Protocol Summary

Rejuvenation of Aged Muscle Stem Cells Through Exercise

BACKGROUND AND SIGNIFICANCE: Continuous physical exercise is a potent method of preserving muscle mass with aging. In terms of muscle stem cell (referred to as satellite cells) health, exercise rescues the aging-associated loss of satellite cells and improves the ability to regenerate muscle after injury. The mechanisms for exercise-induced improvements in satellite cell function are currently unclear. However, exercise has been shown to induce AMPK activity, autophagy and decrease apoptosis in muscle. Moreover, autophagy regulates satellite cell mitochondrial function and proliferative capacity in young and old satellite cells. We have preliminary data showing an exercise-induced increase in autophagy and proliferation in aged satellite cells in the mouse. Based on these findings it is justifiable to investigate the effect of exercise training on satellite cell autophagy/apoptosis in young and aged human's populations to ensure translational capacity to our research program. We hypothesize that exercise will increase muscle stem cell function in young and older individuals through induction of cellular autophagy and survival mechanisms.

FUNDING SOURCE: Dr. James White, Ph.D. will be the Principal Investigator for this study. Dr. William Kraus, M.D. and Dr. Kim Huffman, M.D. will be the physicians and Co-Investigators on this study. The study will be funded by the National Institutes of Health/National Institute on Aging.

PURPOSE OF STUDY: The proposed study will establish a newly-identified signaling pathway regulating both autophagy and apoptosis in human satellite cells. These findings will allow for novel pharmacological targets in satellite cells to return regenerative capacity to elderly individuals.

DESIGN AND PROCEDURES: Up to 80 adults, 21 years of age and greater, will undergo a consent visit followed by three blood draws and muscle biopsy visits- baseline (pre), after the first exercise bout (post-acute), and after the 12 week training program (post-chronic). Blood will be obtained and tested for inflammatory (IL-6/TNF/IL-1) and endocrine markers (Testosterone/Estrogen/Thyroid hormone). As part of the intervention, participants will undergo moderate intensity treadmill exercise three times per week for 10-12 weeks or serve as sedentary controls. The specifics of the biopsy visits are described in the consent and have been repeated here:

- **Visit 1:** The consent visit will take place at the Duke Center for Living and will last one hour. We will explain the study and the consent form in detail and on an individual basis.

- **Visit 2:** This 1-hour visit will take place at the Center for Living.
  - A brief medical history including list of medications will be performed.
  - A blood sample of up to 20 mL will be collected from consented participants through venipuncture.
  - A baseline muscle biopsy procedure will be performed by either Kim Huffman M.D. or William Kraus M.D., both of whom have extensive experience with this procedure in previous studies. The subject will be told to avoid products such as aspirin medication up to five days before this visit to reduce the risk of excess bleeding from the biopsy incision site (also avoid ibuprofen, naproxen the day before this visit). After local anesthesia (xylocaine, 2%) is injected, a small incision will be made in the left thigh. Four to six small pieces of muscle about the size of a pea will be surgically removed. The incision site will be closed using steri-strips. This part of the visit takes approximately 30 minutes. All muscle samples will be stored in a -80ºF freezer at the Duke Molecular Physiology Institute.

The Exercise Training Program: After Visit 2, subjects will be randomized to one of two intervention groups. Group 1 will be required to attend 3 supervised exercise sessions per week for 10-12 weeks. They will be fitted with a heart rate monitor around the chest to maintain running intensity. Each exercise session will begin with a short warm-up before performing submaximal (70% age predicted heart rate) exercise on a treadmill or stationary bicycle for 30 minutes. Group 2 will be the sedentary control group. This group will be asked to maintain their baseline level of inactivity.

- **Visit 3**: This 1-hour visit will take place at the Center for Living. For those participants assigned to exercise intervention, this will take place approximately 2-hours after the first bout of exercise. For the control participants, this visit will occur approximately 1 week after the baseline visit.
    - This visit will follow the same procedures as Visit 2, described above.
- **Visit 4**: This 1-hour visit will take place at the Center for Living. For those participants assigned to exercise intervention, this will take place approximately 2-hours after the final bout of exercise. For the control participants, this visit will take place approximately 10-12 weeks after Visit 3.
    - This visit will follow the same procedures as Visit 3, described above.

STUDY DURATION: The study timeline for this protocol is 14-18 weeks depending on participant and study staff availability. In addition to the study visits listed above, the participants may be asked to come to the Center for Living to exercise on a treadmill or stationary bicycle three times a week for 10-12 weeks.

SELECTION OF SUBJECTS: Study participants will include adults 21 years and older who are not currently participating in regular physical exercise or weight reduction dieting. Inclusion/exclusion criteria are listed below.

The following **INCLUSION** criteria must be met:

- Age 21 years or greater.
- No medication changes within the last 3 months.
- Not participating in regular physical exercise (more than 60 minutes of moderate intensity or 30 minutes of vigorous intensity exercise per week).
- Able to decide if you want to take part in the study.

The following criteria will **EXCLUDE** potential participants from participating in the study:

- Smoker: Tobacco use within the last 12 months.
- Dieting or intending to diet.
- Use of potential confounding medications, e.g. using ticlopidine, clopidogrel, dipyridamole, warfarin, heparin, enoxaparin and other blood thinners.
- Coronary stents or any other medical condition for which aspirin cannot be temporarily withheld.
- Use of hormone replacement medications.
- Absolute contra-indications to exercise: Recent (<6 months) acute cardiac event unstable angina, uncontrolled dysrhythmias causing symptoms or hemodynamic compromise, symptomatic aortic stenosis, uncontrolled symptomatic heart failure, acute pulmonary embolus, acute myocarditis or pericarditis, suspected or known dissecting aneurysm and acute systemic infection.
- Relative contra-indications to exercise: Left main coronary stenosis, moderate stenotic valvular heart disease, outflow tract obstruction, high degree AV block, ventricular aneurysm, uncontrolled metabolic disease (e.g. diabetes, thyrotoxicosis, myxedema), uncontrolled pulmonary disease (e.g. severe COPD or pulmonary fibrosis), mental or physical impairment leading to inability to exercise adequately.
- Have a confounding medical condition that is progressive and unstable such as HIV, Hepatitis C, active cancer, and/or taking medications for those conditions.

- Unwillingness to be randomized to any one of two intervention groups, submit to skeletal muscle biopsies and all other study testing or continuously participate in a randomly assigned exercise training or program for three months.
- Orthopedic limitations, musculoskeletal disease and/or injury.
- Allergic to xylocaine.
- Unwillingness to exercise at the Duke Center for Living during staff supervised times.

SUBJECT IDENTIFICATION, RECRUITMENT AND COMPENSATION: Study recruitment will occur primarily through local newspaper advertisements in the Herald Sun, Inside Duke, Duke Healthline, the Duke Clinical Research website, and word-of-mouth. Interested participants will be directed to contact the study office via phone. Following an initial phone or personal screening questionnaire, eligible and interested participants will be scheduled for a consent visit and a member of the PI's study staff will obtain informed consent prior to scheduling testing visits. Participants will receive $400 total compensation for completion of the study. Participants will receive $100 for completion of Visit 2, and $150 each for Visits 3 and 4.

SUBJECT COMPETENCY: Subjects must be able to speak and understand English to participate in this study.

RISK/BENEFIT ASSESSMENT: Participation in this study may lead to increased risk for the following side effects. Participants may discuss these with the study doctor and your regular health care provider if they choose. There may be risks, discomforts, or side effects that are not yet known. The known risks are:

- Thigh Muscle biopsies may result in momentary pain and discomfort, burning or bleeding, numbness, and rarely fainting or infection. Please notify Dr. James White or study staff if you have taken any product containing aspirin within five days of the muscle biopsy as aspirin can increase bleeding risk. The incision site may leave a scar and muscle soreness may be present up to ten days after the biopsy.
- Xylocaine: While the local numbing medicine xylocaine is almost entirely free from allergic properties (such as causing hives), an allergic reaction is possible, and the participant will not be given xylocaine if they have a history of such a reaction. The xylocaine will be given by a small injection into the skin at the site of the muscle biopsy.
- Risks associated with a standard blood draw include momentary discomfort and/or bruising. In addition, there is a minimal risk of infection, excess bleeding, clotting, and fainting.
- An exercise program may result in muscle, bone and/or joint soreness, discomfort and/or injury.

Any adverse events will be reviewed and signed off on by the PI as they occur. Adverse events will then be reported to the IRB in accordance with HRPP policies.

Participants may benefit from the exercise portions of this study through improved physical function and cardiorespiratory fitness

COST TO THE SUBJECT: The subjects will not incur any costs as a result of participation in this study. Immediate necessary care is available if an individual is injured because of participation in a research project. However, there is no provision for free medical care or for monetary compensation for such injury. During this study, hospitalizations or additional care beyond the scope of this study will be the responsibility of patients and/or their insurance company.

DATA ANALYSIS AND STATISTICAL CONSIDERATIONS: Muscle stem cell function will be compared across age groups at each time point using a One Way ANOVA or t test for specific comparisons. An alternative analysis will be a longitudinal design following muscle stem cell function from each individual before and after exercise training. This analysis will be performed with repeated measure ANOVAs. Data will

be analyzed using GraphPad Prism 6 statistical software. Significance will be accepted as $p<.05$.

DATA STORAGE AND CONFIDENTIALITY: All collected data will be stored in a locked file to be accessed only by Dr. White and his study staff. Study records that identify subjects will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, they will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS). For records disclosed outside of DUHS, they will be assigned a unique code number. The key to the code will be kept in a locked file in Dr. White's office.