Study Protocol: Nutritional Biomarkers of Sarcopenia
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Study Pro
University of Arkansas for Medical Sciences (UAMS) Clinical Protocol

Study Title: **Nutritional Biomarkers of Sarcopenia**

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informed consent	x	
medical history	x	
list of medications	x	
measure height	x	
measure weight	x	
DEXA scan[1]	x	x
In Body scan[1]	x	x
Dominant handgrip strength	x	
5-rep chair stand	x	
10-meter gait speed	x	
SARC-F questionnaire	x	
periodic blood sampling		x
frequent breath sampling		x
ingest study product		x

[1]If subject meets the requirements to perform the DEXA scan and In Body scan on visit 1, these tests will be performed at that time. If they do not, these tests will be performed at Visit 2.

1.0 Background and Rationale

1.1 Introduction:

Sarcopenia increases the risk of a number of deleterious health conditions and represents a major financial cost to our healthcare system. Among older adults who are hospitalized, those with sarcopenia on admission are 5-fold more likely to incur higher hospital costs than those without (1). The operational definition of sarcopenia is defined as meeting the criteria for all of the following: low muscle strength, low muscle quantity or quality, and low physical performance. The diagnosis of sarcopenia requires techniques that are both expensive and operator-dependent. Simple measurements, such as BMI, do not necessarily identify sarcopenia. Importantly, current techniques can only identify sarcopenia after a physical/functional impairment has occurred. Skeletal muscle amino acid kinetics predict muscle health and functionality (2). Altered amino acid kinetics lead to decrements in muscle

mass, quality, and performance. Our laboratory, as well as others, have documented that the muscle response to circulating essential amino acids (EAA) determines muscle amino acid kinetics (2). Thus, we propose to characterize skeletal muscle amino acid kinetics to an EAA challenge, i.e., an oral amino acid tolerance test (OATT), in order to determine the state of muscle health. Analogous to the oral glucose tolerance test (OGTT) used to characterize alterations in glucose metabolism, the proposed OATT represents a potential low-cost solution to classifying patients' skeletal muscle health. The extrapolation of this work is the development of a simple analytical tool that would provide clinicians the ability to discern alterations in muscle amino acid kinetics prior to a loss of function.

1.2 Previous research on the topic

The rate of loss of muscle with aging in any given individual is variable and may be influenced by genetic factors, level of activity, amount of protein in the diet, total body weight, and occurrence of serious diseases that directly affect muscle mass and function. Nonetheless, the estimate from cross-sectional data that between 0.5-1.0 % of muscle mass is lost per year after the age of 70 years is reasonable (3). The physiological basis for loss of muscle over time results from an imbalance between the rates of muscle protein synthesis and breakdown. Consumption of dietary protein results in the absorption of amino acids and the activation of protein synthetic processes throughout the body, particularly muscle protein synthesis (4). There is a direct relationship between the amount of dietary protein intake and the net gain in body protein in young healthy individuals (5). Anabolic resistance refers to the circumstance in which dietary protein has a diminished stimulatory effect on the net production of new muscle protein. Critical illness (6) or the response to a major burn injury (7) provide clear-cut examples of anabolic resistance. In anabolic resistance associated with these pathological conditions, the normal linear relationship between dietary protein intake and net protein synthesis is capped, meaning that beyond a certain level of protein intake there is no further gain in net protein balance. For example, beyond a level of protein intake of 1.1 g protein/kg/day there is no net gain in body protein in critically ill patients (6). Once the maximal rate of protein synthesis is reached, there is no further gain in net protein synthesis, as the rate of protein breakdown is not affected by dietary protein consumption in severely catabolic patients. The

consequence of exceeding the amount of dietary protein intake that maximizes the net gain in body protein is the oxidation of the “extra” amino acids. As a result of the oxidation of a large percentage of dietary amino acids, urea and ammonia production increase significantly (7). Since this clinical population often has multi-organ failure, including impaired kidney function, an increase in urea and ammonia production can have adverse physiological consequences. Aging-related anabolic resistance is different than anabolic resistance associated with critical illness. Differences in the basic metabolic responses of protein metabolism in the two circumstances can explain the basis for the different nature of anabolic resistance. In critical illness, the loss of muscle protein is driven by a high rate of protein breakdown, which may be as much as 3 fold above normal (8). The high rate of protein breakdown floods the intramuscular pool with free amino acid precursors for protein synthesis, driving up the rate of protein synthesis. As a result, although protein synthesis is significantly less than the accelerated rate of breakdown in critical illness, protein synthesis is already driven close to the maximum rate without any intake of protein or amino acids (8). Since precursor supply is already high because of accelerated protein breakdown, provision of dietary protein or amino acids in critically ill patients has only a small effect on precursor availability and thus muscle protein synthesis. In healthy elderly individuals, basal rates of muscle protein synthesis and breakdown are not measurably different from younger individuals. As a result, protein synthesis can be increased by consumption of even small amounts of amino acids (9). In contrast, older adults are less responsive to the anabolic stimulus of low doses of amino acid or protein intake compared to younger individuals (10). Limited responsiveness of muscle protein synthesis at lower levels of intake but a similar maximal protein synthetic response suggests that the slope of the line relating the increase in muscle protein synthesis relative to increasing amounts of dietary protein consumption is less in elderly, but ultimately reaches the same maximum value as in younger individuals. However, behavior change, in this case increasing dietary protein to maximize muscle protein synthesis to blunt age-related losses of skeletal muscle, are difficult in elderly adults (11). Therefore, identification of individuals at risk for sarcopenia sooner in life are required. Considering changes in muscle protein kinetics precede functional outcome changes (e.g. muscle strength), assessment of postprandial amino acid metabolism represents a likely solution. The relationship between protein intake

and muscle protein synthesis has been demonstrated to be driven by the change in plasma essential amino acid concentrations (2). Stated another way, the changes in plasma essential amino acid concentrations following the ingestion of dietary protein drive the increase in muscle protein synthesis. The analogous parallel to amino acid and muscle is the use of the oral glucose tolerance test used to classify individuals with prediabetes and type-2 diabetes. Therefore, demonstrating that the essential amino acid response to an oral amino acid challenge can differentiate between elderly and young populations is a worthwhile first step in developing a new clinical diagnostic test to identify sarcopenia.

2.0 Hypotheses

We hypothesize that plasma amino acid response will be altered in elderly sarcopenic adults as compared to young and elderly healthy subjects.
We also hypothesize that the pharmacokinetics of plasma amino acids can be used to distinguish sarcopenic elderly from young and elderly healthy.

3.0 Study Design and Procedures

We will enroll up to 35 subjects with a goal to study up to 30 healthy male and females: a group of 10 whose age is between 18 and 30 years, a group of 10 whose age is 70-89 years without the presence of sarcopenia, and a group of 10 whose age is 70-89 years with sarcopenia. We will use a 5-hour (one hour prior and 4 hours post-consumption of an amino acid supplement) period to completely characterize the plasma amino acid response to 10 grams of free-form essential amino acids of which 10% of the leucine content has been enriched with L-[1-^{13}C] leucine. The principal end-point will be plasma amino acid response over the 4 hours following the consumption of the study product.
Secondary endpoints include body composition and total body water measurements. Small shifts in fluid from the extracellular to intracellular space likely correspond with EAA appearance and disappearance in plasma. BIA is a non-invasive, rapid assessment that is sensitive to acute shifts in fluid that are associated with disease and malnutrition states, such

as sarcopenia. As an exploratory analysis, we will measure acute shifts in total body, intracellular, and extracellular fluid using multifrequency BIA throughout visit 2. This will serve as preliminary data on a potential non-invasive method to evaluate muscle quality, anabolic resistance, and a proxy indicator of sarcopenia.

3.1 Study Visits

Visit 1: subjects will come to the UAMS TRI for informed consent discussion. Once consent is obtained, subsequent study procedures will be performed. A medical history including allergies and list of current medications will be obtained. Females of child bearing capacity will be asked to provide a urine sample for pregnancy testing prior to the DEXA scan. Exceptions will be made for women who have recently (past week) or currently are menstruating, have an implanted uterine device, or women who are celibate. Urine samples will be sent to LabCorp. Subjects will be asked to perform the following tests and measurements at visit 1:
Standing height without shoes (in cm),
Body weight without shoes (in kg),
Handgrip strength testing of their dominant hand (3 reps, in kg),
10-meter usual gait speed test,
5-rep timed chair stand test,
SARC-F (paper) questionnaire (older subjects only).

If subject meets the requirements (fasted for at least 8 hours, caffeine-free for at least 12 hours, and did not perform strenuous exercise during the past 24 hours) to perform the DEXA scan and In Body scan on visit 1, these tests will be performed at that time. If they do not, these tests will be performed at Visit 2.

Visit 2 (approximately 1 week after visit 1): Subjects return to the UAMS TRI having fasted for at least 8 hours, avoided caffeine for at least 12 hours, and avoided strenuous exercise during the past 24 hours. They will be asked about any adverse events since last visit. The In Body and DEXA scans will be performed if these were not done at visit 1. Prior to the IV insertion, an In Body scan will be performed if not already performed on this visit.

The study nurse will insert an IV catheter into a vein of one of the subjects' arms to use for periodic blood sampling, warming the arm by means of a heating pad or a heated plastic box. After an initial blood sample is obtained, a timer is started and blood samples and In Body scans will be obtained according to the schedule below. The study product (dissolved in ~10 ounces of water along with the stable isotope L-[1-^{13}C] leucine) is served directly after the third blood sample. Subjects are asked to consume the drink within 1 minute.

Using a mask that covers their mouth and nose, subjects exhaled breath will be collected to measure the appearance of the labelled carbon in the leucine isotope in expired CO_2. The mask will be worn for approximately 2 minutes prior to every blood sample time point after the initial sample. A 1-2 minute period of breath analysis will occur at the designated blood sample collection times. The mask can remain off between collection periods.

At the conclusion of 5 hours elapsed time, the IV catheter will be removed and the site dressed with a bandage. A snack and beverage will be offered to the subject. Subject will then be free to leave.

Elapsed time (min., approximate)	Procedure
-0	Blood sample (~4mL into an EDTA tube)
30	In Body scan
60	Blood sample (~4mL into an EDTA tube), , breath analysis, In Body scan
75	Blood sample (~4mL into an EDTA tube), ingest study supplement, breath analysis, In Body scan
90	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan
105	Blood sample (~4mL into an EDTA tube) breath analysis
120	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan

135	Blood sample (~4mL into an EDTA tube) breath analysis
150	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan
165	Blood sample (~4mL into an EDTA tube) breath analysis
180	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan
200	Blood sample (~4mL into an EDTA tube) breath analysis
210	In Body scan
220	Blood sample (~4mL into an EDTA tube) breath analysis
240	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan
270	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan
300	Blood sample (~4mL into an EDTA tube) breath analysis, In Body scan

3.2 Subject Compensation

Subjects will accrue compensation according to the below table. They will be handed a Walmart ® gift card of $25 at the end of Visit 1. They will be handed a UAMS check for their Visit 2 compensation immediately after their participation in visit 2 ceases (whether completed or not). If they were to fully attend every visit, their total amount would be $125. If they stop participating prior to the completion of study visit 2, they will receive prorated pay of $20 per hour.

Visit	Amount

1	$25 (gift card in hand)
2	$100 (check)

3.3 Recruitment

Potential subjects will be recruited from the Little Rock and central Arkansas areas by use of flyer, social media, ARResearch.org registry, and word of mouth. Individuals interested in the study will be scheduled for Visit 1.

3.4 Study Registration

This study will be registered on clinicaltrials.gov website as required.

3.5 Sample Storage

Blood samples will be kept frozen at -80 degrees Centigrade or colder once the initial processing has taken place. Samples shall be stored in appropriate freezers in the PI's laboratory, located in a restricted area inside the UAMS IOA building. Said freezers are monitored continuously for proper temperature and working condition. All blood samples shall be identified using a unique study acronym. None of a subject's personal identifiers shall be present on any biological sample.

3.6 Retention of Samples for Future Use

With explicit permission via the consent form, plasma samples will be kept for future approved research.

- Samples will only be released to investigators within or outside of UAMS who provide proof of appropriate IRB review and/or approval.
- Samples will be kept in the PI's freezers located on the 7th floor of the UAMS Reynolds Institute on Aging. This floor is controlled-access via approved UAMS badges.
- All samples are labelled with an alpha-numeric code. The key to this code is kept on a secure UAMS server to which access is limited to study staff.
- No personal identifiers are on the samples.

- Samples will be kept for up to approximately 10 years, after which they will be placed into biohazard trash receptacles.
- Samples will be retained for further analyses related to metabolic biomarkers (e.g., metabolomics, proteomics, inflammation markers, etc.).
- Subjects can rescind their permission to keep their samples by contacting any of the study staff listed in the consent form.

4.0 Study Population

Up to 35 subjects will be enrolled in order to meet the goal of 10 completers in each of the three groups. Attempts will be made to balance sexes within groups.

4.1 Inclusion Criteria

- Ages 18 – 30 and 70-89 yrs.

4.2 Exclusion Criteria

- History of diabetes
- History of malignancy or chemo/radiation therapy in the 6 months prior to enrollment
- History of gastrointestinal bypass/reduction surgery (Lapband, gastric sleeve, etc.)
- Pregnant females
- Unwilling to wear the breath-collection mask
- Subjects who cannot refrain from using protein or amino acid supplements for 7 days prior to Visit 2
- Concomitant use of oral or injectable corticosteroids
- Concomitant use of testosterone, IGF-1, or similar anabolic agent
- Any other disease or condition that would place the subject at increased risk of harm if they were to participate, at the discretion of the study physician

5.0 Risks and Benefits

There are no direct benefits for the subjects. Expected risks associated with this protocol are described in detail below. All experimental procedures will be performed by appropriately trained and credentialed personnel.

5.1 Blood sampling:

Blood samples will be collected solely for the purpose of experimentation. The blood will be used to measure plasma amino acid concentrations and possibly stable isotope enrichment, glucose, and insulin. The total amount of blood taken will be approximately 60 mL. Subjects should have no noticeable effects from this volume.

5.2 DEXA, In Body scans:

The DEXA scan exposes subjects to approximately ½ of the radiation of one chest x-ray. Subjects will undergo 1 DEXA scan. The In Body scans do not pose any risks.

5.3 Physical and Functional Testing:

The primary risks are fatigue and muscle soreness.

5.4 Study supplement:

The test composition is a patented mixture of 10 grams of amino acids (total of 17.8 grams of commercial product: *XS Muscle Multiplier*) dissolved into approximately 10 ounces of water. It is a commercially available product manufactured by Prinova™ Solutions (Prinovausa.com). All components of the composition have been ruled as Generally Regarded as Safe (GRAS) by the FDA.

5.4.1 Conflict of Interest

UAMS and Dr. Ferrando are listed on the approved patent for this particular amino acid supplement. There is an approved COI plan on file for Dr. Ferrando. He will not have access to raw data, only a summary of the compiled data.

5.5 Stable Isotope Ingestion:

There are no known risks of ingestion of the stable isotope of leucine.

5.6 Confidentiality:

A potential risk is the loss of confidentiality. Measures to protect the confidentiality of study participants will be implemented as described in the Data Handling and Record keeping section below.

5.7 Breath Sampling

The only risk of breath sampling is potential claustrophobia and subsequent anxiety.

6.0 Data Handling and Recordkeeping

Source documents, paper questionnaires and consent forms, and CRFs will be stored in a secure area of the PI's laboratory. Access will be limited to study personnel. Documents containing identifiers will be destroyed by shredding approximately 7 years after data analysis is completed or publication of data; whichever is longest. At no time shall Protected Health Information be released to non-study personnel.

The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. All study subject material will be assigned a unique identifying code or number. The key to the code (the instrument associating the data with subject identity) will be kept on a password-protected UAMS server, located behind locked doors in a restricted access area of the UAMS campus. The code file will contain subject initials, sex, subject ID, enrollment data, and anthropometric data; it will not include any identifiers. Only those individuals listed on the title page of this protocol and their research staff members will have access to the code and information that identifies the subject in this study. This file will be deleted approximately 7 years after data analysis is completed.

7.0 Data Analysis

7.1 Statistical Analysis plan

Means by which experimental data will be analyzed or interpreted: Changes in the plasma amino acids area under the curve (AUC), maximal amino acid concentration, change from basal to maximal amino acid concentrations, rate to maximal amino acid concentration, and the clearance rate of amino acids from the plasma will be assessed with an one-way ANOVA with appropriate post hoc testing. Statistical significance will be accepted at an alpha level of $p \leq 0.05$.

7.2 Sample Size Calculation & Power Analysis

Statistical power and sample size are based on previous studies investigating differences in plasma amino acids in younger and older adults following the ingestion of 10 grams of free form essential amino acids (12). For peak EAA concentrations previous studies have demonstrated an effect size (Cohen's D) of 1.6 between health young and individuals with incipient sarcopenia. This effect size indicates we need 8 individuals in each group to detect a significant difference at an alpha level of 0.05 with 80% power.

7.3 Sample Processing and Analyses

Blood samples will be processed and analyzed as described by PI's lab (2). Blood samples will be analyzed via LCMS to determine the concentration of specific amino acids relevant to the hypothesis. If performed, glucose and insulin concentrations will be performed using a COBAS analyzer. Stable isotope concentrations will be measured using either GC-MS or LCMS methods, if performed. Breath samples will be analyzed in real time via the collection apparatus. After all data has been analyzed and verified, samples will be discarded into a biohazard trash bag and disposed in accordance with UAMS biohazardous waste policy.

7.4 Grouping

Subjects in the older groups will be divided into either sarcopenic or non-sarcopenic based on the results of the handgrip assessment at visit 1 and amount of lean tissue derived from the

body composition measurements. The thresholds (per the Sarcopenic Definitions and Outcomes Consortium) are as follows:
Men whose value is < 35.5 kg are considered to have low muscle strength
Women whose value is < 20 kg are considered to have low muscle strength.

8.0 Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. This consent form must be signed by the subject and the individual obtaining the consent. A copy of the signed consent will be offered to the participant, and the informed consent process will be documented in each subject's research record.

9.0 Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

10.0 References

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