1.0 PROTOCOL TITLE: Evaluation of biomarkers for predicting macronutrient intake

Protocol Version Date: 10/26/2023

☒ Check if this research has U.S. Federal government funding via one or more direct awards or a sub-award. Provide the source of federal support: National Institutes of Health
☐ All other sources of funding:

2.0 PRINCIPAL INVESTIGATOR:

Nicolaas E Deutz, MD, PhD. Professor, Ponder Endowed Chair. Physician Nutrition Specialist. Editor-in-Chief Clinical Nutrition and e-SPEN Journal. Director Center for Translational Research in Aging & Longevity.

3.0 Co- INVESTIGATORS:

Michael McShane, PhD. James J. Cain Professor II in Biomedical Engineering. Head, Department of Biomedical Engineering. Faculty, Department of Materials Science and Engineering. Center for Remote Health Technologies and Systems.

Marielle P Engelen, PhD. Professor, Head Clinical Research. Co-director Center for Translational Research in Aging & Longevity.

4.0 Objectives:

Establish a quantitative relationship between macronutrient intake from fresh meals and the resulting blood and ISF levels of small-molecule metabolites (nutritional biomarkers)

5.0 Background:

Introduction

The increase in plasma concentration of glucose and amino acids (AAs) is directly related to the amount of carbohydrates and protein/free AAs consumed, respectively. However, while glucose dynamics have been studied intensely, the relationship between protein intake and AA levels in ISF is less understood and more complex. Our preliminary results show a different plasma-ISF relationship for individual AAs, with only a few exhibiting strong enough relationships to use ISF levels in predicting plasma levels for liquid meals. It is also unclear whether the ISF concentration is a valid proxy when solid meals are consumed. Therefore, a controlled study with healthy part will be conducted to establish quantitative relationships between compartmental concentrations of macronutrients and metabolites. This study will evaluate the effects of meal composition (macronutrient intake) on the levels of metabolites in blood and interstitial fluid.

Significance

Monitoring and optimizing dietary intake are important for precision nutrition in the treatment of clinical conditions (diabetes, obesity, kidney/liver failure, etc) as well as attenuating loss of muscle function and mass during aging. Processing of macronutrients (carbohydrates, protein, and fat) is believed to be highly personalized. Unfortunately, current methods for personal

health monitoring only allow accurate measurements of physical activity and do not provide reliable measurement of macronutrient intake or availability of metabolites after digestion. Invasive blood samples are inconvenient and only periodic. While minimally-invasive wearable probes for tracking glucose trends are emerging (e.g., CGMs), no tools currently exist to provide on-demand monitoring of multiple macronutrient metabolites (e.g., glucose + amino acids). Thus, there is a technology gap that could be filled with multi-analyte sensing devices.

Innovation
Interstitial fluid (ISF) levels of metabolites are hypothesized to have complex but consistent relationship with their levels in blood/plasma, such that knowledge of ISF levels may be used to predict both blood levels and macronutrient amounts. An innovative combination of insertable optical reporters combined with wearable readers and advanced computational methods are proposed to provide continuous/on-demand measurement of these metabolites in ISF and used them as “nutritional biomarkers” to predict macronutrient intake

6.0 Inclusion and Exclusion Criteria:

Recruitment & Screening
Healthy older adults (50-75y; equal gender) with a BMI between 25 and 35 will be recruited from an existing database of CTRAL. In addition, we will recruit older adults that respond to distributed flyers and advertisements in the newspaper and to radio announcements in the community of the College Station/Bryan area. Informed consent will be obtained on the screening day before any study related procedures will be performed. If inclusion/exclusion criteria are met, participants are invited to take part in the study.

Inclusion criteria

- Ability to walk, sit down and stand up independently
- Age 50-75 years old
- Ability to lie in supine or slightly elevated position for approximately 13 hours
- BMI between 25 and 35
- Willingness and ability to comply with the protocol

Exclusion Criteria

- Established diagnosis of malignancy
- Established diagnosis of Insulin or non-Insulin Dependent Diabetes Mellitus
- History of untreated metabolic diseases including hepatic or renal disorder
- Currently on anticoagulants (i.e., warfarin, factor X inhibitors or direct thrombin inhibitors)
- History of deep vein thrombosis, pulmonary embolisms, or known clotting disorders
- Presence of acute illness or metabolically unstable chronic illness
- Recent myocardial infarction (less than 1 year)
- Known allergy or intolerance to any of the meal components
- Any other condition according to the PI or nurse that was found during the screening visit, that would interfere with the study or safety of the patient
- Failure to give informed consent or Investigator's uncertainty about the willingness or ability of the participant to comply with the protocol requirements

When during the period from enrollment to completion of the study any condition is developed, whether causing the participant to not meet inclusion criteria or to meet exclusion criteria, they will be excluded from the study.

7.0 Number of Local Participants:

Up to 40 individuals will be enrolled in the study. For establishing the quantitative relationship between macronutrient food intake and resulting plasma and ISF values of small molecule markers (Aim 1), our initial focus will be on classical time-series techniques, which can operate well with limited training data. Based on a sample size calculation for the machine-learning model to distinguish a 30% difference in protein and CHO amounts with power of 0.8 and significance level of 0.05, we will need 33 healthy participants, 8 meals per participant. Estimating 20% dropout.

8.0 Study-Wide Number of Participants:
n/a

9.0 Study Timelines:
1 screening visit ~2 hours
4 study visits each ~15 hours over the course of 3-6 weeks

Total time: ~62 hours

10.0 Study Endpoints:
Primary Endpoint

- Prediction models of postprandial plasma glucose in relation to the macronutrient content of predefined meals as assessed by plasma and/or ISF concentrations of amino acids, glucose, and/or triglycerides

11.0 Procedures Involved:
The study will take place at the research facility of the Center for Translational Research on Aging and Longevity (CTRAL), Texas A&M University located in the Human Clinical Research Building. The study involves 1 screening visit lasting up to 2 hours and fasting prior to screening is not required. We will screen participants for any disease or condition that could interfere with the studies. We also will perform Dual-energy X-ray absorptiometry (DXA) to measure body composition. We will also ask participants to answer demographic questions, questions about general health and nutrition, eating habits, etc. Demographic characteristics will be collected at baseline. Prior to completion of the study, all participants will also complete a series of questionnaires. Questionnaires are used to assess the current cognitive and mental well-being. Questionnaires, such as the following, may be completed during the study days:

- 3-day food diary to determine habitual dietary intake
- Questionnaire about gut function and symptoms: The Gastrointestinal Symptom Rating Scale (GSRS)
- Questionnaire about activity: Physical Activity Scale for the Elderly (PASE) & Baeck
- Questionnaires about general well-being: Sf36

On each study day, body weight will be measured. Participants will be fed up to 8 different meals (see **Table 1**) with specific nutritional composition (two per day) and then monitored for four hours after the meal. Meals will be spaced at 8 hours to limit overlapping effects. Meal sets will be randomized across study days (see **Table 2**). At the end of the second monitoring period, catheters will be removed and participants will be given an additional meal. Participants will be able to go to the restroom and drink water ad libitum throughout each study day. See **Figure 1** for an overview of the study day. The microdialysis pump which contains the perfusate solution and catheters will be securely affixed to the subjects forearm using coban. See **Figure 2** for a schematic picture of the microdiaylsis setup and an actual photo of the pump used by CTRAL. This will ensure maintenance of proper placement and collection of samples while out of the

bed. Participants will return 3 times (4 total study days per participant), where the same procedure will be repeated with meals of different compositions.

Table 1. Type and composition of study meals

Meal Number	Carbohydrate (g)	Protein (g)	Fat (g)	Energy (kcal)
1	40	15	15	355
2	40	15	40	580
3	180	40	15	1015
4	180	40	40	1240
5	180	15	15	915
6	40	40	15	455
7	180	15	40	1140
8	40	40	40	680
All meals will be prepared by a local company (Heat Ups) to match the composition of the Frozen Meals.				

Table 2. Meal sets to be randomized across the study day

	AM Meal	PM Meal	Total Calories (kcal)
Meal set A	Meal 1	Meal 4	1595
Meal set B	Meal 6	Meal 7	1595
Meal set C	Meal 2	Meal 3	1595
Meal set D	Meal 5	Meal 8	1595
Meal sets will be randomized across study days for all participants.			

An in-dwelling catheter will be inserted in a peripheral vein of the lower arm or hand to enable blood sampling. The arm will be put in a hot box to allow collecting arterialized venous blood samples. After taking a baseline blood sample (~5 ml), the predefined meal will be consumed within 10 min. Small arterialized venous blood samples (~3 ml) will subsequently be drawn at 30 minutes and then every 60 minutes after for the duration of the study day. This process will be repeated after consumption of Meal 2 on each study visit. Samples will be collected in predefined tubes (15 blood samples, totaling ~60 ml), plasma/serum separated, aliquoted, and stored at -80 Celsius for later assays and/or sent to a local accredited laboratory. A one-time blooddraw (~12 ml) will be collected for measurement of acute clinical chemistry biomarkers.

- Hemoglobin A1C
- Insulin
- Renal function panel (Alb/BUN/Calcium/CO2/Cl/Creat/Glucose/Phosp/Potassium/Sodium)
- Hepatic function panel (ALT, AST, Alb, Alk Phos, Direct bili, Total Bili, Total protein)
- Lipid panel (Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Triglycerides, VLDL Cholesterol)

- CBC (DIFF/PLT) (Hematocrit, Hemoglobin, MCV, MCH, MCHC, RBC, WBC, Platelet count, and percentage and absolute differential counts)
- hsCRP

In the opposite limb, a commercially-available microdialysis catheter will be placed in the forearm following standard operating procedures developed by the European Academy of Allergy & Clinical Immunology Task Force on Skin Microdialysis. Dialysate will be collected in 60-minute aliquots in microvials for the duration of the study day. Microvials will be weighed before and after sample collection. A syringe filled with perfusate containing a mixture of stable tracer-labeled amino acids and glucose will be infused through the syringe pump at a controlled rate (5 µl/min; total fluid perfused 4.5 ml).

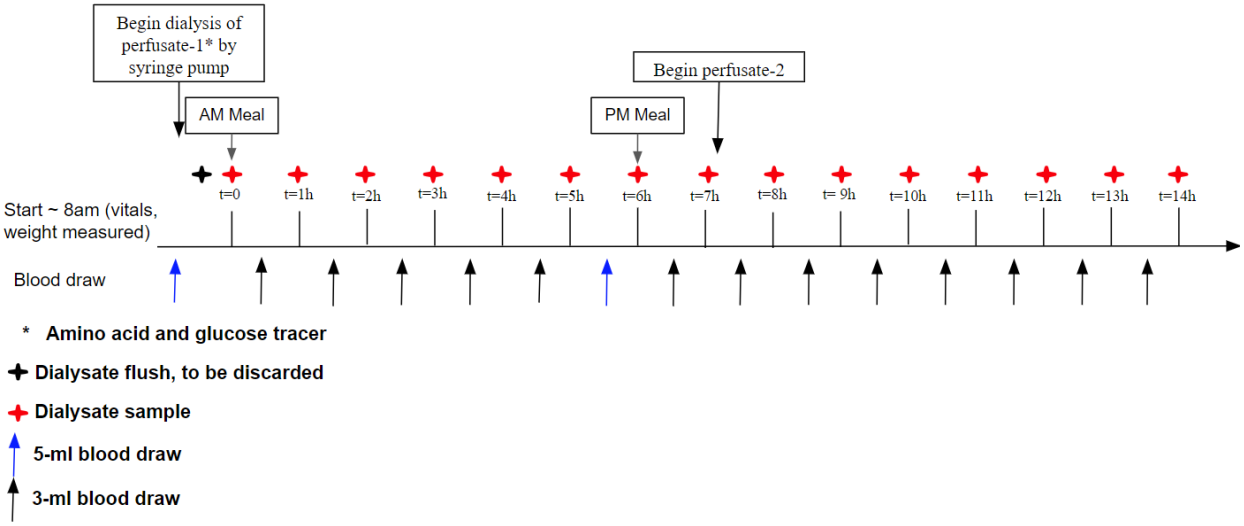

Figure 1. Overview Study Day for 91-Micro2

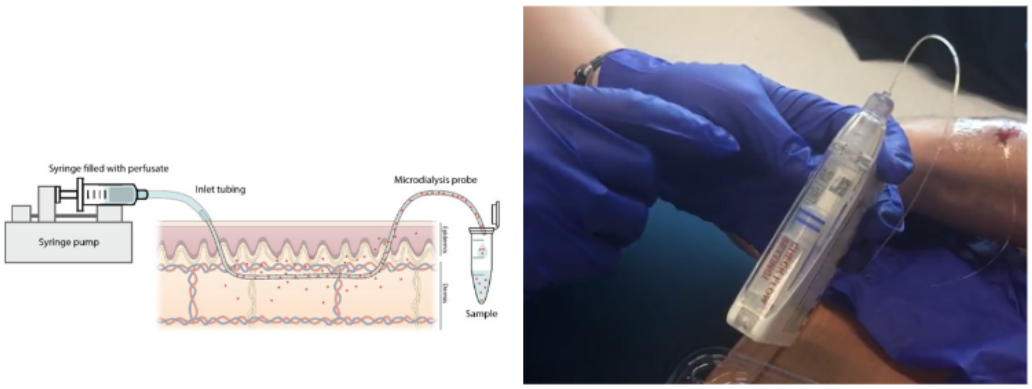

Figure 2. Schematic representation (not drawn to scale) and photo of CTRAL's microdialysis pump

12.0 Data and Specimen Banking:

Laboratory analysis of samples

The concentrations of amino acids and other parameters (fatty acids, glucose, insulin) will be measured. The plasma metabolite concentrations will be measured using a LC-MS/MS with an

ABSciex QTRAP 5500 {Engelen, 2013, 445-53}{Jonker, 2014, 211-20}{Luiking, 2015, 57-67} or a Bruker GC with a SCION Triple Quad Mass Spectrometer. The Center for Translational Research in Aging and Longevity (CTRAL) at Texas A&M University (TAMU) has all the resources to perform the described experiments.

Banking
Participants can opt-in/out of allowing samples to be stored for future use in consent. Samples will be stored in -80 freezer biobank that is continuously monitored for temperature, has controlled and monitored access. Specimens will be stored for a minimum of 7 years. Only authorized members of the CTRAL research group will have access.

13.0 Data and Specimen Management:

The participant's right to confidentiality will be protected at all times and no participant will be identified by name in any publication that results from this research. Participants will be screened in private. Any potentially sensitive information will only be collected in a one-on-one setting. Participants will be informed that this study is also protected under a Certificate of Confidentiality from the National Institutes of Health. The samples will be stored under an identifier, so they are not linked directly to the participant's name. Any personal identifying information that will no longer be used for the research study will immediately be destroyed by the use of an office shredder. All study data collected by the research team, will be recorded on data flow sheets or case report forms (CRFs), and stored in locked file cabinets or databases in a secure area of the PI/Co-I's offices. Each participant will have a binder devoted to filing all pertinent CRFs and all other documents relevant to participation in the study, including printouts of laboratory reports, analytical data (e.g., tracer/tracee ratios, etc.) and any other pertinent information. Any data on a computer will be stored on a secure server with access only to research staff appointed by the PI.

14.0 Provisions to Monitor the Data to Ensure the Safety of Participants:

- All experimental procedures will be performed by appropriately trained and credentialed personnel, following Standard Operating Procedures (SOP). The infusion procedure will be monitored by licensed medical personnel and halted if necessary.
- As part of the safety plan for this study, the PI and/or study coordinator will review individual participant records continuously 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.
- Blood draws: Blood will be withdrawn from a venous line with aseptic technique, which will minimize the risk of infection but will not eliminate it completely. A small volume of saline will be infused during the test days. Participants are not expected to experience any noticeable effects. Expected adverse event due to blood sampling is anemia. Symptoms may include anemia as seen in laboratory tests (low hemoglobin). With the described amount of blood loss the occurrence of anemia is not likely. The hand will be monitored and exposure limited while in a temperature controlled box to minimize risk of scalding. Temperature of the hot box will be monitored and recorded every 2 hours. Cloth or gauze may also be placed over the hand to minimize these risks. The hand may also be removed between blood draws, but must be in the box for 10 minutes prior to the next blood draw.
- Sterile stable isotope solutions will be made as concentrated stock solutions in ready to use working solutions in vials. Sterile compounding of the stable isotope solutions will be completed onsite in United States Pharmacopeia (USP)797 certified cleanroom at the Human Clinical Research Building under the supervision of a licensed pharmacist. Prior

to use, sterility will be determined by following the USP 81 guidelines. The presence of anaerobic bacteria is determined by completely filling the culture-ware (coated with tryptic soy agar) with commercial/certified tryptic soy broth. The presence of aerobic bacteria and fungi is determined by partially filling the culture-ware (coated with tryptic soy agar) with commercial/certified fluid thioglycollate medium. Cultures of isotope solutions and positive and negative controls are placed in an incubator for 14 days at 37°C. After 14 days the isotope cultures are compared to the positive and negative controls to determine sterility. Once stable isotope stock solutions and working solutions pass the sterility testing at the compounding pharmacy, they are stored in a temperature controlled -20°C freezer with an alarm system in compliance with USP 797 guidelines. Prior to use of the stable isotope solutions and working solutions in human studies at our facilities, sterility and pyrogenicity testing is performed. Sterility testing is performed using the same protocol used at the compounding pharmacy. Solutions are tested for sterility every 45 days. Pyrogenicity testing is performed to determine the endotoxin levels in the isotope solutions, using a commercial/certified endotoxin test kit, following the USP 85 guideline. Based on the USP endotoxin limit for common injectables using normal saline (0.5 to 0.9% NaCl), an endotoxin level below 0.5 EU/mL is considered safe whereas levels above 0.5 EU/mL are not safe and discarded appropriately. The expiration on the pyrogenicity testing is 1 year and any solutions kept for 1 year are discarded appropriately. On the test days, the ready to go and stock sterile stable isotope solutions will be withdrawn from the vials within a few hours of administration. The stable glycerol stock solutions will be diluted in a sterile 0.9% sodium chloride infusion bag, in a certified ISO 5 controlled environment in compliance with USP 797. An inline 20 m filter will be used between the syringe or infusion bag and the i.v. (or port) catheter for intravenous administration. Any adverse reactions during the isotope infusion that suggest allergic reaction or infection will be promptly addressed by the nurse and/or study physician. As a precaution, throughout the isotope infusion we will measure body temperature, heart rate, saturation and blood pressure of each participant several times. Additionally, we will perform clinical assessments and check for symptoms and signs possibly related to an allergic reaction or infection caused by the isotope infusion. Participants will be monitored individually for signs (e.g., heart rate, temperature, fatigue, body ache, nausea, vomiting, headache, hives/rash, flushing, sweating, chills, hypo/hypertension, and hyperthermia) to indicate distress during the study day. Depending on the seriousness of any adverse reactions, the infusion study will be terminated and follow-up care provided by nurse and/or study physician. Expected adverse events due to the amount of fluid administered over this time period is fluid retention, which may cause symptoms such as general edema and increased blood pressure. These effects will be monitored carefully and the study will be stopped if necessary.

- In the event of an emergency during the test days proper emergency response protocols (calling 9-911 for serious injury or a medical emergency, calling Biosafety/EHS for cleanup assistance or spill team response, calling UPD for incidents in public areas, retrieving AED located in the lab, performing CPR or other First Aid techniques, etc.) will be followed depending on the severity of the emergency.
    - HCRB equipment (AED, DXA, eye washes) are monitored for expiration and safety checks on a monthly basis.
- As part of the safety plan for this study, the PI and/or study coordinator will review individual participant 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. Participant records include (but are not limited to) consent forms, CRF's, data flow sheets, laboratory specimen

records, adverse event logs and medical charts. An explanation for the omission of any required data should appear as a note to the study record file referencing the appropriate CRF page or other data collection forms. Any change to the information entered on any page in the CRF or other study related forms will be made by deleting the error with a single strike through the incorrect entry, entering the correct entry, and documenting the person making the change by initializing.

- Personnel involved in this activity included:
    - Nicolaas Deutz, MD, PhD, >30 years experience in clinical trials with older adults and chronic disease including metabolism, nutrition, and exercise intervention/rehabilitation
    - Marielle Engelen, PhD, >25 years experience in clinical trials with older adults and chronic disease including metabolism, nutrition, and exercise intervention/rehabilitation
    - Kimberly Coyle, RN, >5 years experience as RN in medical surgery, recovery, education and outreach of patients upon discharge.
    - Laura Ruebush, PhD,
    - Gabe Neal, MD, Chief Clinical Officer, Texas A&M Health School of Medicine. He will be the direct contact person in case of study-induced adverse events
- All participants will be assigned a unique code corresponding with their enrollment in the study. This code will be used on all documents used to collect and enter data. The link to the code is maintained on secure server separate from the data.
- In the event of an emergency during the test days proper emergency response protocols (calling 9-911 for serious injury or a medical emergency, calling Biosafety/EHS for cleanup assistance or spill team response, calling UPD for incidents in public areas, retrieving AED located in the lab, performing CPR or other First Aid techniques, etc.) will be followed depending on the severity of the emergency.
- If an adverse event occurs, during or subsequent to the study, the nurse and PI will be notified immediately. In case of a serious adverse event also the study physician will be notified immediately. All research staff complete American Heart Association HeartSaver training containing skills for first aid, CPR, and AED.
- Research staff are trained and follow standard operating procedures for all clinical measurements, acute resistance exercise, and endurance exercises. This training provides normal range of various safety measurements as appropriate (e.g., blood pressure, O2 saturation, temperature VAS scale for fatigue and soreness). If measurements are outside normal range, staff immediately inform PI to assess potential participant safety and ability to continue protocol. All measurements and action are recorded in CRF. All staff are made aware of potential adverse events and safety measures enacted during weekly staff meetings.
- The frequency with which all available data and adverse events for completed participants will be reviewed based on actual accrual rates of participants (33% of enrollment needed for completion, or every 6 months, whichever comes first) by (co)PI and study coordinator/ key research staff to ensure safety of participants and integrity of data collected.
- Participants will be given contact numbers and told to contact the research staff if they perceive anything unusual. All adverse events will be recorded in the participant's record file and the (S)AE form of the CRF will be completed. The PI will ensure that the information, including onset, duration and nature of event, severity, and action taken, is captured.
- In case of a serious adverse event the study physician will also be involved in the evaluation and attribution on the risk/benefit ratio.

- The PI and nurse and if needed other research staff conducting the study will be responsible for evaluating each AE for relationship to the protocol and products, seriousness and expectedness and will determine attribution as well as the impact of the adverse event on the risk/benefit ratio, using the Grading and Attribution Scale as appropriate. Each event is graded according to the following 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. The PI and nurse and if needed other research staff conducting the study will judge whether or not an adverse event is:
    - 1) not related;
    - 2) possibly related;
    - 3) probably related;
    - 4) definitely related to the study interventions.

In case of a serious adverse event the study physician will also be involved in the evaluation and attribution on the risk/benefit ratio.

- The PI will report, based on HRP-029 Reportable New Information:
    - any serious adverse events that may be considered unanticipated problems to the IRB within 5 business days of the investigator becoming aware of the event. Unanticipated problems that result in a participant's death or are potentially life-threatening will be reported to the IRB as soon as possible, but no longer than 5 business days. The investigator will report an unanticipated problem to the IRB by completing and submitting Reportable New Information Form.
        - If participants are hospitalized, undergo additional treatments, or die as a result of disease progression, or other age-related conditions they may be excluded from the study after evaluation against study in/exclusion criteria.
        - The investigator is responsible for determining the actions that need to be taken to ensure participant safety. The investigator will submit any unapproved actions to the IRB and these actions may not be initiated without IRB review and approval except when necessary to eliminate apparent immediate hazards to the participant. The IRB may identify additional measures that need to be taken.
    - In case a protocol deviation happens which is a failure to follow the protocol due to the action or inaction of the investigator or research staff will be reported within 5 business days.

15.0 Withdrawal of Participants:

Participants are informed and reminded during all research activities that they are able to discontinue or omit procedures at any point without reason.

Participants who are unwilling or unable to complete a full study day will be withdrawn from scheduling the remainder of the visits. They will be compensated for any visits completed.

All completion information including date and reason for early exit is recorded in the REDCap project.

16.0 Risks to Participants:

- Demographic and Socioeconomic Questions: Responding to demographic and socioeconomic questions could cause you to feel uncomfortable or upset. Please tell the study staff if you feel uncomfortable or upset while answering the questions.
- Pain
- Bleeding
- Mild hematoma
- Bruising
- Fluid retention (edema, increased blood pressure) associated with stable tracer ingestion or any adverse reactions during the tracer ingestion that suggest allergic reaction or infection (fatigue, body ache, nausea, vomiting, headache, urticaria, flushing, sweating, chills, altered heart rate, hypo/hypertension, and hyperthermia)
- Anemia (increased need for transfusion)
- Infection associated with peripheral catheter placement repeated blood sampling from the catheter
- Vaso-vagal response associated with peripheral catheter placement
- Skin rash associated with an allergic reaction to tape or other medical disposables used for study purpose
- While using the temperature controlled box, in people with poor circulation or frail skin, scalding may occur after prolonged exposures in the temperature controlled box.
- Gastrointestinal problems like nausea, bloating, diarrhea and vomiting associated with the intake of the test meal(s)
- Health deterioration and hospitalization due to underlying conditions or treatment may be expected events between enrollment and completion of the study. Although the possibility is unlikely due to the strict in- and exclusion criteria and the type of intervention, death and readmission after hospital discharge might occur in these patients related to their underlying condition(s)
- Exposure to low levels of x-ray radiation during DXA scan

17.0 Potential Benefits to Participants:

There are no direct benefits to the study participants.

18.0 Vulnerable Populations:

n/a

19.0 Sharing of Results with Participants:

Results of body composition scan can be shared with participants upon their request. No other research related data will be shared directly with the participants.

20.0 Setting:

All research activities will take place in private rooms within the CTRAL-Clinical Research Unit (CRU). These spaces are only accessible to trained research staff and other enrolled participants who are accompanied at all times by research staff.

21.0 Resources Available:

The **CTRAL-CRU** consists of 12 overnight stay beds (4 private rooms, 4 semi-private rooms, one 4-bedroom), 4 private exam/procedure rooms, 1 room to measure resting energy expenditure, a nurse station, family waiting room, phlebotomy room, offices, and several storage rooms. Two of the private rooms have bariatric size beds. All rooms in the CTRAL-CRU are stocked with required equipment for safely collecting, processing, and disposing of biohazardous materials. Additionally, each bed has oxygen supply, nurse alarm system, emergency power, internet connection, direct point-to point communication possibility with nursing station, TV/DVD, recliner for (overnight) visitors, personal storage for subjects. CTRAL-CRU has 5 custom made hot-boxes to obtain arterialized-venous blood samplings, automatic and manual B/P Machines, pulse oxygen meter, CPR board, blood glucose glucometers (Accu-Check Aviva), as well as continuous glucose monitor system (Abbott Freestyle Pro), 3 manual wheelchairs and an electric wheelchair. Research nursing staff and study physician are responsible for the medical aspects of the outpatient part of the studies. CTRAL is at the Texas A&M campus and is readily accessible with easy parking for study subjects and staff. Wall mounted storage boxes for sample pick-up by commercial laboratories are also available.

A metabolic kitchen (commercial grade) is available 24/7 for food/test diet/food supplement and non-hazardous oral compounding (USP 795) preparations for human subjects, and has a controlled/monitored 4°C and -20°C storage. A clean room unit for clinical preparations is present, containing an IV compounding facility (USP 797 grade) e.g. non-hazardous stable isotope infusate compounding, a controlled/monitored 4°C and -20°C storage, and a sterilizer for clinical tools/instruments. This facility is also used to prepare aseptic syringes and infusion bags on the clinical test days. Additionally, an IV compounding facility is located on-site in the HCRB. Sterility and apyrogenicity of the prepared IV compounded infusates are tested by CTRAL according to guidelines of USP 797 and the standard operation protocol of the pharmacist. Experienced staff is available to ensure appropriate use of stable isotope technology in human subjects. All facilities within the Human Research Support Core are equipped to safely collect, process, and dispose of biohazardous materials. A laboratory is present to facilitate STAT blood/ tissue processing and is equipped with 2 biosafety cabinets, 2 centrifuges and a blood cell counter. A high-density secured data archive system is located in the secured office area. A secured/ controlled/ monitored sample storage/ biobank is available with a CO_2-backup system and emergency power for seven -80°C freezers with a capacity to store 250.000 samples. All human material is stored in secured area controlled via two swipe card entries, and monitored via closed-circuit video monitoring. Swipe access is granted to individuals after confirmation of all training requirements have been met. Temperatures of the freezers for storage are also monitored continuously and alerts are automatically sent if temperature fluctuates outside approved temperature range. Clinical waste management exists consisting of a decontamination room for reusable tools/instruments used in human subjects, a soil room and a waste autoclave to sterilize biohazardous clinical and laboratory waste. The Research Electronic Data Capturing (REDCap) system is used to collect and store all data associated with human subject protocols. REDCap was developed by Vanderbilt University and has become a well-received tool for managing clinical studies and their data collection. Automated reports can be used to monitor the data on an ongoing basis. Additional reports may be used to create variables of interest needed for analysis. Data collected via REDCap will be merged with data housed on encrypted, password protected CTRAL server for final analyses.

22.0 Prior Approvals:
Biohazards-- IBC2018-053, renewal 06/04/2024
Radiation-- #R1042 (DEXA performed by Kim Coyle or Sunday Simbo or Laura Ruebush)

23.0 Confidentiality:
All hard copy data will be stored in locked file cabinets in a secure area of the CTRAL offices. The Texas A&M REDCaps system will also be used to store data. Any data on a computer will be stored on a secure and encrypted server with access only to research staff appointed by the PI.

All documents pertaining to the conduct of the study will be kept on file by the investigator for a period of at least 7 years beyond completion of the study.

24.0 Provisions to Protect the Privacy Interests of Participants:
DESCRIBE CLINIC STAFFING AND INTERACTIONS DURING STUDY VISIST

25.0 Compensation for Research-Related Injury:
If a participant suffers any injury as a result of taking part in this study, nothing is arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment and professional services will be available to participants, just as they are to the community in general.

26.0 Economic Burden to Participants:
none

27.0 Recruitment Methods:

Recruitment will be conducted using the following methods:

- Notices to general public via flyers, email, advertisements, InfoGroup mass mailing, Facebook posts in CTRAL page's newsfeed
- The website of the research group will contain a page with information regarding participating in research projects. When interested, they can contact the PI or research staff by phone or email for further information and will subsequently be screened via direct or telephone contact, or websites (CTRAL and ClinicalTrials.gov)
- Participants who have previously participated in CTRAL studies and who have provided consent for re-contact may also be approached.
- Local support groups/organizations, who have agreed to assist with the recruitment of participants, will briefly mention the research study to their organization and hand out the flyer.
- Potential participants will be screened regarding in and exclusion criteria (height, weight, dob, relevant medical problems). Participants may also self-select to complete a pre-screening questionnaire (providing basic eligibility criteria and Contact information) via Qualtrics/REDCap.

Compensation will be provided as follows:

- $20 for screening
- $200/completed study day
- up to a total of $820

Compensation is provided in the form of a check in the total amount of all completed study activities. Checks will be prorated if they discontinue prior to completing all study days. At the end of each study visit, a free meal will also be provided.

28.0 Study-Wide Recruitment Methods:
n/a

29.0 Consent Process:
The PI or research staff will interface with the participant during a screening visit at their convenience, in private. During the screening visit the Informed Consent process will take place and participants are screened for eligibility. Before giving consent participants are given the time to thoroughly read the Informed Consent. The PI or research staff will conduct the consent process and will discuss appropriateness of the study with the participant. They are prepared to devote 30 minutes (longer if needed) to the discussion. The person obtaining consent will thoroughly explain (including, but not limited to) all study related procedures, outline risks and benefits, duration of the study and compensation. The information will be given in language understandable to the reader. Participation privacy will be maintained. All questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. No research related procedures will be performed prior to obtaining informed consent.

30.0 Process to Document Consent in Writing:
If the participant agrees to enroll as a study volunteer, they will be asked to sign the informed consent form. Signatures will be obtained in the presence of the PI or research staff. A copy of the signed and dated consent will be emailed to the participant via REDCap and stored electronically in the document repository. Participant screening and enrollment will be documented using a screening and enrollment log.

31.0 Drugs or Devices:
n/a

32.0 Waiver of IND or IDE
n/a

33.0 Multi-Site Research:
n/a