Bed Rest in Accelerated Sarcopenia Study
#17-0064

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National Institutes of Health

Version 15.1

January 25, 2023

1. Introduction and Purpose:

Accelerated Sarcopenia. Sarcopenia is a complex geriatric syndrome characterized by the loss of skeletal muscle mass, strength and function with aging[15]. It is a major contributor to the cycle of frailty [38] and increases the risk of falls, physical dependence, disability and mortality in older adults[15,68,82]. While the loss of muscle mass and function occurs gradually with healthy aging, diseases or other insults and injuries can accelerate sarcopenia and lead to catastrophic declines in mobility and independence[74].

Type 2 Diabetes Chronically Accelerates Sarcopenia. Chronic diseases are associated with acceleration of sarcopenia. Among these, Type 2 Diabetes Mellitus (T2DM) has been reported in longitudinal studies to hasten the loss of muscle mass and strength in older persons, even after adjustment for comorbidities, glucose control, disease length and other factors[58,72-74]. Insulin resistance may play a role in this process[62]. However, *we do not know which specific mechanisms are involved in the acceleration of sarcopenia by T2DM*.

Bed Rest Inactivity Acutely Accelerates Sarcopenia. Hospitalization is a well-known risk factor of functional loss in older adults[14]. Profound inactivity is the common denominator of hospitalization in geriatric patients, irrespective of disease or condition that led to hospital admission[36]. Inactivity during the hospital stay is associated with poorer clinical outcomes such as the length of stay[3,51]. More in general, bed rest inactivity is a major contributor to muscle loss and functional decline in older persons[47,60] (Preliminary Data). The deleterious effect of bed rest on muscle size and function is significant and occurs even in the absence of disease[22,60]. However, *the exact mechanisms by which acute inactivity accelerates sarcopenia are not well understood*.

Knowledge Gap. Conditions that accelerate sarcopenia, such as T2DM or acute immobilization, increase the risk of disability and dependency in older adults. However, the fundamental mechanisms by which sarcopenia accelerators induce loss of muscle mass and function are unknown. As a consequence of this knowledge gap, and in the absence of specific therapeutic targets, it is not possible to develop innovative and effective strategies for prevention and treatment of accelerated sarcopenia in older patients.

Significance. The proposed research is expected to spur a vertical advancement in our knowledge of the mechanisms that underlie accelerated sarcopenia in older adults. *This work is significant because it will lead to the identification of novel, specific targets for the development of evidence-based interventions to prevent and treat accelerated sarcopenia and maintain independence in older adults at high risk of physical dependence.*

Benefit. Mobility disability and physical dependence are major concerns for older adults, and important contributors to rising healthcare costs in the geriatric population[53]. In the year 2010, 26.9% of the U.S. adults 65 and older had diabetes[11]. A decline in muscle mass and muscle quality in this patient population is associated with reduced mobility[90] and increased risk of functional disability[91]. Hospitalization and bed rest for any cause are major events that interact with chronic conditions, further reducing function and independence in older adults[14,47]. By identifying the fundamental mechanisms that accelerate sarcopenia in these disabling conditions, we will be able to develop novel treatments for maintenance of independence. Thus, our research has the potential to positively impact not only healthcare outcomes but also costs over the long term.

2. Background:

Low muscle mass is a basic component of sarcopenia[15]. The association between muscle mass and function is not linear in healthy older adults[35]. However, conditions that accelerate sarcopenia decrease muscle mass at the same rate as strength, or faster[3]. A major determinant of skeletal muscle mass is contractile protein content, which is regulated by the balance between protein synthesis and proteolysis[99]. Incorporation of dietary amino acids into newly synthesized proteins is a fundamental process for maintenance and growth of skeletal muscle, because it allows for replacement of the essential amino acids released by proteolysis and lost with oxidation or transport to other tissues[92]. The stimulatory effect of an amino acid/protein meal on skeletal muscle protein synthesis is large and rapid[48,84], and driven by essential amino acids, particularly leucine, which directly stimulate muscle protein synthesis by activating translation initiation via the mTORC1 pathway[18,5]. To activate mTORC1 signaling and protein synthesis, amino acids must first enter the muscle cell via amino acid transport. Amino acid transport is an active process that is emerging as a key regulator of skeletal muscle protein homeostasis and anabolism[55]. Amino acid transport is controlled by several factors [1,55]. We have reported that dietary amino acid intake[27,96], insulin [86] and resistance exercise[26] can independently activate amino acid transporter expression and function in humans, and this effect is associated with increased muscle protein synthesis [19].

There is growing evidence from our group[43,75,87] and others[80] that healthy aging *per se* induces a mild but significant reduction in the response of muscle proteins to anabolic stimulation. This phenomenon has been termed "anabolic resistance" and may account for the slow loss of muscle mass with advancing age. It is characterized by a reduced anabolic response to low amino acid doses[17,56,57,84,94] and insulin[43]. This is a true "resistance" because the maximal anabolic response to high doses of amino acids[83,93,96] or insulin[43] is preserved in healthy older adults and comparable to that observed in younger individuals.

Our preliminary data suggest that a global, underlying mechanism of accelerated sarcopenia is an increase in anabolic resistance associated with a reduction in amino acid transport. With this application we will examine how amino acid transport is impacted by conditions accelerating sarcopenia and identify factors that control it.

In Specific Aim 1 we will determine if a well-known chronic accelerator of sarcopenia, T2DM, reduces the sensitivity of amino acid transport to increasing doses of exogenous amino acids. In Specific Aim 2 we propose that short-term bed rest inactivity will reduce the sensitivity of amino acid transport to exogenous amino acids and that T2DM will worsen this negative adaptation. Upstream regulatory pathways will be explored also in this aim to identify promising therapeutic targets for interventions.

Innovation

The research we propose is innovative, in our opinion, for at least three major reasons:

1. Conceptual Innovation. Our proposed studies will explore for the first time the potential global role of amino acid transport in conditions that accelerate sarcopenia chronically and acutely. By using a well described and common model of accelerated sarcopenia, T2DM, this research may also rapidly yield practical innovation that could be rapidly translated in the clinical setting.

2. Methodological Innovation. Innovation also stems from our novel integrative and interdisciplinary translational approach that combines state of the art and novel methodologies to determine amino acid transporter expression and function and their upstream regulation. These methodologies include: a 3-pool model tracer methodology to measure amino acid trafficking across vascular and intracellular compartments[20]; molecular techniques to measure regulation of muscle protein synthesis and amino acid transport[26,27]; and near infrared spectroscopy (NIRS) to determine the role of endothelial function and microvascular muscle perfusion[86,87]. Inactivity is a well-established means to induce muscle atrophy. Bed Rest inactivity also reduce amino acid transport and transporter expression and leads to anabolic resistance[22]. In this application, we will *innovatively use Bed Rest as a tool to modify the response of amino acid transport and transporter expression* with the purpose of testing our hypotheses and identifying new therapeutic targets.

3. Discovery of Novel Molecular Targets. Our secondary outcomes will include exploration of the role of novel regulatory proteins in relation to the changes in amino acid transport associated with T2DM. The basic assumption is that chronic and acute sarcopenia accelerators may reduce amino acid transport with different mechanisms. Thus, we will determine the potential role of newly identified amino acid sensing pathways such as the Sestrins-GATOR1/2 and the lysosomal vATPase/SLC38A9/Regulator complex in the activation, or lack of thereof, of mTORC1 signaling and amino acid transporter expression and function.

Our approach — unique and substantially different from the status quo — is expected to unravel the key underlying mechanisms responsible for accelerated sarcopenia due to T2DM. The knowledge generated by our studies is needed to develop future evidence-based clinical trials of interventions.

3. Concise Summary of Project:

Subjects. We will test our central hypothesis in community dwelling, independent older adults with non-insulin dependent T2DM. Since data previously gathered on healthy subjects will serve as controls for T2DM subjects, who are expected to be overweight or obese, we will limit inclusion to subjects with BMI 23-35 kg/m^2. Those who have a BMI ≥ 35 kg/m^2 will be considered for inclusion on a case-to-case basis, depending on whether or not thigh adiposity would impair muscle biopsy. Morbidly obese, frail or exercise trained subjects will be excluded, as these conditions can significantly impact muscle protein metabolism[13,79,89], confounding the results. Subjects will be weight stable, as active weight loss in obese older adults can temporarily improve the response of muscle protein synthesis to anabolic stimuli[88]. The study will enroll 80 subjects who will be recruited from the UTMB Pepper Center Volunteer Registry, the Diabetes Clinic for Seniors directed by Dr. Volpi, the UTMB Stark

Diabetes Center (Dr. Belalcazar), referral from other UTMB physicians, and advertisement in local media. To aid in subject retention and encourage study adherence, transportation to and from study visits via Lyft will be offered to subjects at no cost to them. We will use the UT System Lyft contract and desktop app, so that subjects will not need to own or use a private smartphone to benefit from this service.

Screening: After carefully explaining procedures and risks, informed consent will be obtained from all subjects. Eligibility will be assessed with clinical history, physical exam, questionnaires, functional testing, DEXA scan and a number of laboratory tests including blood count, metabolic panel, uric acid, lipid profile, coagulation panel, hemoglobin A1c, Hepatitis B and C, HIV test, urinalysis and urine toxicology drug screening. The subjects will arrive fasting for their screening visits for blood measures. An OGTT will be performed, which may involve the use of U-^{13}C glucose tracer to better characterize glucose metabolism. Habitual physical activity will be measured using both StepWatch Activity Monitors (SAM) and pedometers for 1 week during the screening process. Rescreening of subjects who did not meet inclusion criteria for reasons that may resolve over time as determined by the investigator is allowed (e.g. dehydration, acute infections, medications, etc.). Subjects will be rescreened within 3 months and only 1 rescreen will be allowed per subject.

4. Study Design. Subjects will undergo a two acute metabolic experiments, one prior to and one immediately following the completion of 5 days of Bed Rest, in which we will test the effects of 7 or 21 g of EAA in older healthy and T2DM patients. The study will last a total of 7 consecutive days, including the bed rest staying and the following rehabilitation. Note that the rehabilitation period could be prolonged according to the physical therapist evaluation. If this will be the case, the physical therapist will provide extra rehabilitation treatment as usually occur during hospital provider.

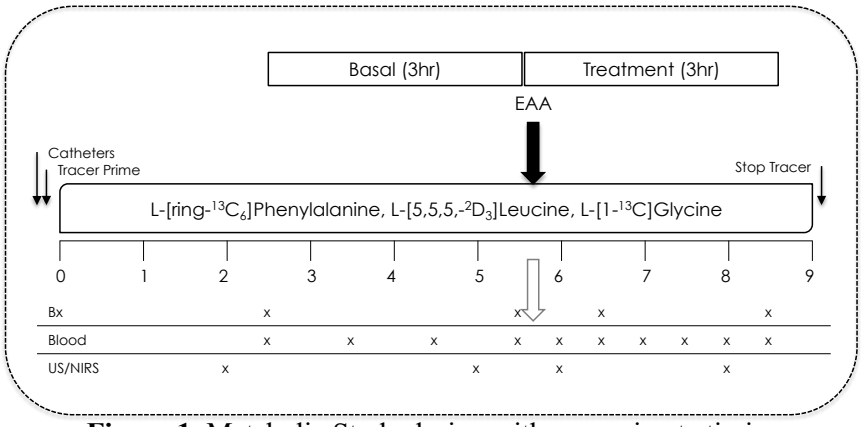

Figure 1. Metabolic Study design with approximate timing.

1) <u>Run-In</u>: Subjects will report to the Institute for Translational Sciences Clinical Research Center (ITS-CRC) the day prior to the experiment, in this occasion we will:

- Follow current institutional COVID-19 testing policy. If positive the subject will be referred to own PCP. If negative we will:
- Admit subjects to the ITS-CRC.
- Draw safety labs including blood count, metabolic panel, coagulation panel, urinalysis. Additional labs may be ordered by the study clinical provider, as clinically appropriate, if changes in the medical history have occurred between screening and admission.
- Provide standardized meals (see above).
- Hold vasodilators, monitor blood pressure, and manage hypertension with other medications if necessary [43,75,87].

2) <u>Metabolic Experiment</u>. The experimental procedure is designed to measure skeletal muscle amino acid transporter expression and function, whole body and muscle amino acid kinetics, muscle protein synthesis and proteolysis is depicted in *Figure1.* The subjects will be studied in the post-absorptive state, after an overnight stay in the ITS-CRC. After admission, subjects will be given dinner at approximately 18:00 and a snack at approximately 21:00, after which they will be allowed only water ad libitum. At ~06:30 we will measure body composition by DEXA. At approximately 07:00 we will insert: an antecubital forearm venous catheter for systemic infusions; a retrograde venous catheter in the opposite hand placed in a heating pad for arterialized blood draws. At approximately 0900, we will place if possible a 3 French/ 8 cm or 4 French/ 12 cm pediatric central catheter in the femoral vein for blood draws. If not possible the femoral catheter will not be placed and the metabolic parameters that need a femoral venous blood sample will not be measured. Subsequently, we will start a primed-continuous infusion of L-[ring-$^{13}C_6$]-phenylalanine, L-[5,5,5-$^{2}H_3$]-leucine, and [1-^{13}C]-glycine to measure muscle

protein turnover as well as the amino acid transport rates through LAT1 (Phe, Leu), SNAT2 (Gly), and PAT1 (Gly)[55,93]. To reach the intracellular space, amino acids have to first be transported across the endothelium and then across the muscle fiber membrane[66]. To reduce risks we will use arterialized blood samples, rather than arterial blood, to measure arterial amino acid concentrations and enrichments. This method has been previously validated for amino acid and other metabolite concentrations[8], and we have preliminary data indicating that it is also valid for amino acid enrichments. Whenever possible we will also perform a Flow Mediated Dilation test to detect endothelial function using Doppler Ultrasound and Near Infrared Spectroscopy. After an approximately 4-hr baseline period, subjects will ingest the assigned amino acid mixture, as a bolus. The mixture will contain EAA in the pattern found in animal protein[22] and will be enriched with L-[ring-$^{13}C_6$]-phenylalanine, L-[5,5,5-$^{2}H_3$]-leucine to maintain the isotopic steady state[22] (labeled glycine will not be added because it is not included in the oral mixture). Large vessel blood flow will be measured by Doppler[86,87]. Microvascular nutritive flow is emerging as a potential regulator of muscle amino acid and protein metabolism[85-87]. Thus, we will measure it in the basal state (see above) and after amino acid ingestion using Near Infrared Spectroscopy (NIRS) and measuring blood flow using Doppler Ultrasound. Frequent blood samples will be collected during the last hour of the basal period and the first and third hour of the amino acid period to measure amino acid concentrations and enrichments, glucose, insulin, cytokines and endothelin-1. T2DM is characterized by hyperglycemia, hyperinsulinemia, inflammation and endothelial dysfunction, which may have an impact on muscle protein metabolism [69,86]. Additional serum samples will also be collected and stored for future analyses (e.g. other hormonal assays, proteomics, metabolomics). Two muscle biopsies will be taken in each period to measure all pertinent amino acid and protein kinetic parameters, cross-sectional area, cell signaling, novel regulatory pathways, and gene expression (see Analytical Methods). A portion of the biopsies will be collected for immunohistochemistry. A third incision may be made should the need arise. At the end of the experiment catheters will be removed and subjects will be given a meal. The metabolic study will take approximately 8 hours.

3) Physical Inactivity (Bed Rest). After the Metabolic Experiment is completed, subjects will start bed rest inactivity. UTMB has a long tradition of safely performing bed rest studies of any length (from a few days to up to 90 days) in adults of all ages, including seniors (see for example refs.[22,31,32,70,77]). The UTMB ITS-CRC is equipped with specialized rooms for bed rest studies, which include CCTV cameras for continual monitoring and recording of subject to ensure that bed rest is effectively maintained throughout the experimental period. Recordings may be retained for a period not to exceed 45 days for review and will then be erased. Highly trained nursing staff and other patient care personnel is available at all times. The following standard procedures will be implemented for subject safety and comfort:

- A slight bed-back elevation will be permitted and subjects may raise their shoulders with two pillows.
- Periodic change of position in bed to alleviate positional discomfort and to eat.
- Serial compression devices (SCDs) and compression stockings will be worn to decrease DVT risk.
- Low molecular weight heparin (day 1-4)
- Standard of care in-bed physical therapy for bedridden patients involving passive range of motion of the lower extremity major joints.
- In-bed bathing, hygiene activities and diuresis.
- Bedside commode allowed for bowel movements.

The total energy content of the subjects' diet during bed rest will be reduced to account for inactivity. However, the protein content will remain at the pre-bed rest RDA level (0.8 g/kg/day)[32,37]. T2DM patients will continue their anti-hyperglycemic treatment with glinides and/or short acting insulin, adjusted as necessary. Blood glucose will be continuously monitored during bed rest using a device called Continuous Glucose Monitor (CGM).

4) End of Bed Rest. On day 5 of bed rest body composition by DEXA, Metabolic Experiment and functional tests will be repeated. After the tests, subjects will be allowed to de-ambulate with staff help.

5) Inpatient Rehabilitation. Our pilot data indicate that three days of traditional rehabilitation is sufficient to restore physical function (measured by SPPB score) to pre bed rest levels in older subjects (see *Figure 12* in Human Subjects). All subjects will be screened for glucose tolerance and postural hypotension prior to the resumption of weight-bearing activities. On day 1 they will focus on gradual re-mobilization with mild stretching, walking, and cycle ergometry. Days 2 will consist of graded aerobic exercise with 30 min of treadmill walking at a moderate rate of perceived exertion and resistance exercise focusing on the lower body, core stability and postural control. Rehabilitation will be delivered by a trained staff member and overseen by Dr. Steven Fisher, PT, PhD., who will discharge you (based on his evaluation of your progression) with prescription for a home exercise program to maintain your functional level. During this period you will remain in the CRC and continue to receive prepared meals. Dietary energy intake will be increased to account for the higher physical activity level.

5. Sub-Study Procedures:

Specimens obtained during the study will be stored in the principal investigator's laboratory and may be used in future research. For example, from time to time, new laboratory tests and procedures are developed that could use better understanding of the human body during metabolism studies.

The extra muscle tissue/blood left over after the initial research study tests are done will be stored and used in future research. Samples will be kept for a maximum of 10 years and then destroyed. The samples will be used to look at muscle growth related pathways. DNA will not be stored and no genetic testing will be done. Any future research will not involve additional specimens being taken or require any extra involvement from subjects. We will not need to contact the subjects again. The specimens will be labeled with a code number and will not directly link any of the subjects who partake in the study.

6. Criteria for Inclusion of Subjects:

- Age 60-85 yr
- Body mass index: 23-35 kg/m^2
- Or BMI>35 if thigh adiposity does not impair muscle biopsy
- Score ≥ 26 on the 30-item Mini Mental State Examination
- Stable body weight for at least 3 months
- Normal, non-diabetic (normal response to a 2-hr 75 g oral glucose tolerance test per American Diabetes Association criteria)[4]

  OR
- T2DM

7. Criteria for Exclusion of Subjects:

- Insulin therapy that cannot be converted to non-insulin therapies, significant diabetic complications, or A1c > 8%. Those excluded based on A1c will be eligible for re-evaluation.
- Impairment in Activities of Daily Living
- ≥ 2 falls/year or weight loss ≥ 10% in the past year
- Significant Cardiac abnormalities considered exclusionary by the study physician (e.g., , CAD,)
- Vascular disease
- Use of anticoagulant therapy. (e.g., warfarin, heparin, Pradaxa)
- Uncontrolled blood pressure: systolic >160 mmHg or diastolic > 100 mmHg
- History of stroke with motor disability
- Significant Chronic kidney disease
- Anemia: Those excluded for anemia may be counseled and rescreened (CBC) in 30 days
- Liver disease (AST/ALT 2.5 times above the normal limit)
- Significant Respiratory disease (acute upper respiratory infection, chronic lung disease)

- Active cancer
- History of mastectomy with axillary lymph node dissection
- Recent (within 3 months), chronic treatment with anabolic steroids, systemic corticosteroids or estrogen.
- HIV or active hepatitis B or C
- Alcohol (CAGE questionnaire) or drug abuse (urine screening)
- Any other condition or event considered exclusionary by the PI and faculty physician

8. Sources of Research Material:

Participants will provide verbal and written data specifically for research purposes. Study physician will have access to UTMB EPIC for subjects Measurement equipment (DEXA, stress test, food diary, timers for functional tests) will capture numerical and qualitative data. The SAM device will collect data on steps. Only trained study staff will have access to written study data. These data will be confidential.

9. Recruitment Methods and Consenting Process:

Subjects will be recruited through an institutional review-board approved advertisement that will be posted at UTMB, local newspapers, and social centers as the library, recreation centers, university announcements, flyers, and by word of mouth. Subjects will also be recruited by patient recruitment letters by mail, in-person at UTMB's outpatient clinics, by physician referral, on-line recruitment using UTMB's calendar, newsletters and websites, targeted Facebook and Twitter ads, and Trialfacts recruitment services. Referring providers sole responsibility is to refer potential subjects. Referring physicians will NOT be involved in recruitment, consenting or any other aspect of the study. They will only give us permission to contact any potential eligible patient in their care. Subjects will be met at the Institute for Translational Sciences- Clinical Research Center. Each subject will have a private room. The door will be closed during the consenting process. We will take all measures to ensure that subject privacy is maintained. After responding to ads, subjects will be pre-screened and asked to come in for a screening visit at the ITS-CRC. We will include all subjects who respond to our advertisement and invite them for a screening interview. If they qualify, they will be offered an opportunity to participate in the study. This will happen on a "first come first served" basis. We will study a normal healthy population between the ages of 60-85 representative of the ethnic makeup of the United States. the details of the experimental procedures, including risks and benefits, will be explained by a research team member at the time of initial screening at the ITS-CRC. At this time, the subject will be given ample time to read the consent form, ask questions and discuss any concerns. After the subject has been satisfied, the subject will be given an opportunity to sign the consent form. At every study encounter, each subject will be asked if they are still willing to participate in the study. Each subject will be asked if they have any questions, concerns, complaints, or comments. If the subjects have any of those listed, they will be appropriately addressed by the PI.

Lyft service will be provided to those subjects that don't have a car and need to reach the ITS-CRC site for screening and studies.

10. Potential Risks:

Possible risks and discomforts a subject might be exposed to during the course of the study include:

Positive screening tests: One or more of the screening tests might be positive of for a medical condition. This information might be upsetting for the subject. The PI and study physician, Dr. Elena Volpi or our research team nurse practitioner will provide counselling to the subject in the event that a screening test is positive for a medical condition or a blood level is abnormal. The likelihood of this is low.

Fasting: Risk of fasting is hypoglycemia, dizziness, nausea, and light headedness. Risk is low.

Blood draws: The subjects will undergo blood draws during the course of the study. The total amount of blood drawn will be approximately 170 ml for each of the two metabolic study days. The risks are pain, bruising and infection. People with borderline anemia might become more anemic with the repeated blood draws. Fainting is also a risk as some people faint at the sight of needles and/or blood. The risk of this event is low.

COVID-19 rapid test: It involves a nasal swab, which may be uncomfortable and rarely cause a nose bleed. Risk is low.

Anemia: Repeated blood draws or excessive sampling volume increases the risk of anemia. This is unlikely as the blood collected is less than a blood donation.

Muscle biopsies: The risks of this procedure are pain, bleeding, bruising, infection, and a little scar at the site of the incision. The risks are low. The biopsy scar is in the shape and size of this "_" Some people may also faint at the sight of needles and/or blood. Up to 50% of subjects experience some soreness at the site of the biopsy up to 48 hours after it is done.

Stable Isotopes Infusion: The only possible risk is sepsis (blood infection) and fever, but the risk is so small that the precise number is unknown. The isotopes are tested in order to assure their sterility (absence of microorganisms, such as bacteria, that may cause infections and fever). Antibiotics can treat these infections should they occur.

DEXA Scan: Each DEXA scan will expose the subject to a small amount of radiation. Exposure to radiation can increase the risk of cancer and birth defects. The amount of radiation that a subject will be exposed to is so small that the exact risk is unknown.

Femoral Venous Catheter: We will insert only a pediatric catheter in the femoral vein. The risks are the following: (A) Trauma to structures adjacent to the targeted vein (hematoma, nerve injury) is the most commonly recognized complication/error during the cannulation process (1.9-3.6% incidence during central venous cannulation are generally reported). Traumas rarely are serious, but the experience of the operator and the use of ultra-sound assistance can bring the risk to virtually 0%. (B) Complications related to the presence of the catheters, i.e., infection, thrombosis and thromboembolism. For patients with central venous catheters (CVC), an infection rate of 1.4 per 1,000 catheters is reported. However, most patients with CVC are immunosuppressed because of their underlying disease, therefore having a higher risk of infections than our research volunteers. Infection in the immediate post-insertion period (3-5 days) is commonly due to infection of the subcutaneous track caused by intraoperative contamination and as such are more easily diagnosed and treated than the feared systemic infections more prone to appear later. Catheter-related thrombosis occurs in 3.7-10% of patients. This complication is more likely to occur in patients with specific thromboembolic risk. Factors that contribute to thrombosis in patients are hypercoagulability (malignant tumors), venous irritation caused by chemotherapeutic agents and hyperalimentation fluids. The risk is moderate.

Strength Testing: The subject will experience soreness and tenderness in the muscles for 2 to 3 days following exercise. This is normal and is one of the ways in which the human body responds to exercise in order for the muscle to grow. A cardiovascular event such as a heart attack is a very rare potential risk.

Near Infrared Spectrometry (NIRS): No additional protection is necessary. The effect of heat due to near infrared light absorption is low – less than 0.5°C.

Deep Venous Thrombosis (DVT): While there have been no reported DVT events associated with previous bed rest studies, there is a small risk of a DVT as a result of reduced physical activity or bed rest. We will use leg compression devices and low molecular weight heparin to further reduce this risk.

Loss of Muscle Mass and Functional Capacity*:* We expect that the subjects on bed rest will experience a loss of muscle mass and function during bed rest. The magnitude of this loss has been previously described by our group[22,60], and can be reversed by 3 days of intensive in-hospital rehabilitation (see preliminary data in *Figure 12*).

Confidentiality: There is a general risk of disclosure of personal sensitive data in a clinical investigation. The risk is minimal.

11. Subject Safety and Data Monitoring:

Data Safety and Monitoring Plan. The ultimate responsibility for data and safety monitoring rests with Dr. Volpi. The risk level associated with this study is estimated to be moderate.

Our data safety and monitoring plan will include:

1. Review during of screening results and any other available data at the weekly investigators' meeting.
2. A quarterly review of data safety and interim data analysis performed by the PI.
3. An annual review performed by the UTMB IRB.
4. Semi-annual patient safety review by a Medical Monitor, Mukaila Raji, MD, Chief of the Division of Geriatric Medicine, UTMB.

Careful monitoring of the recruitment, enrollment, retention, adverse events, and study procedures will help protect the safety of study subjects, the quality of data, and the integrity of the study. As part of the safety plan for this study, Dr. Volpi, who is ultimately responsible for the medical management of the subjects, will review each subject's records to ensure that appropriate mechanisms to protect the safety of study participants are being followed; that protocol requirements are being adhered to; and that data is accurate, complete, and secure. Subject records include consent forms, case report forms, flow of data forms, laboratory specimen records, inclusion/exclusion forms, adverse event logs, and medical charts.

Non-serious, anticipated adverse events (AE) include hematoma, pain, infection, syncope, and loss of subject confidentiality. If an anticipated adverse event occurs, Dr. Volpi will be immediately notified and a note will be entered into the subject's CRC chart. Dr. Volpi will be responsible for evaluating each AE and determining attribution as well as the impact of the AE on the risk/benefit ratio.

All unanticipated, serious, fatal and/or life-threatening adverse events will be reported to the IRB within 24h of occurrence. The IRB and the PI will be responsible for determining whether modifications to the protocol and consent form are required. If a determination is made that participants are found to be exposed to excessive risks in relation to anticipated benefits, the study will be immediately suspended. Studies will not resume until modifications are made that are deemed to result in an acceptable risk/benefit ratio by the PI and the IRB. Aggregate reports of adverse events will be prepared as mandated and forwarded to the IRB and ITS-CRC for review.

Plan for Adverse Event Reporting. The PI will be responsible for evaluating each adverse event and determining attribution as well as the impact on the risk/benefit ratio, using the following Grading and Attribution criteria:

Mild – transient laboratory test alterations that do not suggest injury; discomfort noted but no disruption of daily activities; no therapy, or only symptomatic therapy required.

Moderate – Laboratory test alterations indicating injury without long-term risk, discomfort sufficient to modify normal daily activity, specific therapy required (i.e., more than symptomatic).

Serious – laboratory tests indicating a serious health threat or permanent injury, incapacity, inability to work, or to perform normal daily activity, hospitalization required or prolonged, emergency treatment required, life-threatening events, death.

The attribution scale assesses the relation of the event to the study procedures. Dr. Volpi will judge whether an adverse event is: 1) *not related*; 2) *possibly related*; 3) *probably related*; or 4) *definitely related to the study interventions*.

Table 1. Groups for Aim 1. Older adults with T2DM and healthy controls will be randomized to two groups (n=8/group) receiving 7 g or 21 g of EAA.

	Essential Amino Acid Dose	
	7 g	**21 g**
Control	C7	C21
T2DM	D7	D21

12. Procedures to Maintain Confidentiality:

All study data will be collected in REDCap by the research team. Paper data flow sheets or case report forms will be stored in locked file cabinets in a secure area of the PI's recruitment office. Interim data will be reviewed by the PI. Any appearance of a Conflict of Interest by the research team will be avoided by the periodic monitoring by UTMB's Conflict of Interest Committee. The subjects' right to confidentiality will be protected at all times and no subject will be identified by name in any publication that results from this research.

13. Potential Benefits:

The only potential benefits for the participation in this protocol are related to the knowledge of the results of the screening tests that will be released to the volunteer. The knowledge collected from the present studies may be of benefit to society, as these studies will help develop the scientific basis for designing treatments for reducing loss in muscle mass and function in older subjects with T2DM.

Importance of the Knowledge to be Gained. These studies will allow us to identify the biological mechanisms by T2DM accelerate sarcopenia in older individuals. Since T2DM is quite common in older adults and increase the risk of physical dependence, the knowledge to be gained will be very important to develop interventions to maintain independence in older adults.

13. Statistical Analysis:

Primary outcome variables will be measures of amino acid transport. All other variables will be treated as secondary outcomes. We will first compute the basal-to-acute treatment change for each variable in each subject. Experiment to test Aim 1 is designed using a *2×2* factorial approach with no repeated measures (see Table 1). A standard *2×2* ANOVA model will be used to test the main effects, treatment by group interactions, and contrasts of interest, particularly the interaction effect of EAA dose (Aim 1) on challenges to the metabolism by T2DM.

Experiment for Aim 2 is designed using a *2×2* factorial approach with repeated measures across time (see Table 2). We will then use a standard repeated measures *2×2* ANOVA model to test the main effects, treatment by group interactions, and contrasts of interest, particularly the interaction effect of amino acid dose (Aim 1)/exercise (Aim 2) on the effect of bed rest. For each variable we will also test if there are basal differences between groups using one way ANOVA. If basal differences are found, we will then use the basal values as covariates. Factors such as age, sex, and BMI will also be considered as covariates. The same analytic plan will be used for secondary outcome variables, and the results will be used for the overall interpretation of our study. To further elucidate potentially important mechanistic relationships between the primary and secondary outcomes, along with their relationship to demographic characteristics such as sex, we will also use multiple regression models and other techniques, such as principal components analysis. Differences will be considered significant at $p<0.05$. Post-hoc testing will be carried out using the Tukey-Kramer test. All calculations will be conducted in SAS (version 9.2 or later; SAS Institute Inc., Cary, NC). As necessary, responses will be transformed to assure that model assumptions of normality and constant group variance are met. Results will be presented as mean ± SD.

Table 2. Groups for Aim 2. Healthy and T2DM older adults will be randomized into two physical therapy groups: standard of care (Control) and Exercise. In all groups we will measure the effect of 7 g of EAA before and after 5 days of bed-rest, as described in Aim 1.

*same group randomized to 7 g of EAA in Experiment 2.

	PHYSICAL THERAPY	
GROUP	**Control**	**Exercise**
Control	BR-C7*	BR-ExC7
T2DM	BR-D7*	BR-ExD7

Sample Size and Power Calculation. Sample size and power have been calculated for the primary endpoints for the planned ANOVA model (see Statistical Analysis). All calculations were conducted using our preliminary data and the nQuery software package. We have not powered the experiments to measure sex differences because in our experience, and under similar experimental conditions, muscle protein metabolism does not behave differently in women and men[44], regardless of age[64]. However, since there are no data on the effect of gender on skeletal muscle protein turnover and amino acid transport in bed rest and T2DM, we will balance groups by sex. In *Table 3* we report the expected differences as original or log transformed units, the SD, the calculated n/group and the n/group including subjects to offset the expected attrition of 12%.

Table 3. Sample size (n) per group, based on the primary outcome, to detect the expected smallest differences between groups (∆min) given the measured standard deviation (SD) of the difference, alpha=0.05 and beta+0.8. n= subjects/group, n+ = n/group including expected attrition rate.

Outcome Variable	∆min	SD	n	n+
Amino acid transporter expression	0.52	0.29	6	8
Leg Lean Mass	0.40	0.20	3	8

Group Randomization. All recruited subjects who had signed the consent form before screening will be randomized to receive 7g or 21g of EAA to test Aim 1. To contain experimental costs and improve efficiency, subjects in C7 and D7 will be the control group BR-C7 and BR-D7 in Aim 2. Subjects in BR-C7 and BR-D7 will then be randomized to receive standard of care (BR-C7 and BR-D7) or intense physical therapy (BR-ExC7 and BR-ExD7) during Bed Rest.

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