SAEs, as well as semiannual reports in study progress. IMM reports will be included in annual continuing reports to the IRB.
- All phlebotomy will be performed by trained study staff holding a current certificate in the appropriate jurisdiction.
- Emergency numbers are posted near all lab telephones.
- All serious adverse events and adverse events will be reported to the IRB within the mandated period as follows:
    - For any adverse event, the PI will immediately notify and consult with the Study Physician and come to an opinion as for the relatedness of the event and the study procedures as described in the protocol.
    - Adverse events will be evaluated by the Study Physician using the following criteria:
        - Grade 1, Mild: Awareness of symptom, but easily tolerated; usually transient requiring no special treatment; does not interfere with usual status or daily activities
        - Grade 2, Moderate: Might be ameliorated by simple therapeutic measures; may interfere with, but not keep the participant from performing usual daily care or participating in daily activities.
        - Grade 3: Incapacitating event, inability to perform usual activities
        - Grade 4, Life-threatening/Disabling: Patient is at risk for death, or worsening disability or impairment as existed at the time of the event
- For the first two grades, the Investigators will observe the participant and as necessary institute standard medical or therapeutic care. Repeated occurrence of Grade 1 and 2 events may be cause for notification of the IRB by the Investigators that stoppage is appropriate. The Investigators may take this action, and the IRB will be so notified.

- Grade 3 and 4 events will be individually evaluated. Any occurrence of Grade 3 and 4 events may be cause for notification of the IRB by the Investigators that stoppage is appropriate. The investigators may take this action, and the IRB will be so notified. Upon a determination that the (S)AE was related to the protocol, the study will stop and undergo evaluation for continuation.

Withdrawal of Subjects

Subjects may withdraw from the study without prejudice to medical treatment at the center or involvement in future studies at the discretion of the PI. The ICF advises students and employees of the rights with respect to study withdrawal. We may end a subject's participation in the study if s/he experiences adverse effects or in case of an incidental pregnancy. Subjects who experience AEs (nausea, bloating, for example) will be referred to the Study Physician for appropriate medical care and will have their dose decreased by 25% (total dose of 300mg or 3 capsulse in the morning plus 3 capsules at night). This lower dose has been reported to be efficacious and may help alleviate the AEs. If symptoms do not improve after 1-2 days, subjects will be withdrawn from the study. Subjects that become upset by answering questions about their SCI will be referred to the Study Physician for appropriate medical care. Compliance to UA dosing will be calculated monthly and subjects who fall below the 80% adherence threshold will be withdrawn from the study.

Risks to Subjects

- Subjects may become upset by having an investigator ask questions about their SCI.
- The risks of blood drawing include fainting, the occurrence of temporary discomfort and/or bruise at the site of puncture; rarely, infection or the formation of a small clot or swelling to the vein and surrounding area may occur. There may be slight discomfort in the arm or hand during the venipuncture. Occasionally, a small accumulation of blood (hematoma) may form at the point of insertion of the needle. This may result in a small lump that will disappear. Occasionally, a small amount of bleeding may occur around the catheter site. On rare occasions, a local infection may occur around the venipuncture site.
- You may find yourself becoming hungry when you fast to have your blood drawn.
- Answering questions about your injury may make you feel nervous or upset. If you do not wish to answer a question, you can skip it and go to the next question.
- DXA scans may cause anxiety and additional radiation exposure. Radiation exposure is less than two weeks of natural background radiation or like the exposure during a round-trip cross-country flight.
- CT Imaging may cause anxiety and an excess exposure to radiation. Exposure to low-levels of radiation does not cause immediate health effects, but can cause a small increase in the risk of cancer over a lifetime.
- MIP procedure is non-invasive and relatively quick procedure. Subjects may feel the need to cough or may feel short of breath during or after the test. On rare occasions, subjects can become lightheaded.
- Muscle soreness from the exercise regimen that is part of this study is a possibility
- Ursolic Acid - naturally occurring triterpene compound - is an herbal-like isolate obtained from the leaves of various plants (rosemary, marjoram, lavender, thyme, and organum), fruits (apple fruit peel), and berries. Some of the common side outcome
    - Nausea
    - Stomach distention or bloating
    - Trace quantities of blood within the urine
    - Skin rash

Small safety studies indicate UA is tolerable with mild to moderate side effects [10, 11, 12]

<u>In addition to these risks, this research may hurt you in ways that we do not yet know</u>

Potential Benefits to Subjects

Direct benefits are not promised. It is likely that participants in exercise groups will improve fitness and health and learn to lead a healthier lifestyle.

14. Setting

- Consent will take place at the following sites:
  - The Miami Project to Cure Paralysis located at the Christine E. Lynn Rehabilitation Center on the University of Miami Miller School of Medicine Medical Campus.
  - Blood samples will be analyzed in the Biomarker and Immunoassay Laboratory of the DRI (3rd. Floor).

15. Resources Available

- Mark S. Nash, PhD is a Professor in the in the Department of Neurological Surgery and Physical Medicine and Rehabilitation, and a Principal Investigator (Applied Physiology Research) for the Miami Project. He will serve as a Principal Investigator on the protocol and assessments, and will assist with data interpretation and dissemination; Product accountability.
- Alberto Martinez, M.D.: He will serve as the study physician: primary responsibilities will be to assess potential AEs throughout the trial and determine reporting requirements.
- Armando Mendez, PhD: He will oversee all lab testing involving subject samples
- Gary Farkas, PhD: He will serve as a Sub-Investigator and will assist with protocol, assessments, data analysis and interpretation
- David McMillan, PhD: Primarily responsible for outreach and participant recruitment. He will assist with study assessments
- Patricia Graham, MS, CCRC: She will be responsible for regulatory management and will assist with the conduct of the study, including consenting, assessments, product dispensation and accountability.
- Francisco Diaz: He is in charge of the lifestyle center at LRC. He will assist with the exercise intervention.

16. Compensation for Research-Related Injury

Details regarding research related injury compensation will be included in the FE agreement. The following language was added to the ICF: *You may be exposed to risk of injury from participation in this study. If injury occurs, treatment will in most cases be available. If you have insurance, your insurance company may or may not pay for these costs. If you do not have insurance, or if your insurance company refuses to pay, you will be expected to pay. Funds to compensate for*

pain, expenses, lost wages and other damages caused by injury are not routinely available.

17. Economic Burden to Subjects

Subjects that sign consent and are eligible to participate will be reimbursed by check (sent by mail) after each completed visit.

Group 1 (total $200):

- $50 for completing the first or screening visit
- $150 ($50 every month) for completing the 12-week intervention (exercise and daily UA).
- Re-dispensing of a new UA bottle will occur once a month at a scheduled visit
- Monthly pregnancy tests for all women of child-bearing potential
- $50 for completing the post-study assessments at the final visit

Group 2(total $200):

- $50 for completing the first or screening visit
- $150 ($50 every month) for completing the 12 week intervention (daily UA)
- Subjects will be required to return to the study site once a month for re-dispensing of the UA.
- Monthly pregnancy tests for all women of child-bearing potential
- $50 for completing the post-study assessments at the final visit

18. Consent Process

Consent will take place at each Center in a private office at the Miami Project to Cure Paralysis located at LRC, 2nd Floor. We will abide all regulatory mandates and suggestions of the FDA '**A Guide to Informed Consent - Information Sheet'** www.fda.gov/RegulatoryInformation/Guidances/ucm126431.htm

Before signing the ICF study subject candidates will be encouraged to share the ICF with family, a trusted family friend or their physician. Subject candidates will be required to read the approved consent form. The person obtaining consent will ask several questions to assure they understand the study procedures and risks. A statement on the consent form will advise university employees that their participation (or failure to participate) will not affect their employment or academic status. The statement in the consent will advise individuals with spinal cord injury that their participation (or decision to not participate) will not influence their treatment or candidacy for other studies in any way. Individuals will be given the right to withdraw at any time without prejudice.

Process to Document Consent in Writing

- Subjects will be presented with and asked to review the consent form. They will be queried whether they have any questions and if so, these questions will be addressed by the principal investigator or individual approved to obtain consent. Once the consent form is signed (subject may provide a mark if unable to sign), witnessed, and signed by the principal investigator (or other designated person obtaining consent) a copy of the consent will be provided so that the subject can review their rights.
- A copy of the consent will be provided so that the subject can review their rights at any time in future. The original copy of the ICF will be placed in the participant file and secured as described in section 21.b

Authorization for Use and Disclosure of Protected Health Information (HIPAA)

Waiver of Authorization for access to medical record for subject identification/recruitment.

19. REFERENCES:

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