Enhancing Adaptations to Exercise

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Title of the study: Enhancing Adaptations to Exercise
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ABSTRACT

Many older adults suffer from derangements in skeletal muscle, including mitochondrial impairments, oxidative stress, insulin resistance, weakness, and fatigue. Despite evidence implicating inflammation in muscle dysfunction, a mechanistic link in the context of human aging is in early stages of investigation. The objective here is to determine how chronic inflammation influences exercise responsiveness, mitochondrial physiology, and metabolic function in skeletal muscle of older adults. Our central hypothesis is that chronic inflammation originating from inflamed adipose tissue triggers local inflammatory responses within skeletal muscle, leading to oxidative stress, reduced mitochondrial capacity, compromised muscle quality, functional and metabolic impairments, and attenuated adaptive responses to exercise. We will test this hypothesis through cross-sectional comparisons of older humans grouped by inflammation status. We will also perform a blinded, placebo-controlled study to determine if suppressing chronic inflammation using omega-3 fatty acids (n3-PUFA) restores skeletal muscle function and exercise responsiveness in older adults.

RESEARCH PLAN

A. Specific Aims

Aim 1. To determine the impact of chronic inflammation on skeletal muscle physiology.

Hypothesis 1: chronic inflammation triggers local inflammation within skeletal muscle, which provokes mitochondrial dysfunction, oxidative stress, protein damage, and functional impairments.

Approach: Skeletal muscle biochemical and functional parameters will be compared in older adults grouped into quartiles based on a composite systemic inflammatory score (CRP, IL-6, TNF-α). Outcomes will be measured again following 26 weeks of placebo or n3-PUFAs to reduce adipose tissue inflammation.

Aim 2. To determine the impact of chronic inflammation on responsiveness to acute exercise.

Hypothesis 2: Acute exercise responsiveness is blunted in older adults with chronic inflammation, but restored when inflammation is reduced.

Approach: We will combine conventional molecular techniques with tracer-based approaches to evaluate anabolic and metabolic responses to a single bout of exercise in older adults grouped by inflammation status before and after inflammation is reduced by n3-PUFAs. Muscle and blood samples collected before and after a single bout of exercise will be used to measure anabolic and metabolic responsiveness to exercise from mRNA of exercise-responsive genes, activation of signaling proteins in muscle, and whole-body amino acid kinetics. Exercise-stimulated anabolic and metabolic responsiveness will be evaluated again after reducing inflammation in older adults (placebo vs. n3-PUFAs).

B. Background

Many older adults suffer from derangements in skeletal muscle, including mitochondrial impairments, oxidative stress, insulin resistance, weakness, and fatigue. Exercise forestalls disease and disability in older adults, but many (15-20%) fail to demonstrate favorable adaptations even when interventions are painstakingly controlled (1-4). This so-called exercise resistance is particularly evident in older adults, who exhibit blunted anabolic responses to exercise and nutrition (5-7), which likely contributes to sarcopenia and attenuates adaptive responses to exercise. Early evidence shows that clearance of inflammatory cells in adipose tissue restores mitochondrial function and muscle mass in mice. In humans, we show that responsiveness to *acute* exercise is enhanced by omega-3 fatty acids (n3-PUFAs), putative suppressors of chronic inflammation, particularly in adipose tissue (8, 9). **Knowledge gap:** Despite evidence implicating inflammation in muscle dysfunction, a mechanistic link in the context of human aging is in early stages of investigation.

C. Significance of Proposed Research

A goal of Healthy People 2020 is to improve the quality of life of older adults. The initiative underscores the importance of physical activity for metabolic health and preventing frailty. In this regard skeletal muscle health is critical, but muscle mass and strength are clearly compromised in older adults (10, 11). Sarcopenia is a major factor contributing to physical impairment with aging and predictive of disability (12). Exercise is a proven strategy to prevent and reverse sarcopenia, insulin resistance, and related complications (13, 14), but the recommended 150 minutes per week as a lifestyle goal is often met with poor adherence or is impractical for people with physical disability or other contraindications to exercise. There is also emerging evidence that the adaptations to exercise training are blunted in some people. For example, there is considerably variability in the improvement in cardiorespiratory fitness in response to a standardized aerobic training program (1, 2). These findings are in line with the concept of “anabolic resistance” whereby some individuals, particularly older adults, exhibit blunted increases in muscle protein synthesis in response to exercise or nutritional stimuli (3, 5, 6, 15, 16) and attenuated transcriptional responses to acute endurance exercise (4). Through a better understanding of the factors that attenuate exercise responsiveness, it may be possible to enhance adaptations to exercise in people who are “exercise resistant” or are unable to engage in exercise at sufficient intensities or volumes required to induce therapeutic benefit. Chronic inflammation appears to be a factor in exercise resistance based on observations that anabolic responses are restored when inflammation is reduced (17, 18). Altogether, precedent literature and our own preliminary data suggest that chronic systemic inflammation is detrimental to skeletal muscle and may limit adaptative responses to exercise. Inasmuch, targeting inflammation holds promise for enhancing responses to exercise, particularly under conditions of chronic inflammation such as sarcopenia, insulin resistance, type 2 diabetes, rheumatoid arthritis, and cancer cachexia. *The following contributions will emerge from the proposed work:*

1) *Identify mechanistic links between systemic inflammation, adipose tissue inflammation, and local inflammatory responses within aging muscle.*
2) *Determine the influence of inflammation on biochemical and functional parameters in aging muscle.*
3) *Determine if reducing inflammation can enhance the adaptations to acute exercise in older humans.*

This contribution is significant because it will provide new fundamental knowledge to help understand the origins of metabolic and functional impairments in skeletal muscle

of older people and how chronic inflammation may be an effective target to enhance therapeutic benefits of exercise. Such knowledge would be expected to have a positive impact on health and quality of life of older adults.

D. Research Design and Methods:

Study Design: Men and women between the ages of 20-35 years (N=30)and 65-85 years (N=120) will be recruited for this study. Participants in the older age group will be randomly assigned to receive n3-PUFA or placebo (corn oil) in a double-blind manner. Before and after the intervention, all participants will complete an outpatient study day (body composition, blood draw, treadmill test, strength test) and an inpatient study day (muscle biopsies, fat biopsies, indirect calorimetry, exercise test, mixed meal test) as described below in more detail. The DHA/EPA and placebo softgels will be supplied by Sancilio and Company, Inc.,Riviera Beach, FL) and stored in the Mayo Clinic Research Pharmacy at room temperature. During the intervention phase of the study, participants will be instructed to swallow 2 softgels twice per day with meals (morning and evening) for a total of 4 softgels per day. The DHA/EPA softgels will each contain 675mg of EPA and 300mg of DHA for a total daily dosage of 3.9g/day. The total omega-3 content of each capsule is 1050mg, which includes an additional 75mg of "non EPA/DHA" omega-3 fatty acids. The total fat content of the capsule will be 1200mg. This dose of EPA?DHA was choosen because it has been shown to augment the anabolic response of skeletal muscle [24]Every 4 weeks, participants will report to the Clinical Research and Trials Unit (CRTU) to pick up a new prescription and return any remaining capsules from the previous prescription. Remaining pills will be counted to determine compliance. The pharmacy will maintain records of receipt, dispensation, and return pill counts for compliance. Unused drug will be incinerated at the end of the study. On the day they pick up prescription refills, participants will report to the CRTU for a fasting blood sample to measure liver function (ALT, AST), coagulation (INR), blood lipid profile, glucose, and insulin. Participants will be withdrawn from the study if INR values exceed 2.0, indicating a high chance of bleeding. Participants will also be withdrawn from the study if ALT levels exceed 165IU/L or AST levels exceed 144IU/L. The duration of the intervention will be 6 months.

Participants: Our plan is to complete the study on 120 men and women age 65-85yrs and 30 young adults who will only be studied at baseline We are anticipating a target accrual of 179 subjects to account for an anticipated dropout rate of 20% in the intervention group.

Description of recruitment methods:
We will use lists of prior research participants in our lab who have given permission to be contacted. We will use brochures, flyers, radio ads, television ads, and advertisements on the Research Studies section of the Mayo Classifieds. A member of the study team will contact participants by telephone or email with a brief description of the study and to determine if they are interested in participating. Brochures, flyers and radio ads will include the telephone number of a study team member. Participants will be asked to call the contact person. When the call is received, the member of the study team will perform a brief screening questionnaire to determine eligibility to proceed with enrollment. The screening questionnaire may also be sent by email in accordance with the Research Policy Manual Policy on Electronic Communications with Research

Subjects. The information gathered from the screening questionnaire will not be stored or used for data analysis if participants do not qualify for the study.

Exclusion criteria:

1) Regular use of omega-3 nutritional supplements
2) Diabetes or fasting plasma glucose ≥126 mg/dL
3) Anemia (female subjects hemoglobin of ≤11 g/dl and male subjects hemoglobin ≤12 g/dl)
4) Active coronary artery disease or history of unstable macrovascular disease (unstable angina, myocardial infarction, stroke, and revascularization of coronary, peripheral or carotid artery within 3 months of recruitment)
5) Renal failure (serum creatinine > 1.5mg/dl)
6) Chronic active liver disease (AST>144IU/L or ALT>165IU/L)
7) Oral warfarin group medications or history of blood clotting disorders.
8) INR >2.0
9) Smoking
10) Pregnancy or breastfeeding
11) Alcohol consumption greater than 2 glasses/day or other substance abuse
12) Untreated or uncontrolled hypothyroidism
13) Debilitating chronic disease (at the discretion of the investigators)
14) Fish or shellfish allergy

Consent and screening: Potential participants will report to the Clinical Research and Trials Unit at Mayo Clinic Hospital St. Marys Campus the morning after an overnight fast. A member of the study team will meet the potential participant and the consent form will be read and discussed in private area. After receiving consent, the CRTU staff will measure vital signs including blood pressure, heart rate, temperature. Height and weight will be measured. The participant will undergo urine pregnancy test (if applicable) and screening blood work that will include CBC, lipid profile, creatinine, liver enzymes, TSH, glucose, insulin, blood clotting time (INR), c-reactive protein, and interleukin-6. Participants will be instructed on the use of a physical activity monitor (Actigraph), which they will wear for waking hours for a period of two weeks to quantify habitual physical activity levels. Participants will return for two additional blood draws and weight approximately 2 and 4 weeks later to repeat measurements of inflammatory markers (CBC, c-reactive protein, interleukin-6). RReconsenting may take place if study is stopped due to COVID closure. The subject will be withdrawn from the study at the point where the study stopped. The subject will be reconsented and start over from the beginning of the study.

Withdrawn and Reenrollment

Two participants were withdrawn from the study due to COVID-19 related closure of the clinical research unit. One participant completed a consent and screening visit (baseline blood testing). Another participant completed consent, screening, and an outpatient visit involving DEXA and exercise testing. Neither participant completed baseline inpatient study visits or were randomized to study arms. These participants will be re-enrolled, starting from the beginning given the amount of time that passed since the initial testing and potential drift in measured outcomes.

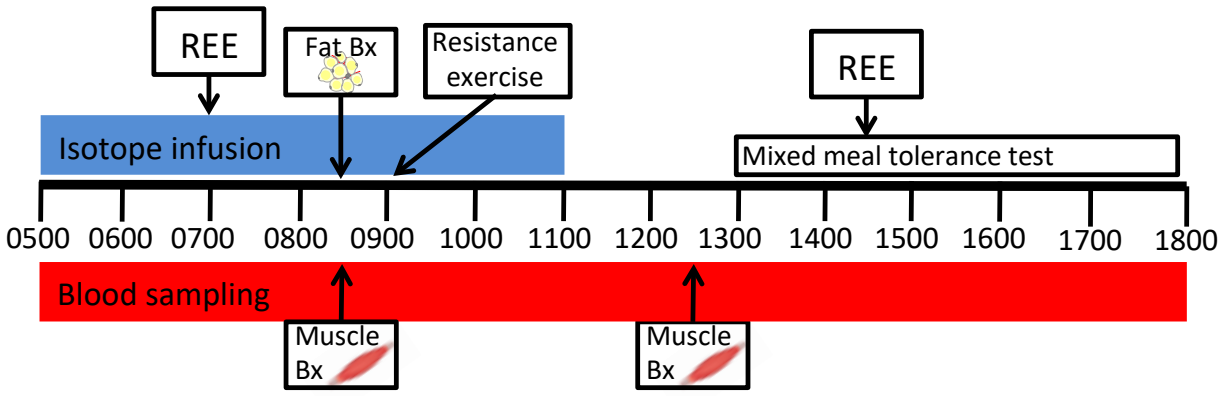
Outpatient testing: At least 4 weeks following the screening visit, participants will return for an outpatient testing session when they will provide a third blood sample to confirm inflammatory status. Participants will meet with a dietician to discuss food preferences. Participants will undergo measurements of body composition by dual-energy X-ray absorptiometry (DEXA), resting energy expenditure (REE) by indirect calorimetry, and a graded exercise test on a treadmill. Participants will also be habituated to the single-leg exercise that will be used to evaluate acute exercise responsiveness. Blood tests, REE, DEXA, and treadmill tests will be repeated after 26 weeks of the n3-PUFA/placebo intervention.

Inpatient study day: Participants will be admitted to the CRTU on the evening of the 3rd day of a weight-maintaining diet provided by the CRTU metabolic kitchen. The weight maintenance meals (diet composition: 20% protein, 30% fat, 50% carbohydrate) will be monitored daily to assure correct calorie level and that the participants are weight-stable. After a standardized meal (10kcal/kg with 50% carbohydrate, 20% protein, and 30% fat) at 1800 hours on the evening of admission to the CRTU, participants will remain fasting except for water until the noon on the following day.

At 0500h infusions of $^{13}C_6$ phenylalanine at 1.0 mg/kg FFM/h and [^{15}N] tyrosine at 0.5 mg/kg FFM/h will be started after bolus of 1.0 mg/kg FFM [$^{13}C_6$] phenylalanine, 0.5 mg/kg FFM [^{15}N] tyrosine, and 0.5 mg/kg FFM [$^{13}C_6$] tyrosine. These are stable (non-radioactive) isotopes. Muscle biopsies from one *v. lateralis* will be obtained at 0830hrs for measuring baseline gene expression, protein expression, and mitochondrial function. Muscle biopsy samples will be collected under local anesthesia (2% lidocaine buffered with sodium bicarbonate) using a modified Bergstrom needle. A single abdominal subcutaneous fat biopsy will be performed at 0830 hrs under local anesthesia using sterile technique. Following the muscle and fat biopsies (~0900hrs), participants will perform a bout of unaccustomed unilateral knee extension exercise using only the leg without prior biopsies. Following a warm-up set, subjects will complete 8 sets of 10 repetitions at 70% of their 1-repetition maximum, determined at least 7 days prior. A second muscle biopsy will be obtained from the exercised leg 3 hours following exercise (~1230hrs).

Following the second biopsy (~1300hr), participants will consume a mixed liquid meal containing 15% protein, 55% carbohydrate, and 30% fat, approximately equal to 35% of resting energy expenditure determined from the REE on the outpatient study day. Arterialized blood samples will be taken from a heated hand vein for glucose, insulin, and c-peptide measurements at intervals over 5 h after the test meal, which will be consumed over 10 min. Blood samples will be collected at 30, 20, and 10 minutes before the meal and at 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 260, 280, and 300 minutes following the meal. We are collecting and washing red blood cells for storage for future testing. Resting energy expenditure and respiratory quotient will be measured by indirect calorimetry for 30 minutes under postabsorptive conditions (0700hrs) and postprandial (1430hrs). Participants will be given a meal from the hospital kitchen at approximately 1800hr before discharge.

The inpatient and 48 hour study day will be repeated following the 6 month intervention.



48 hours post-exercise biopsy:
Participants will return to the CRTU for an outpatient study visit involving an additional muscle biopsy from the exercised leg 48 hours following the single bout of exercise.

Additional Dexa scan
One subject will have an additional Dexa scan due to unforeseen Covid circumstances. Participant was withdrawn from the study due to COVID-19 related closure of the clinical research unit. Patient completed consent, screening, and an outpatient visit involving DEXA and exercise testing. The participant will be re-enrolled, starting from the beginning given the amount of time that passed since the initial testing and potential drift in measured outcomes.

Statistical analyses and Power:
For all variables in aim 1, the interest is in assessing if the change from baseline to post treatment differs by treatment arm. We will determine the influence of chronic inflammation on these outcomes in older adults grouped according to quartiles of a composite score including serum CRP and IL-6. The initial analysis will be stratified by inflammation composite score quartiles. Within a subgroup, the Wilcoxon rank sum test will be applied to test for difference in the change of outcome value pre and post treatment between the two treatment arms, placebo and n3-PUFAs. Additionally, generalized linear models will be used to assess the effect inflammation has on modifying the treatment effect by including interaction terms of a smoothing spline functional fit of the composite score with the treatment and time variables. The residuals from the regression will be used to assess distribution assumptions and variables will be transformed if required for appropriate statistical inference. All participants with at least a pre-treatment measurement will be included in the primary analysis utilizing the as-randomized study arm assignment for comparison. Efforts will be made to minimize missing data, but in the event of missing values, characteristics between those with observed values and with missing values will be compared. If no observed differences are identified, multiple imputation will be used with the primary analysis. Power calculations were performed for hypotheses related to mitochondrial physiology. We used the preliminary data where we measured effects of EPA on mitochondrial capacity. With mean JO2 = 28.9 pmol/s/tissue and σ = 4.891, a sample size of 15 per treatment group within an inflammation quartile gives 80% power (α=0.05) to detect a treatment difference in the difference from baseline to post treatment of 5.18 pmol/s/tissue (15% change). With 4 inflammation quartile groups, the total sample size will be 120, with

balanced randomization that gives 60 for each treatment arm in total. We believe that this expected difference is meaningful and realistic because our target population of older adults is known to exhibit reductions in mitochondrial capacity, and this degree of improvement would be physiologically important.

The main outcome variables for specific aim 2 are: 1) mRNA expression of anabolic and metabolic signaling genes, 2) expression and activation (phosphorylation) of signaling proteins, and 3) whole-body protein turnover. Hypothesis 2a will be addressed using the pre-treatment measurements, including a baseline and 3 post-exercise time points for all individuals. For each outcome, linear random effects models with time point and the inflammation score interaction terms plus a random intercept for each individual will be estimated. The statistical analysis for hypothesis 2b will be similar to specific aim 1, wherein regression models will be estimated for each outcome variable and include an interaction term between assigned treatment arm and the inflammation score. Missing data and loss to follow-up will be handled as described above in aim 1. Power calculations were performed using the preliminary data in which protein synthesis was measured using the same exercise protocol proposed in this aim. We utilized a linear random intercept model with a baseline and 3 post intervention time point and assuming an exchangeable correlation matrix with pairwise correlation of 0.7 within an individual between time points. With $\sigma = 0.025$ %/hr, a sample size of 30 per inflammation group gives 80% power ($\alpha=0.05$) to detect a 0.028 %/hr slope change between inflammation groups in muscle protein synthesis in response to exercise. We believe that this minimal detectable difference is meaningful, realistic, and conservative.

Human Subjects

1. Risks to the subjects

Human Subjects Involvement and Characteristics: All protocols and all techniques to be used will be approved by the Mayo Clinic Institutional Review Board prior to initiation of any studies. Subject characteristics and exclusion criteria have been clearly specified above. All women of child bearing potential will have a negative pregnancy test within 24 hours of the study and will be advised to refrain from conception for at least two months after the study. No children will be studied. All subjects will be weight stable before enrolling in the study. Our plan is to complete the study on 135 participants.

Sources of Materials: Samples of blood, skeletal muscle, and adipose tissue obtained during the study will be used exclusively for research purposes. Data on body composition will be used only for research. No use will be made of pre-existing specimens.

Muscle tissue: Two or three muscle biopsies (~300mg each) will be obtained during each inpatient study visit.
Blood: 1 blood draw will occur during the screening visit (approximately 15ml). Each inpatient study will require 48 blood draws (approx 407mL blood). All specimens will be processed internally and stored in the study team laboratory.
Adipose tissue: One subcutaneous abdominal fat biopsy (2-3g) will be obtained during each inpatient visit.

Potential Risks: The following are the potential risks for this study:

a) Blood will be withdrawn. The total blood withdrawn will not exceed 550mL in a 12-week period. Hemoglobin measured before starting the study will be within safe limits as decided by the investigators. All subjects will refrain from giving blood twelve weeks prior to the study and until twelve weeks after the completion of the study.

b) DEXA will be completed on all subjects for all specific aims. The radiation exposure from this technique is within the safe limits as determined by the Radiation Safety Committee of our institution. Scans are done on the Lunar iDXA, software version 11.4 (General Electric Company, GE Healthcare Technologies, Madison, WI). This can be done with sufficiently low radiation exposure that pregnancy testing is not required.

c) Vascular catheters will be placed. Catheter insertion, intravenous infusion and blood withdrawal are associated with a small risk of phlebitis. This will be minimized by careful attention to sterile technique. If phlebitis occurs, it will be treated conservatively with heat and when appropriate, with antibiotics. In all experiments, “arterialized – venous” blood will be obtained by placing a hand in which a catheter has been inserted in a heated box during the study. The temperature inside the box is maintained at ~55°C. With prolonged exposure to continuous heat, there is a potential risk of local skin irritation or a minor burn. If this occurs, it will be treated appropriately. However we have used this technique for the past 18 years and have had no instances of hot box-related burns or injuries. The potential risks of catheters and hot box use will be discussed with the volunteers prior to obtaining consent for the study.

d) Muscle biopsies will be performed with a percutaneous biopsy needle by the PI or an appropriately-trained member of his study team in accordance with approval from the Research Resources Executive Committee. Local subcutaneous injection of 2% lidocaine buffered with 8.4% sodium bicarbonate will be used for analgesia. A small incision will be made through the skin and fascia. After the biopsy, pressure will be held over the incision until hemostasis is achieved. The incision is closed with sterile strips, gauze and Ace wrap. Risks of this procedure include hematoma, infection, and pain. Hematoma likelihood is minimized by holding pressure after the biopsy to ensure hemostasis, followed by a pressure dressing. Risk of infection is minimized by using sterile surgical techniques. Pain is managed by local analgesia during the procedure and Tylenol following the procedure.

e) Whole-body cardiorespiratory fitness will be evaluated by a graded exercise test on a treadmill. Risks include chest pain, arrhythmias, myocardial infarction, and hyperventilation syndrome. We will monitor these risks by having only trained personnel with CPR training performing the testing. Continuous ECG and blood pressure monitoring will be performed a minimum of every 2 minutes during the test and 5 minutes following the test. Pretest history and physical examination will be performed with emphasis on signs and symptoms of cardiac, vascular or pulmonary disease. Levels of perceived exertion with be asked every 2 minutes. Emergency resuscitation equipment and drugs and telephone to activate code team are in testing room. Attention to exercise test end points will be made.

f) Fat biopsies will be taken from the abdomen. The risks of these procedures include pain, hematomas, bruising, infection, and scaring of the biopsy site. The risk of pain and bruising from adipose tissue biopsies is high, and the volunteers are given this information. We reduce the risk of infection by performing all biopsies under sterile conditions. Nonetheless, each volunteer is told of the signs symptoms of infection and is told to call the study coordinator immediately should any of they occur. A record is kept of all adverse reactions to biopsies. If an increase in the number of adverse reactions relative to historical controls is observed during the course of a particular study a careful assessment of technique and approaches is made. The protocol might then be modified or additional instruction or supervision of the individual performing the biopsies could be undertaken. The subsequent results are then audited to determine how effective the intervention has been.

2. Adequacy of Protection against Risks

Feasibility, Recruitment and Informed Consent: Volunteers will be initially recruited from individuals who have previously indicated a desire to participate in research. Volunteers will be recruited from advertisements placed on the Mayo Clinic and appropriate Mayo Health System (MHS) bulletin boards, electronic bulletin boards, in local newspapers and the Mayo Health System Newsletter. Each willing participant will meet one of the investigators who will explain the scientific rationale of the study, the procedures and potential risks involved in the study. The consent form will be approved by Mayo IRB prior to use. Informed written consent from the participant will be obtained by one of the investigators prior to participation. An electronic note will be entered in each participant's medical record regarding the consent and a copy of the consent will be electronically kept with the participant's medical record. A copy of the consent will be given to the subject and the original kept with the investigators records.

Protection against Risk: All protocols and all techniques to be used will be approved by the Mayo Clinic Institutional Review Board prior to initiation of any studies. All participants will have the Mayo Clinic paging operator available to them 24 hours a day to contact the investigators for any problems. The Clinical Research Unit also has an on-call physician available 24 hours a day to contact for problems or concerns. The following protection will be taken for the risks identified above:

1. Intravenous risks will be minimized by having experienced CRTU staff place the lines and infusing 0.9% normal saline throughout the study to decrease the risk of phlebitis or occlusion.
2. All infusions will be prepared in the pharmacy or by a trained nurse or doctor to ensure accuracy of all infusion concentrations and rates.
3. The amount of blood drawn is within the amounts acceptable by the institution. There is an institutional electronic tracking system to ensure that a patient is not in more than one study at a time.
4. The Radiation Safety Committee will approve the study for radiation safety of the Lunar iDXA scan.
5. A DSMP will be utilized for patient safety. A DSMB is not necessary for the study. Investigators and the CRTU have had extensive experience in all procedures and aspects of the study. The investigative team will review any participant safety issues as delineated in the DSMP.
6. Subject protection is as listed in the above section. Confidentiality of all medical records is strictly maintained by established procedures. The original study data are kept in the principal investigators' laboratory/office and are entered into a secure computer database password protected under a secure server space behind the Mayo firewall allocated for use by only the study team. All data are reviewed by the principal investigator. All investigators carry pagers. Volunteers have access to the Mayo Clinic paging operator 24 hours a day.
7. Risk for infection from muscle and fat biopsies is minimized by using sterile surgical technique. Participants will be educated on proper post biopsy care to further minimize infection risk.
8. The risks to the subjects are small being primarily those of blood withdrawal, catheter insertions and muscle biopsies.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Muscle health has a tremendous impact on physical function and metabolic health. Although exercise is an effective strategy to enhance muscle and metabolic health, there is emerging evidence that some people exhibit blunted responsiveness to exercise. Chronic systemic inflammation may be a factor that contributes to this so-called exercise resistance. The proposed studies will determine the relationship between systemic inflammation and salient parameters of skeletal muscle function. Such information will help identify effective strategies to manage sarcopenia, diabetes, and other chronic diseases that influence muscle physiology. The risks to the patients are minimal compared to the knowledge gained from the study.

5. Protection of Human Research Participants

The personnel identified in this application have completed the required education on the protection of human research participants. The institution has established a formal program entitled the Mayo Investigator Training Program or MITP. The MITP is a web

based educational course designed to provide all personnel involved in human subject research with training about human subject protection. All Mayo personnel engaged in human subject research are required to complete the course and recertify themselves every two years. The primary objectives of the course are to provide the historical framework for current human subject protection regulations and to explore the evolving issues related to human subject research. The course is divided into four sections:

1. Course introduction and general overview
2. History section which explores examples of unethical behavior in human subject research
3. Review of major human subject protection issues
4. Discussion of the various roles and responsibilities of individuals involved in human subject research.

At the conclusion of the instruction, individuals are required to complete a thirty-question assessment.