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

**Study Title:** **Whole Body Protein Metabolism of a Whey/EAA Supplement Using Continuous Oral Stable Isotopes**

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Table of Contents

## List of Abbreviations

| | |
|---|---|
| CRF | Case Report Form |
| CTRAL | Center for Translational Research in Aging and Longevity |
| DXA | Dual-energy X-ray Absorptiometry |
| EAA | Essential Amino Acid |
| EDTA | Ethylenediaminetetraacetic acid (a blood preservative) |
| FDA | Food and Drug Administration |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| GRAS | Generally Regarded as Safe |
| RIOA | Reynolds Institute on Aging (UAMS) |
| IRB | Institutional Review Board |
| LC-MS | Liquid chromatography-mass spectrometry |
| LOA | Limits of agreement |
| NB | Net balance |
| PB | Protein breakdown |
| PI | Principal Investigator |
| PS | Protein synthesis |
| SP | study product or placebo |
| TE | Total error |
| SEE | Standard error of the estimate |
| UAMS | University of Arkansas for Medical Sciences |
| IV | Intravenous catheter |

## Study Schema

Study staff will recruit subjects via word of mouth to friends, family and colleagues.

Informed consent process takes place during Visit 1, followed by DXA scan.

Randomized subjects undergo two separate 7-hour study visits.

Visits 2 and 3 entail serial blood sampling from an IV catheter, ingestion of stable isotopes of amino acids every 10 minutes, and ingestion of SP (study product or placebo).

Blood samples analyzed at completion of study visits.

**Study Calendar**

| Procedure | Visit 1 | Visit 2 | Visit 3 |
|---|---|---|---|
| Informed consent | x | | |
| Fasted prior to visit | | x | x |
| Blood sampling | | x | x |
| IV | | x | x |
| Ingest stable isotopes | | x | x |
| Ingest SP | | x | x |

## 1.0 Protocol Summary

This will be a double-blinded, randomized, crossover pilot study to evaluate the validity of a continuous oral sip-feeding of stable isotope tracer method for the evaluation of whole-body protein metabolism. This method will be evaluated using two different amounts of an essential amino acid (EAA) + whey protein supplement on up to ten younger, healthy subjects. Stable isotope preparations will be continuously consumed by subjects throughout visits 2 and 3 to enable whole body protein metabolism to be measured. A DXA scan will be performed to quantify subject's lean mass.

## 2.0 Background

Stable isotope tracers are routinely used to study protein metabolism in free-living individuals in the fields of nutrition and exercise (1, 2). Whole-body rates of protein synthesis (PS), breakdown (PB), and net balance (NB) in response to nutrition and exercise interventions are commonly evaluated using a primed-constant infusion of essential amino acid (EAA) tracers, typically $^{2}H_{5}$-phenylalanine and $^{2}H_{4}$-tyrosine (1). This method has many advantages. The primed-constant infusion allows for determination of basal protein kinetics, in addition to the acute physiological response to an intervention, which allows for each subject to serve as their own control. Although an effective method, administration of the primed-constant infusion requires involvement from multiple trained individuals, including research pharmacists and nurses who compound and administer the EAA tracer. This limits the ability to apply these methods in field-type settings outside of a hospital or lab. The cost associated with purchase and

compounding of the tracer, in addition to involvement from multiple specialists, makes these types of studies very expensive and difficult to conduct.

An alternative to primed-constant infusion is to orally ingest amino acid tracers in sip fashion to achieve a similar plateau enrichment. Oral bolus ingestion of amino acid tracer has been used to quantify 24-hour whole-body protein metabolism in a variety of healthy and clinical populations for over 50 years (3). This method is one of the most conducive field-methods for determining whole-body protein metabolism in free-living subjects and has minimal participant burden. However, full integration of the tracer from a single bolus ingestion into the entire protein pool takes at least 10 hours. This limits the ability to quantify acute changes in protein kinetics of acute exercise or nutritional interventions. However, ingestion of a priming bolus, followed by continuous ingestion of EAA tracer may achieve steady-state equilibrium similar to constant infusion of the isotope. Thus, oral ingestion may enable the determination of acute changes in protein kinetics in the same respect as constant infusion. If shown to be valid, this method would significantly reduce the rigor and expense of pharmacy-prepared infusions, and more importantly, allow for quantification of acute physiological protein responses within the field setting.

## 3.0 Specific Aims

SA1: To evaluate the validity of a continuous oral sip-feeding method of stable isotope tracer for the evaluation of whole-body protein metabolism in response to two different doses of an EAA/whey protein supplement (Low=6.3g and High=12.6g). We will compare kinetic responses with oral isotope ingestion to those previously determined by constant infusion with the same SP (4).

## 4.0 Study Population

Up to 10 subjects of any gender or ethnicity will be enrolled with a target of n=10, 50% female for study completion. Subjects' duration of participation is approximately 2 weeks.

### 4.1 Inclusion Criteria

1. Aged 21-40 years.
2. Body weight of ≤ 80kg (176 pounds).

### 4.2 Exclusion Criteria

1. History Chronic kidney disease.
2. History of dairy allergy.
3. History of lactose intolerance.
4. History of whey allergy.
5. History of gastric reduction/bypass surgery.
6. Pregnant females.

### 4.3 Subject Recruitment and Consent

Study staff will recruit subjects via word of mouth to friends, family and colleagues. Those who are interested will schedule Visit 1 with study staff, where the informed consent discussion and determination of eligibility will take place. Those who are eligible will have Visit 2 scheduled.

### 4.4 Subject Compensation

Subjects will be compensated $15 for Visit 1 and $100 each for Visits 2 and 3. They will be mailed a check through UAMS SAP for their amount based upon visits attended.

### 4.5 Study Visits

Visit 1: This visit will take place at the UAMS RIOA.

At this visit, informed consent discussion is held. Study staff will use appropriate PPE with surgical mask and gloves. Subjects will also be required to wear a mask and if they do not have one, a mask will be provided to them by the study staff. If a subject enrolls, their eligibility will be determined. Eligible subjects will be randomized and be scheduled for Visit 2. A whole body DXA scan will be performed by study staff using the PI's machine for quantification of lean mass.

Visit 2: This visit will take place at the UAMS IOA. Subjects will be instructed to fast (consuming only water) from 10:00 P.M. the evening prior to this visit. Study nurse will insert an IV into the subject's preferred arm. At scheduled intervals, study staff will draw a ~2mL blood sample from the IV for research purposes, warming the arm by means of a heating pad or a heated plastic box. Blood will be transferred into a blood collection tube that contains EDTA, cooled on wet ice, centrifuged at ≥ 3000 RPM for ≥ 6 minutes, and have the resultant plasma transferred to a labelled cryo tube for freezing at ≤ -80 degrees Centigrade.

After the initial blood sample is drawn, subjects will begin ingesting their oral isotope preparations, beginning with a priming dose (per study-specific stable isotope calculations). A timer will start when the priming dose is ingested. At 10-minute intervals, subjects will ingest a smaller volume of stable isotope preparations that will continue throughout the duration of the study visit (approximately 7 hours).

Approximately three hours after study initiations, subjects will ingest their randomly chosen amount of SP. Blood sample collection will continue at specific intervals throughout the remainder of Visit 2.

At the conclusion of the 7-hour study visit, the IV will be removed and a bandage placed over the site. Subjects will be provided with snacks and a beverage and be free to leave the site. Visit 3 is scheduled.

Visit 3: Subjects will come back to the RIOA having fasted overnight as for Visit 2. All study procedures undertaken during Visit 2 will be repeated at Visit 3, with subjects ingesting the remaining SP amount.

### 4.6 Blinding

This will be a double-blinded study. Both SP amounts are dissolved into equal volumes of water so that subjects will not know which amount they are ingesting. Blood samples are coded so that lab staff and investigators will not know which amount of SP was consumed at each visit. Investigators will be unblinded after all samples have been processed and data analysis has taken place.

### 4.7 Randomization

SP grouping will be predetermined before the first enrollment takes place. Study staff will document enrollment information into a spreadsheet that contains the SP group designations. SP labels will contain these designations. In the event that subject dropouts or screening failures skew the balance of groups, the groups will be modified toward the end of the enrollment period so that balance will be regained at the conclusion of the study.

### 4.8 Study Products

SP arrives packaged in a small tub. It is stored at room temperature. Study staff will weigh the doses of SP: 6.3g and 12.6g. Prior to ingestion, SP is dissolved into approximately 120mL of cool water. This product has been approved and used in previously approved studies (IRB#217658). It is provided at no cost by Adesso, LLC (Delaware).

## 5.0 Data and Specimen Collection and Management

### 5.1 Blood Sample collection

Study staff will collect approximately 2mL of whole venous blood at each occasion and place it into an EDTA tube. A total of 15 blood samples per study visit will be drawn for this study. Approximately 60 mL of blood will be drawn per subject if they complete both visits 2 and 3.

### 5.2 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 PI's laboratory, located in a restricted area inside the UAMS RIOA building. Said freezers are monitored continuously for proper temperature and working condition. Samples will be destroyed only after all data has been analyzed. All blood samples are coded using a unique identifier. None of a subject's personal identifiers are present on any biological sample.

### 5.3 Sample Analysis

Plasma samples are analyzed for amino acid concentrations and stable isotope levels (enrichment). The analyses are performed in the CTRAL by study staff.

### 5.4 Data Analysis & Statistics

Separate independent sample t-tests will be used to evaluate the difference in average whole-body protein metabolism measured using stable isotope infusion versus oral sip-feeding method for both the low- and high-dose supplement. To assess validity of the oral method compared to the infusion method, total error (TE), standard error of the estimate (SEE), and Pearson's correlation coefficient, in addition to mean difference and 95% limits of agreement (LOA), will also be evaluated for PS, PB, and NB. Data using the infusion method was collected on a previous cohort of participants of similar demographics (n=8; 60% female; Age=21.4 ± 0.5 years; Weight=73.8 ± 4.8 kg; percent body fat=21.1 ± 2.2%) using the same supplement product and dosing (4).

### 5.5 Dissemination of Data

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

### 5.6 Data Handling and Recordkeeping

Source documents and paper CRFs will be stored in a secure area of the PI's laboratory. Access will be limited to study personnel. When eventually destroyed, paper documents will be shredded per UAMS disposal guidelines. At no time shall Protected Health Information be released to non-study personnel. Coded data (results from blood sample analyses) will be stored in an Excel file kept on the UAMS/RSCOA server (requires secure access). These data files do not contain PHI or identifiers; the code to subjects is also kept in an Excel file on the UAMS/RSCOA server. These files will be kept indefinitely.

### 5.7 Data Access

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. 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 seven years after data analysis is completed.

## 6.0 Risks and Benefits

There are no guaranteed benefits for the subjects. Anticipated risks associated with this protocol are described in detail below. All experimental procedures will be performed by appropriately trained and credentialed personnel. The PI and/or study physician will be responsible for oversight of study procedures and evaluation of adverse events.

### 6.1 COVID-19 Protections

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 isotopes/SP. 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.

### 6.2 Blood sampling/IV insertion

Blood samples will be collected solely for the purpose of experimentation. The total amount of blood taken will be up to 60 mL. Subjects should have no noticeable effects of this volume of sampling. Brief discomfort due to the IV insertion is common. There is also a risk of possible bruising, redness, and swelling at the IV site. There is also a possibility of feeling lightheaded when the IV is inserted or, less commonly, fainting. Finally, there is the rare risk of infection due to the IV. The subject's heated arm will be monitored for signs of skin injury. Subject's arm will be inside a pillowcase when using the hot plastic box method, with its thermostat set no higher than 55 degrees Centigrade. Heating pad will be used on the highest setting available.

### 6.3 Confidentiality

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

### 6.4 Study Products

The SP contains whey protein and may contain a small amount of lactose. Subjects with a known allergy or intolerance to whey protein, milk or lactose should not participate. In subjects with these allergies/intolerances, side or adverse effects of SP would include: abdominal pain, bloating, rash or hives, red and watery eyes, a runny nose, sneezing and coughing, diarrhea, nausea, and intestinal cramps.

The amino acids are naturally occurring required nutrients with no known adverse effects, even at much higher doses than will be used in this protocol. They are classified

as GRAS by the FDA. Study staff are responsible for accountability and dispensing of SP.

### 6.5 Adverse Events

- Study staff will ask subjects about the occurrence of adverse events during visits 2, 3, and the follow-up calls after these visits.
- Possible side effects of the SP listed above will be documented as an adverse event. Any allergic reaction to the SP will be treated as an adverse event.
- The PI and study physician are responsible for reviewing and evaluating adverse events.
- Adverse events will be recorded in the CRF and on an excel spreadsheet that is submitted to the IRB at required intervals.
- Serious adverse events will be reported to the IRB within 24 hours of their discovery.

### 6.6 DXA Scan

A whole body DXA scan will be performed one time per subject. The radiation exposure for a single scan is approximately half that of a chest x-ray.

## 7.0 Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences (UAMS) 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 given to the participant, and the informed consent process will be documented in each subject's research record.

## 8.0 References

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3. Waterlow, J.C., Garlick, P.J., and Millward, D.J. 1978. *Protein turnover in mammalian tissues and in the whole body*. Amsterdam ; New York New York: North-Holland Pub. Co.; sole distributors for the U.S.A. and Canada, Elsevier North-Holland. viii, 804 p. pp.
4. Park, S., Church, D.D., Azhar, G., Schutzler, S.E., Ferrando, A.A., and Wolfe, R.R. 2020. Anabolic response to essential amino acid plus whey protein composition is greater than whey protein alone in young healthy adults. *J Int Soc Sports Nutr* 17:9.