**Study Title:** Role of protein matrix in the anabolic response to a ground beef patty as opposed to the Impossible (vegi-) Burger

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**University of Arkansas for Medical Sciences (UAMS) Clinical Protocol**

**Study Title:** **Role of the protein matrix in the anabolic response to a ground beef patty as opposed to the Impossible (vegi-) Burger**

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| measure weight | x | | |
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| DEXA scan[1] | x | x | |
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| ingest study food | | | 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:

According to the USDA Economic Research Service Food Availability Data, beef consumption has been on a general decline for the past 40 years, with current per capita consumption approximately 35% lower in 2020 than in 1980. Some of this decline over the past few years can be attributed to politically motivated claims that carbon emissions from cattle represent a major threat to the global environment. However, the major reason for declining beef consumption is the widely promulgated claim that beef is unhealthy. The hamburger in particular has become a poster child for the notion that beef consumption is responsible for the increasing occurrence of obesity in the United States. The trend of decreasing beef consumption due to perceived health concerns, particularly obesity and associated health problems, has led major fast-food chains such as Burger King and McDonalds, to offer vegetarian burgers as healthy alternatives to the traditional beef burger. However, examination

of the components of the macronutrient matrix of a typical vegetarian burger gives reason to question the health benefits as compared to a traditional beef patty-hamburger.

### 1.2 Previous research on the topic:

One of the principal nutritional benefits of dietary protein in a food source is the stimulation of muscle protein turnover and the net gain of body protein (the anabolic response) (1).

**Table 1** shows key aspects of the macronutrient matrix of the Impossible Burger and an 80/20 percent beef patty, with particular emphasis on the protein/essential amino acid (EAA) content.

**Table 1.** Proposed Experimental Groups

| | 80/20 Beef Patty | Impossible Burger | 2 Impossible Burgers |
|---|---|---|---|
| Amount (oz) | 4 oz | 4 oz | 8 oz |
| Protein (g) | **19 g** | **19 g** | 38 g |
| EAAs (g) | ***8.9 g*** | 4.2 g | ***8.4 g*** |
| Calories | 287 | 240 | 480 |
| Calories / g EAA | 32 | 57 | 57 |

The Impossible Burger was chosen as a representative vegetarian burger because soy, which is the highest quality plant-based protein, is the principal protein source (2). Four-ounce servings of a beef patty and of an Impossible Burger each provide 19 g of protein. However, a closer inspection of the macronutrient matrix of the two food sources reveals that the 4 oz beef patty contains more than twice the amount of essential amino acids (EAAs) as compared to a 4 oz Impossible Burger. We recently completed an NCBA-supported study in which the anabolic responses to seven different “ounce equivalents” of protein food sources, as described by the USDA My Plate, were quantified using stable isotope tracers. Rather than being metabolically equivalent, as implied by their designation by My Plate as “ounce equivalent” protein food sources, we found that there was a linear relation between the net gain in body protein and the EAA content of the protein food source (**Figure 1A**) (3). Moreover, the slope of the relation between EAA content and increase in net protein balance was approximately 1, meaning that any difference in EAA content directly corresponded to an equivalent difference in net body protein gain (**Figure 1B**).

These results support the notion that the EAA content of a protein food source is principally responsible for the magnitude of the anabolic response. This fundamental concept is the basis for the scoring of protein quality by the Digestible Indispensable Amino Acid Score (DIAAS) (4). With relevance to the comparison of a beef patty and an Impossible Burger, we found that consumption of 2 ounces of beef resulted in an anabolic response that was approximately 4 g net gain of protein greater than the response to consumption of 2 ounces tofu (Figure 1) (3). The difference in anabolic responses corresponds to the difference in the EAA content of the two protein food sources (Figure 1A). Muscle protein fractional synthesis rate was also greater in those consuming beef as opposed to tofu. Further, compilation of our in-house data set across various populations and food sources indicates that the EAA content of a protein food source explains 80.4% of the variance in the total amount of body protein gained (5). This

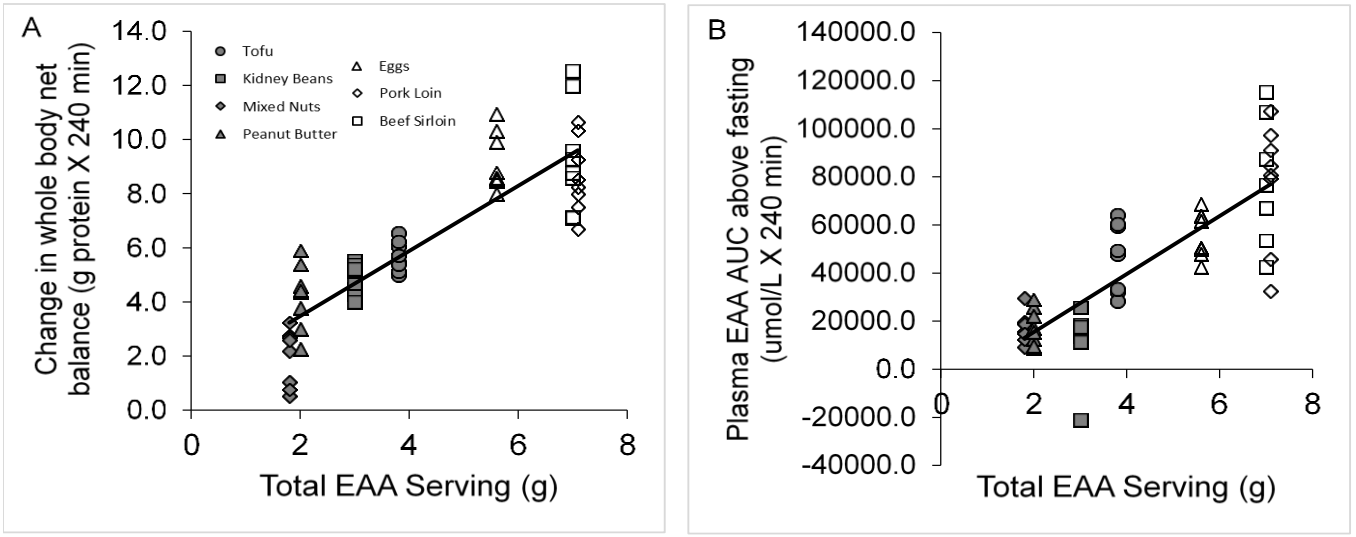


**Figure 1.** Relation between EAA serving and net gain in body protein of a variety of protein food sources.

large dataset has allowed us to develop a predictive equation that indicates the total amount of body protein gained will be 7.6 g from 4.2 g of EAA (the Impossible Burger) and 16.5 g from 8.9 g of EAA (beef patty). Stated differently, the greater EAA content of a 4 oz beef patty is predicted to elicit more than double the anabolic response to the same amount of an Impossible Burger.

Plant-based burgers such as the Impossible Burger are being marketed as a one-for-one replacement for beef-based burgers, but the disregard for protein quality is misleading and creates a false perception of equivalent nutritional benefit of the two protein food sources. In

addition to the greater EAA content per gram protein, the profile of EAAs in beef protein more closely reflects the dietary requirements for individual EAAs, as reflected by a DIAAS approximately 40% greater than soy protein. In addition, the overall macronutrient matrix of a protein food source can potentially influence the anabolic response by affecting the temporal EAA response. Both the peak concentration of EAAs after consumption as well as the time to reach the peak concentration affect the anabolic response (5). The Impossible Burger contains various binders and starches that could potentially interfere with the absorption of the EAA from the protein matrix, with a resultant slowing of the rate of absorption and blunting of the maximal concentrations of EAAs in the blood and the speed at which the peak concentrations are reached. These factors likely have a corresponding negative impact on the magnitude of the anabolic response. Since no such issues impede the digestibility of beef protein, it is reasonable to propose that the plasma EAA levels will reach higher peak EAA concentrations, and achieve the peak concentrations more rapidly, than ingestion of the same amount of EAAs in the matrix of the Impossible Burger. As a result of both the advantageous profile of EAAs in beef protein and the more rapid absorption of EAAs, we propose that a beef patty will not only provide a greater anabolic response than an iso-nitrogenous amount of Impossible Burger, but that also consumption of 4 oz of beef patty will also elicit a greater anabolic response than consumption of two Impossible Burgers.

The caloric value of the Impossible Burger and a beef patty is also relevant when considering the relative nutritional value and nutrient density of the two protein food sources. The caloric value of the Impossible Burger is almost twice that of a beef patty when normalized for the amount of EAAs (Table 1). We therefore anticipate that differences in anabolic responses between a beef patty and Impossible Burger will be amplified when normalized by the caloric values of the two protein food sources.

## 2.0 Hypotheses

1. Consumption of a 4 oz beef patty will result in a greater anabolic response than consumption of a 4 oz Impossible Burger, including greater increases in plasma EAA

concentrations, a greater balance between whole body protein synthesis and breakdown, and a greater increase in muscle protein fractional synthetic rate (FSR).
2. Consumption of a 4 oz beef patty will result in a greater anabolic response than consumption of two 4 oz Impossible Burgers, including greater increases in plasma EAA concentrations, greater balance between whole body protein synthesis and breakdown, and greater increase in muscle protein FSR.

## 3.0 Study Design and Procedures

We will enroll up to 28 subjects with a goal to study up to 24 healthy male and females. We will use a 10-hour period to measure the protein metabolism in response to the study food (one beef patty, one vegi-burger, or two vegi-burgers).

### 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 questionnaire including allergies and list of current medications will be completed. Females of child bearing capacity will be asked to provide a urine sample for pregnancy testing. About 4mL of venous blood will be drawn to send for complete blood count for study criteria. Blood and 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),
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.
Run-in meals will be discussed with subjects to elicit food allergies or intolerances.

Visit 2: Subjects return to the UAMS TRI. If they will undergo the DEXA and InBody scans, the must have 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, DEXA, ultrasound scans will be performed if these were not done at visit 1. Two days' of run-in meals will be dispensed with instructions on their preparation. Meals will be consumed for the two days immediately preceding Visit 3.

Visit 3: Subjects return to the UAMS TRI having fasted overnight. Study staff will ask about adverse events since their last visit. Vital signs (heartrate, blood pressure and oral temperature) will be measured. 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. A second IV catheter will be placed into a vein on of the subject's other arm to use for stable isotope infusion.

After an initial blood sample is obtained, the priming dose of isotopes are given IV push, the constant infusions are begun, and a timer is started. Blood and muscle samples are obtained according to the schedule below.

The study food is served after the 4:00 blood/muscle sample are obtained. Subjects are asked to consume the study food within 10 minutes.

At the conclusion of 10 hours elapsed time, the IV catheters are removed and the sites dressed with a bandage. Vital signs (heartrate, blood pressure and oral temperature) will be measured. 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) |
| 120 | Blood sample (~4mL into an EDTA tube), ingest study supplement, muscle sample from vastus lateralis |
| 150 | Blood sample (~4mL into an EDTA tube) |
| 180 | Blood sample (~4mL into an EDTA tube) |

| | |
|---|---|
| 210 | Blood sample (~4mL into an EDTA tube) |
| 240 | Blood sample (~4mL into an EDTA tube), muscle sample from vastus lateralis serve study food |
| 270 | Blood sample (~4mL into an EDTA tube) |
| 300 | Blood sample (~4mL into an EDTA tube) |
| 330 | Blood sample (~4mL into an EDTA tube) |
| 360 | Blood sample (~4mL into an EDTA tube) |
| 390 | Blood sample (~4mL into an EDTA tube) |
| 420 | Blood sample (~4mL into an EDTA tube) |
| 450 | Blood sample (~4mL into an EDTA tube) |
| 480 | Blood sample (~4mL into an EDTA tube) |
| 510 | Blood sample (~4mL into an EDTA tube) |
| 540 | Blood sample (~4mL into an EDTA tube) |
| 570 | Blood sample (~4mL into an EDTA tube) |
| 600 | Blood sample (~4mL into an EDTA tube), muscle sample from vastus lateralis |

### 3.2 Subject Compensation

Subjects will accrue compensation according to the below table. They will be handed a $20 gift card at the end of visit 1. They will accrue $20 for visit 2 and up to $300 for visit 3. A check will be mailed via UAMS SAP for their accrued compensation approximately 3-4 weeks after their participation ceases (whether completed or not). If they were to fully attend every visit, their total amount would be $340. If they stop participating prior to the completion of study visit 3, they will receive prorated pay of $25 per hour.

| Visit | Amount |
|---|---|
| 1 | $20 |
| 2 | $20 |

| | |
|---|---|
| 3 | $300 |

### 3.3 Recruitment

Potential subjects will be recruited from the Little Rock and central Arkansas areas by use of UAMSHealth social media, word of mouth, the ARResearch.org registry, and contacting prior subjects who indicated they wanted to be contacted about future studies. UAMS students and employees will not be targeted for participation but will not be refused. Study staff will ask enrolled subjects to recruit others if they are comfortable doing so, and study staff will seek subjects within their social circles. Individuals interested in the study will be scheduled for Visit 1.

**Facebook Script:**

The Center for Translational Research in Aging and Longevity at UAMS is recruiting healthy individuals ages 18-40 for a study about measuring how blood and muscle proteins respond to either a beef patty or vegi-burger(s).

Participants will be compensated for their time. If interested, please contact us:
Email sseale@uams.edu or call 501-526-5734 for more information.

**Twitter Script:**

Our research team is recruiting individuals ages 18-40 for a study about how blood and muscle proteins respond to a beef patty vs vegi-burger.
Participants will be compensated! Contact us for more info: email sseale@uams.edu, or call 501-526-5734

During the initial conversation (in person or via phone), the following items will be discussed in non-technical language:

- this study is completely voluntary.
- the goal of the study: to see if beef or vege-burgers are more efficient at making proteins in our body.
- the number of visits to UAMS study site and their duration: 2 visits that are less than an hour, and 1 visit that lasts a bit over 10 hours.
- study procedures: must have 2 IV catheters placed and undergo 3 muscle biopsies from one incision on their outer thigh, eat our food for almost 3 full days, fast overnight twice,

have an x-ray for body composition, must get an infusion of stable isotopes (and define what they are).

- risks of procedures: pain, bruising, bleeding, infection, possible fever/chills, discomfort from fasting, small amount of radiation from x-ray, amount of blood taken (approx.. 5 tablespoons), skin numbness and scar formation from biopsy incision.
- compensation for time and travel: up to $340 if all 3 visits are completed.

ARResearch.org:

Using the age criteria, study staff will setup the study in the registry. The registry gatekeeper will send the announcement to a predetermined number of registrants (as determined by study staff, e.g. 250 for the initial volley). The submitted language will be emailed to the filtered registrants by the registry's software. Interested registrants (respondents) will click a button to either allow the study staff to contact them via their preferred means or decline to be contacted. If/when a respondent replies to study staff's contact, a brief discussion of the study overview will take place. Respondents who are still interested will schedule visit 1 with study staff. Recruitment metrics will be updated on the registry's website through completion of enrollment.

### 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.

With explicit permission via the consent form, muscle and plasma samples will be kept indefinitely for future approved uses. No samples will be released to other investigators.

### 3.6 Randomization

Subjects will be stratified by sex and then randomized equally to one of three groups (one beef patty, one Impossible Burger, or two Impossible Burgers).

### 3.7 Muscle Biopsy Procedure

At Visit 3, qualified and credentialed study staff (M.D.) will perform three separate vastus lateralis muscle biopsies of the subject's preferred outer thigh using 1% plain lidocaine for anesthesia. One incision will be used for all 3 biopsies. Approximately 100mg of muscle tissue per biopsy will be obtained using a Bergstrom needle. The tissue will be rinsed with normal saline and flash frozen using liquid nitrogen. The sample will be transferred into a labelled cryo tube and frozen at < -60 degrees C in the PI's lab. Processing and analyses will take place in batches according to the procedures mentioned in 7.3 below.
the incision will be closed with a medical glue, then dressed using a transparent adhesive bandage followed by a 6" wide elastic wrap that will protect the site from incidental contact and assist with hemostasis. Subjects are provided verbal and written instructions for care of the biopsy site, along with the phone number of the study nurse should they have any questions or concerns once leaving the study site.

## 4.0 Study Population

Up to 28 subjects will be enrolled in order to meet the goal of 8 completers in each of the three groups. Attempts will be made to balance sexes within groups.

### 4.1 Inclusion Criteria

- Ages 18 – 40 yrs.
- Body Mass Index of 20-32 inclusive.

### 4.2 Exclusion Criteria

- Unwilling to eat animal proteins
- History of diabetes that requires medication for control of blood glucose

- 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
- Hemoglobin less than 12 g/dL at screening
- Platelets less than 150,000 at screening
- Subjects who cannot refrain from using protein or amino acid supplements for 7 days prior to Visit 3
- 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, stable isotope enrichment, glucose, and insulin. The total amount of blood taken will be approximately 72 mL (~5 tablespoons). Subjects should have no noticeable effects from this volume.

### 5.2 DEXA, In Body, ultrasound 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 and ultrasound scans do not pose any risks.

### 5.3 Study foods:

The run-in meals are comprised of frozen, ready-to-eat portions that are commercially available at most grocery stores (e.g. Stouffers). The 'test' foods are:

- one 4 ounce (cooked weight) patty of 80/20 beef (from Kroger e.g.)
- one 4 ounce Impossible Burger (https://impossiblefoods.com/products/burger)
- two 4 ounce Impossible Burgers

The risk of eating any of these foods is that of a food allergy. Subjects will be shown the list of foods and asked if any of them are intolerable.

### 5.4 Stable Isotope Infusion:

The primary risks of infusion of the stable isotopes are fever, chills, infection (from contamination). A 0.2 micron in-line filter will be used during the infusion. The isotopes will be purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA) and sent to the Center for Translational Research in Aging and Longevity at Texas A&M University (College Station, TX) for contractual compounding. Finished products will be shipped to the UAMS Research pharmacist who will store and dispense the isotopes to study staff as needed. Isotopes will be stored in glass vials and frozen until use. Two solutions will be produced: a mixture of L-[ring-$^{2}H_{5}$] phenylalanine + U-13C9-15N tyrosine (for constant infusion IVPB), and a mixture of L-[ring-$^{2}H_{5}$] phenylalanine + U-13C9-15N tyrosine + L-[ring-$^{2}H_{4}$] tyrosine (for priming doses IVP).

Isotope rates of infusion:

- L-[ring-$^{2}H_{5}$] phenylalanine: priming dose: 3.6µmol/kg. Constant infusion: 3.6 µmol/kg/hour.
- U-$^{13}C_{9}$-$^{15}$N-tyrosine: priming dose: 0.113µmol/kg. Constant infusion: 0.113µmol/kg/hour.
- L-[ring-$^{2}H_{4}$] tyrosine: priming dose: 0.31µmol/kg.

| IVP vials contain: | IVPB vials contain: |
|---|---|
| L-[ring-$^{2}H_{5}$] phenylalanine | L-[ring-$^{2}H_{5}$] phenylalanine |
| U-$^{13}C_{9}$-$^{15}$N-tyrosine | U-$^{13}C_{9}$-$^{15}$N-tyrosine |
| L-[ring-$^{2}H_{4}$] tyrosine | [redacted] |

**5.5 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.

**5.6 Intravenous Catheter insertion:**

The risks of inserting an IV catheter are pain, bruising, bleeding, and infection.

**5.7 Muscle Biopsy:**

The risks of the muscle biopsy procedure include pain, bleeding, bruising, infection, residual numbness from the incision, scar formation, soreness lasting >48 hours, and hematoma.

**5.8 Covid-19 Related risks:**

Subjects will be COVID-19 negative and/or asymptomatic. Subjects will undergo the current screening procedures in place for UAMS patients at the time of their visits. Visitors will not be permitted unless UAMS policy changes permit them. Subjects will be required to wear a suitable facemask for the entirety of their study visits except when ingesting their meals. Study staff will wear their mandated masks and use universal blood-borne precautions while handing blood samples. Study visit areas are disinfected after study visits as per protocol.

**5.9 Safety Monitoring Plan**

Study staff will monitor for adverse events and protocol deviations though the end of the study. The PI and study physician (if necessary) will be notified of adverse events shortly after their discovery. If a subject has an adverse event while on site, study staff will help get the care the subject needs. This may include first aid, emergency care, and/or follow-up care.
Adverse events and protocol deviations will be recorded on an Excel spreadsheet that is filed with the IRB at specific intervals. Any “unexpected problems involving risks to subjects or others” (UPIRTSO) will be reported to the IRB within 24 hours of discovery.

## 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

The post-prandial change in net protein balance (i.e., protein synthesis minus protein breakdown) relative to the baseline in each group will be the primary outcome. Responses of the rates of protein synthesis and breakdown will be secondary outcomes. Additional secondary endpoints will be muscle protein synthesis, plasma EAA and insulin area under the curve above baseline values, and protein kinetics normalized by caloric and EAA intake. Correlation analysis of the relation between protein kinetics and the EAA content of the protein food source will be additional secondary endpoints.

One-way ANCOVA will be used to compare differences in protein kinetics (FSR, NB, PS, and PB) and protein kinetics normalized by caloric and EAA intake. Plasma EAAs and insulin area

under the curve above fasting valueswill be assess by a one-way ANOVA. All significant main effects of group will be followed with Bonferroni-adjusted pairwise comparisons. Partial Pearson's correlation coefficients controlling for protein source (beef patty or Impossible Burger) will be used for the correlation analysis. Statistical significance will be accepted at $p < 0.05$.

### 7.2 Sample Size Calculation & Power Analysis

A group size of 8 per group was a priori determined based upon an ANCOVA model to compare the protein sources and amounts with respect to mean response of net protein synthesis after adjusting for baseline measures. With this sample size, the ANCOVA model has 80% power to detect effect sizes of $f = 0.484$ or larger. This estimate assumes that the baseline covariate explains 50% of the variation in the response. A 5% α-level will be used to determine statistical significance.

### 7.3 Sample Processing and Analyses

Calculation of protein kinetics: The calculation of whole-body protein kinetics (protein synthesis, protein breakdown, and net protein balance) is based on the determination of the rate of appearance (Ra) of phenylalanine (Phe) and of tyrosine (Tyr) into plasma and the fractional Ra of endogenous tyrosine resulting from phenylalanine hydroxylation. A two-pool model has been previously described and discussed in detail (6). Briefly, an isotopic steady state will be established in the baseline/fasted period, and protein kinetics are calculated accordingly (6). For the 6 hours after ingestion of the patty, the area under the curve (AUC) of plasma enrichments of phenylalanine and tyrosine tracers will be calculated. Ra of Phe reflects protein breakdown in the fasted state; the total appearance of Phe over the 6 post-prandial state reflects both protein breakdown and the appearance of protein from the ingested meal. The appearance of exogenous Phe in the peripheral circulation must be subtracted from total appearance of Phe to determine the rate of endogenous protein breakdown. The total appearance of exogenous Phe in peripheral blood is estimated from the amount of Phe in the dietary protein and the amount of protein consumed, the published value for the true ileal digestibility of Phe in the test protein, and the measured fraction of absorbed Phe hydroxylated

to tyrosine (6). We have recently discussed in detail the validity of assumptions underlying this model of protein kinetics (6). Most importantly, this model does not introduce any systematic errors that might affect the comparison of the anabolic responses to the burgers (6). The rate of muscle protein synthesis will be calculated by dividing the increase in tracer enrichment in muscle protein over time by the precursor enrichment, taken to be the intracellular free phenylalanine enrichment.

Analytic methods: Plasma samples will be processed as previously described for determination of enrichment by gas chromatography-mass spectrometry (7). Plasma amino acid concentrations will be determined by liquid chromatography-mass spectrometry using the internal standard method as described previously (3). Plasma insulin concentrations will be measured by commercially available human insulin ELISA kit (Alpco Diagnostics, Salem, MA). After all data has been analyzed and verified, samples for which permission was not granted for indefinite retention will be discarded into a biohazard trash bag and disposed in accordance with UAMS biohazardous waste policy.

## 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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