University of Arkansas for Medical Sciences (UAMS) Clinical Protocol

Study Title: Effect of an essential amino acid/protein composition on protein metabolism.

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Principal Investigator: Arny A. Ferrando, Ph.D.
University of Arkansas for Medical Sciences
4301 West Markham St., Slot #806
Little Rock, AR 72205
Office Telephone: 501-526-5711
Email: aferrando@uams.edu

Sub-Investigators: Gohar Azhar, M.D.
Sanghee Park, Ph.D.
University of Arkansas for Medical Sciences
4301 West Markham St.
Little Rock, AR 72205

Study location: Donald W. Reynolds Institute on Aging @ UAMS
629 Jack Stephens Drive
Little Rock, AR 72205

Support (Funding): Adesso, LLC.

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Physical exam	x[2]		
vital signs	x[2]	x	x
DEXA scan	x[3]	x[3]	
stable isotope study		x	x
periodic blood sampling		x	x
muscle biopsies		x	x
ingest study product		x	x
follow-up call		x	x

*Only for subjects who have enrolled into the crossover arm of the study.
[1] For women of child bearing potential, sent to LabCorp.
[2] Can be done at Visit 1 or Visit 2.
[3] DEXA scan will be performed on female subjects of childbearing potential after it is confirmed they are not pregnant.

1.0 Background and Rationale

1.1 Product to be tested

The product to be tested is designed to stimulate the net synthesis of body protein, including muscle protein. The composition is protected by intellectual property and is based on a specially formulated mixture of essential amino acids (EAAs) and whey protein. The overriding metabolic goal of the composition is to maximally stimulate the rate of net muscle protein synthesis. The stimulation of protein synthesis is the metabolic basis for increases in muscular strength and function, and is crucial for increased biogenesis of mitochondria.

1.2 Product composition

The product composition is protected by intellectual property. It contains a combination of ingredients designed to stimulate muscle protein synthesis and to ultimately induce long-term improvements in muscle function and size.

Muscle Protein Synthesis: EAAs are the key to rapid recovery of muscle. EAAs are the “active” components of dietary protein, meaning that they are the amino acids contained in dietary protein that actually stimulate the production of new muscle protein. In fact, EAAs are the only dietary macronutrients that are required for survival. They are “essential” because the body cannot produce them. There are 9 dietary EAAs. There are 11 more dietary non-essential amino acids that are not required in the diet because they can be produced in the body. The composition is based on a blend of 8 of the 9 EAAs. In addition to being the “active” components of dietary protein, EAAs also have the advantage over intact protein because free EAAs are absorbed from the intestine more quickly and more completely than amino acids contained in protein, which require digestion before absorption. Finally, the relative amounts of each EAA can be tailored for any specific metabolic function. In the case of the composition to be tested, the EAA formulation is designed to maximally stimulate muscle protein synthesis in healthy individuals under the age of 50 y.

Intact Protein: Intact proteins are popular dietary supplements to stimulate muscle protein synthesis. While EAAs have a distinct advantage over intact protein in terms of the amount of muscle protein produced following exercise, the response to intact protein is sustained over a longer time than free EAAs because of the slower absorption. The composition combines the

benefits of free EAAs as well as intact protein. The unique combination of EAAs and intact protein cause an immediate increase in muscle protein synthesis (due to the EAAs), and the response is sustained by the slower entry into the body of the EAAs contained in the intact protein.

1.3 Previous research on the topic

The primary goal of the composition is to promote muscle protein synthesis and the balance between synthesis and breakdown. Muscle protein synthesis is most effectively stimulated by EAAs. The response of protein synthesis following EAAs is more than twice as large as a comparable dosage of whey protein (1). Oral ingestion of 2 x 6 g doses of EAAs, taken one hour apart after exercise, induces a greater response of muscle protein turnover than any other nutritional strategy (2). The magnitude of response to EAAs after exercise is dose dependent. The anabolic response to 6 g of EAAs was twice that of the response to 3 g (2,3). Addition of non-essential amino acids to EAAs provides no extra benefit in terms of muscle protein synthesis (4,5).

We assert that the optimal profile of EAAs for young, healthy subjects differs from that for elderly individuals. In contrast to the case for older individuals, there is no requirement for a disproportionate amount of leucine above and beyond to fulfil its requirement as a precursor from protein synthesis. Whereas a disproportionately large dose of dietary leucine relative to the other EAAs amplifies the stimulation of muscle protein synthesis in metabolically-challenged circumstances such as aging (6), a large dose of leucine is not necessary in younger individuals. A large dose of leucine activates mTOR and other factors that are involved at the molecular level in regulating the rate of muscle protein synthesis (7). While this action of leucine is helpful when the activity of mTOR and associated factors are blunted, such as occurs in aging. However, mTOR activity is not rate-limiting in young, healthy individuals. Therefore, a high proportion of leucine in the composition would be redundant in terms of activation of mTOR. By reducing the proportion of leucine in the composition, the profile of the EAAs in the composition more closely reflects the profile of EAAs in muscle protein. This is advantageous since the limiting step in muscle protein synthesis in healthy young individuals is the availability of EAA precursors in proportion to the profile of EAAs in muscle protein (8).

The profile of EAAs of the composition is specifically designed for maximal stimulation of muscle protein synthesis following exercise.

The use of free EAAs is advantageous in terms of being able to determine the exact profile of the amino acids. Also, unlike intact protein, EAAs are rapidly and extensively absorbed (9). The rapid peak response in plasma EAAs is a key reason for their effectiveness (10). On the other hand, the total duration of the response is limited, because just as the concentrations of EAAs in the blood rise rapidly, they fall rapidly as well (2). For this reason, the composition contains protein in addition to the EAAs to prolong the anabolic response in the recovery period.

EAAs are the “active” components of dietary protein that are primarily responsible for the stimulation of protein synthesis. Non-essential amino acids (NEAAs) are not required in the short term. This is because the NEAAs are normally produced in the body at fast enough rates to avoid deficiencies. On the other hand, studies performed in livestock suggest that maximal long-term animal growth and development is achieved with a balance of about 20-30%% NEAAs and 70- 80%EAAs (11). The addition of a small amount of intact protein to a mixture of EAAs is the most efficient way to incorporate the appropriate amount of NEAAs into a dietary supplement designed to stimulate muscle protein synthesis over the long-term. A number of studies have determined that whey protein is the most effective intact dietary protein in terms of stimulation of muscle protein synthesis after exercise (12,13). Whey protein is approximately 50% EAAs and 50% NEAAs. By combining a small amount of whey protein with EAAs, the necessary intake of NEAAs is provided in a format (I,e,. intact protein) that, while not as effective as the EAA mixture, nonetheless has its own stimulatory effect on muscle protein synthesis. The combination of free EAAs and intact protein give an immediate response (from the free EAAs) which is prolonged over time by the slower digestion of the intact protein.

The idea of combining a specific formulation of EAAs with an intact protein to obtain both an immediate as well as a more sustained response of protein synthesis is novel. Previous studies have added leucine to whey protein, but the inventor’s laboratory found this approach to not be effective (14). If only leucine is added to intact protein, an imbalance in the availability of the other EAAs is created that limits precursor availability for protein synthesis. The same limitation is true in the case of providing only branched chain amino acids. A unique aspect of

the composition to be investigated is that a balanced mixture of all the EAAs is added to the whey protein source. With this approach, the rapid response of muscle protein synthesis to the free EAAs is sustained by the more gradual absorption of the EAAs from the whey protein. The unique combination of whey protein and a specially formulated EAA mixture that coincides with the proportionate demand for precursors for stimulated muscle protein synthesis provides a rapid and sustained response.

The stimulation of both the number and function of the mitochondria is central to improved functional capacity of muscle. The EAA leucine in the composition acts as a nutraceutical to stimulate mitochondria biogenesis (15), and the free amino acids and protein stimulate the synthesis of new mitochondrial proteins (16). Therefore, the combination of free EAAs increases the ability of the muscle mitochondria to produce energy.

2.0 Hypothesis

We propose that consumption of the EAA/protein composition will stimulate the net gain (net protein synthesis) of body protein in a dose-dependent manner. We further propose that the magnitude of increase in net protein synthesis will be greater than induced by the consumption of the same amount of whey protein isolate, which is currently the most popular supplement on the market targeting the stimulation of muscle protein synthesis.

3.0 Study Design and Procedures

We will study a total of up to 20 healthy male and females between 18 and 50 years of age. We will use a randomized, two-period, stable isotope (Cambridge Isotope Labs, Tewksbury, Mass.) infusion study: 4.5-hour basal fasted period and 4-hour post-meal period (total 8.5-h time period). The principal end-point will be the total anabolic response (whole body protein synthesis minus breakdown) and the secondary end point will be muscle protein fractional synthetic rate (FSR) over the 4 hours following the consumption of either the test composition or whey protein. The plasma amino acid response will also be a secondary end point.

We will utilize two distinct arms within this protocol. Arm 1 will consist of a group of subjects who will perform a randomized, single blind cross-over (two stable isotope studies) between the two doses of the EAA/protein study supplement, with a ≥ oneweek washout period between stable isotope studies. Arm 2 will consist of a group of subjects (with similar gender composition) who will undergo one stable isotope study during which they will ingest the whey protein isolate study supplement. Subjects will be enrolled into the double stable isotope study arm, utilizing the specific consent form. After Arm 1 enrollment is completed, unique future subjects will be enrolled into Arm 2 – the single stable isotope arm, using the specific consent form.

3.1 Study Visits

Visit 1: subjects will come to the UAMS IOA 3rd floor for informed consent discussion. Once consent is obtained, subsequent study procedures will be performed. A medical history including allergies and list of current medications will be obtained (printed from Epic if they are a UAMS patient). Subject height and weight will be measured. A blood sample will be drawn for complete blood count [CBC]. Females of child bearing capacity will be asked to provide a urine sample for pregnancy testing. Blood and urine samples will be sent to LabCorp for the screening tests. A physical exam and vital signs will be performed at this visit or at visit 2. A DEXA scan for whole-body analysis will be performed in the PI's lab at UAMS (or at visit 2). Subjects can get a copy of their CBC and DEXA scan upon request. Any abnormal findings will be discussed with them by the study nurse and/or physician. Based upon the results of the screening blood sample, visit 2 will be scheduled.

Visit 2: Subjects return to the UAMS IOA having fasted overnight from 10:00 P.M. They will be asked about any adverse events since last visit. If not performed at visit 1, the physical exam and/or DEXA scan will be performed at this visit. For subjects who enrolled into the double infusion arm, randomization will be performed as to the order of supplement dose ingestion. After vital signs are measured, the study nurse will insert an IV catheter into a vein on each of the subjects' arms. One catheter is used to infuse the stable isotopes L-ring-D5 phenylalanine and 2H2-tyrosine. The other is to allow for periodic blood sampling, warming the arm by means

of a heating pad or a heated plastic box. After an initial blood sample is obtained, the study nurse will infuse the priming doses of the two above isotopes as well as a priming dose of 2H4-tyrosine. Constant infusion of isotopes commences immediately after the priming doses are completed. A timer will be started, and blood/muscle samples will be obtained according to the schedule below. The appropriate study supplement will be served directly after the second muscle biopsy procedure. Subjects will be asked to consume their supplement within 2 minutes.

At the conclusion of the 8.5-hour infusion study, the IV catheters will be removed and the sites dressed with a sterile bandage. Written and verbal instructions regarding the care of the muscle biopsy site will be provided. A snack and beverage will be offered to subjects. Vital signs will be measured, and subjects will then be free to leave. A follow-up phone call will be made within 72 hours after subjects have left the facility to assess for adverse events related to the stable isotope study visit.

Elapsed time (min., approximate)	Procedure
-0	Blood sample (~6mL)
120	Blood sample (~6mL)
150	Blood sample (~6mL), muscle sample (subject's choice of left or right vastus lateralis ~150mg)
180	Blood sample (~6mL)
210	Blood sample (~6mL)
240	Blood sample (~6mL)
270	Blood sample (~6mL), muscle sample (~150mg), ingest study supplement
290	Blood sample (~6mL)
310	Blood sample (~6mL)
330	Blood sample (~6mL)
360	Blood sample (~6mL)
390	Blood sample (~6mL)

420	Blood sample (~6mL)
450	Blood sample (~6mL)
480	Blood sample (~6mL)
510	Blood sample (~6mL), muscle sample (~150mg)

3.2 Second study (visit 3, for EAA/protein Arm)

After approximate minimum 1-week washout period, subjects who enrolled into the EAA/protein arm will undergo a second stable isotope study as described above. The order of consumption of each beverage will be randomized. The opposite leg will be used for muscle protein synthesis measurements. No further DEXA will be performed.

3.3 Subject Compensation

Subjects will accrue compensation for every visit according to the below table. They will be mailed a check for their total compensation approximately 2-3 weeks after their participation ceases (whether completed or not). If they were to attend every visit, their total amount would be $325 for the whey arm and $625 for the EAA/protein arm. If they stop participating during a stable isotope study visit, they will receive prorated pay of $25 per hour.

Visit	Amount
1	$25
2	up to $300
3 (if applicable)	up to $300

3.4 Randomization for EAA/protein Arm

Study staff will strive to maintain gender balance between the two arms. A randomization list will be implemented to reveal which dosage of EAA/protein will be tested first in the case of the cross-over study (Arm 1).

3.5 Blinding

This will be blinded to the extent feasible, given that different beverages will look and taste different. Since the subject has no conscious control over their rate of muscle protein synthesis, total blinding is of no concern. Nonetheless, subjects will not be told which dose of the product they are consuming. The data analyst will need to know the amount and nature of the product being consumed to calculate the rate of protein breakdown.

3.6 Sample Storage

Blood and muscle 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. With explicit permission via the ICF, all biological samples will be kept indefinitely for future IRB-approved uses. All blood and muscle samples shall be identified using a unique study acronym. None of a subject's personal identifiers shall be present on any biological sample.

4.0 Study Population

Subjects will be recruited using these methods: 1) past subjects that indicated they wanted to be contacted about future studies will be called by study staff to elicit their interest in this study, and 2) the study staff will place IRB-approved flyers around the Little Rock community. Once a potential subject has agreed to come to UAMS for an informed consent discussion, an appointment will be made for them to meet with study staff in the research area of the 3rd floor of the Reynolds Institute at UAMS.

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4.1 Inclusion Criteria

- Ages 18-50 yrs.

4.2 Exclusion Criteria

- History of diabetes
- History of malignancy in the 6 months prior to enrollment
- History of gastrointestinal bypass/reduction surgery (Lapband, gastric sleeve, etc.)
- History of chronic inflammatory condition or disease (Lupus, HIV/AIDS, etc.)
- Pregnant females
- Subjects who report regular resistance training exercise > once per week
- Hemoglobin < 9.5 g/dL at the screening visit
- Platelets < 150,000 at the screening visit
- Subjects who cannot safely stop using aspirin for 7 days prior to muscle biopsies
- Concomitant use of corticosteroids (ingestion, injection or transdermal)
- 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 determine eligibility and to measure glucose, insulin, plasma amino acid concentrations and stable isotope enrichment. The total amount of blood taken will be approximately 100 mL for subjects in the whey protein isolate arm, and 190 mL for subjects in the EAA/protein arm. Subjects should have no noticeable effects from these volumes.

5.2 DEXA scan:

The DEXA scan exposes subjects to approximately ½ of the radiation of one chest x-ray. They will undergo 1 DEXA scan.

5.3 Muscle samples:

Briefly, the risks related to the biopsy procedure include pain, bleeding, bruising, small scar formation at the biopsy site, Infection, and vaso-vagal response. Muscle biopsies are performed by a physician utilizing local anesthesia (using 1% plain lidocaine) for pain management and strict sterile procedures. Subjects must meet platelet criteria prior to biopsy. See appendices for SOP and related documents.

5.4 Study supplements:

The test composition will be provided by Adesso, LLC. It consists of a proprietary blend of eight essential amino acids and whey protein. It is produced by Prinova, Inc, Carol Stream, Il. This product is not currently commercially available. All components of the composition have been ruled as Generally Regarded as Safe (GRAS) by the FDA. They are natural components of the normal diet. Prinova, Inc, has all necessary certifications for making products for human consumption. Two doses of the product will be tested (approximately 6.3 and 12.6 g of active components). The product comes as powder and will be dissolved in water (8-16 oz) for consumption.

The whey protein isolate is available commercially. The dosage (12.6 g) will be dissolved in water (12 oz). There are no known risks of adverse effects of either the test composition or the whey protein in the dosages used in this study. All components are Generally Regarded as Safe by the FDA, and Prinova Inc has all the necessary certifications for producing and packaging products for human consumption

5.5 Confidentiality:

A potential risk to study participants is the potential for 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 Stable Isotope Infusion:

Briefly, the risk related to the isotope infusion is pyrogenic response. All isotope infusions are compounded and prepared by a licensed pharmacist at UAMS. An SOP for infusion procedures will be utilized (see Appendix). All stable isotopes are tested for sterility and pyrogenicity. The stable isotopes will be filtered during infusion through a sterile 0.22 micron (Millipore) filter placed in the infusion line. Stable isotopes are naturally occurring compounds and are not radioactive and are already present in the body in varying amounts. The infusion will be in a 'tracer' dose, i.e., a dose only detectable by GCMS. Infusion of these molecules will increase the level of naturally occurring isotopes by 7-10%. Any adverse reactions during the isotope infusion that suggest infection (urticaria, flushing, nausea, vomiting, sweating, chills, altered heart rate, hypo/hypertension, and hyperthermia) will be promptly addressed by the study physician. Depending on the seriousness of the reaction, the infusion study will be terminated.

6.0 Data Handling and Recordkeeping

Source documents and CRFs will be stored in a secure area of the PI's laboratory. Access will be limited to study personnel. Documents containing identifiers (except the signed ICF) will be destroyed by shredding approximately 7 years after data analysis is completed or publication of data; whichever is longest. The original, signed ICF will be kept indefinitely. 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. 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.

Sponsor (Adesso, LLC) will have monitoring and audit access to source documents and all collected and/or analyzed data. Any data sent to them will remain coded with the unique subject alphanumeric identifier. No identifiable information will leave UAMS.

7.0 Data Analysis

7.1 Statistical Analysis plan

The purpose of this study is to estimate and compare the effect of two different nutritional beverages on total anabolic response (TAR) and muscle protein synthesis (MPS). To this end, an analysis of covariance (ANCOVA) model will be employed to compare the protein sources with respect to mean response (either TAR or MPS) after adjusting for basal measures. If a significant protein group effect is detected, then pairwise comparisons will be performed to determine if the two sources or two doses differ. In this case, the Tukey-Kramer method will be used to adjust for multiple comparisons. A 5% will be used to determine statistical significance.

7.2 Sample Size Calculation & Power Analysis

A total of N=16 subjects will be required for this study, 8 in each arm. With this sample size, the ANCOVA model will have 80% power to detect effect sizes of ***f*** = 0.484 or larger (Table 1). This estimate assumes the basal covariate explains 50% of the variation in the response and a 5% α-level is used to determine statistical significance. (Note that the effect size, f, is simply the ratio of the variation between the three group means and the between-subject variation). In the event the effect of the covariate is mis-specified, the following table presents effect sizes that can be detected as the magnitude of variation explained by the covariate increases from 10% to 90%.

Table 1. Expected Effect Size by Covariate Variation

Variation Explained by Covariate (%)	Detectable Effect Size, *f*
10	0.649
30	0.573
50	0.484
70	0.375
90	0.216

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 given 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. Adesso LLC, will have 90 days to review potential publications before submission. The ultimate decisions on content of publications will reside with UAMS investigators. The publications will not contain any identifiable information that could be linked to a participant.

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11.0 Appendices

11.1 CTRAL Muscle Biopsy SOP

Policy: The following information is written to serve as a resource and guideline for the percutaneous vastus lateralis muscle biopsy procedure. This SOP will ensure that the procedure is performed in a consistent manner that protects participant safety. This procedure can be performed concurrently with a fat biopsy, provided that the protocol calls for samples at the same time-point. In this case, the fat bx shall be performed first so that the muscle bx site does not bleed during the performance of the fat bx.

Purpose: The muscle biopsy procedure is conducted to obtain a sample (~100-150mg) of muscle tissue for metabolic (and possibly structural) analyses.

Responsibility and Accountability:

Principal Investigator: Will oversee, direct, and be responsible for assuring adherence to the entire procedure.

Study Physician: Responsible for overall medical supervision and performance of the procedure including handling of subject complaints or adverse events related to muscle biopsies.

Study/staff nurse: Will assist study physician with their performance of muscle biopsy procedure. Will educate subject about post-biopsy care per the written instructions.

Materials:

Equipment: sterile 5mm (or 6mm) Bergstrom biopsy cannula.

Supplies:
- 1 betadine 3-swab pack, commercial preps, or chlorhexidine solution
- 2 drape sheets
- 1 fenestrated drape sheet
- 1 laceration tray

1	lidocaine 1% w/o epinephrine (from Rx)
2pr	sterile gloves
2	packs non-adhesive dressing sponges
1	Opti-lock IV extension tubing
1	sterile IV extension tubing ~24” long
1	#11 scalpel
1	Dermabond glue
2	clear Tegaderm-type dressing
1	50 or 60 mL syringe for suction
1	5mL syringe for rinsing muscle
1	18g needle for rinsing muscle
1	5mL sterile saline for rinsing muscle
2	6” ace bandage

Pre-Procedure:

1. Verify subject has already completed the consent and screening process.
2. Nurse verifies that subject has not taken any blood-thinning medications recently.
    a. Prohibited medication classes: antiplatelet agents, anticoagulant agents. If subject uses these medications as prescribed by their physician, they will not be allowed to undergo a muscle biopsy.
    b. Aspirin or aspirin-containing compounds: subjects should wait 7 days after the last dose of aspirin was ingested before they undergo a muscle biopsy.
3. Staff will set up a sterile field containing the above supplies and bx needle.
4. Explain the procedure to subject in terms that they understand, including expected sensations and side effects.

Procedure:

1. The biopsy site (lateral thigh, 1/2 way between hip and knee) will be prepped with one of the prep solutions to ensure disinfection.
2. Physician performing the bx and nurse will properly don sterile gloves.
3. The prepped area will be draped using the supplied sterile materials.

4. Sufficient 1% lidocaine solution will be used to anesthetize the skin, subcutaneous tissue and fascia at the biopsy site using supplied syringe and needles. Limit lidocaine volume to less than 20mL per biopsy. Wait approximately 6-10 minutes after the initial injection before testing area for sensation.
5. Using aseptic technique and sterile scalpel, a small incision (approximately ¼ inch) will be made in the skin after adequate anesthesia has been confirmed.
6. The Bergstrom needle will be inserted through the incision site approximately ¾ to 1" past the fascia, and a muscle biopsy will be taken with the aid of suction.
7. After the bx needle has been removed, a person wearing sterile gloves will apply firm compression for at least 7 minutes to the biopsy site.
8. Muscle sample will be handed to study staff for appropriate processing.
9. After hemostasis has been achieved, the biopsy site will be cleaned, and if this is the only or final biopsy to be performed, a medical adhesive will be used to close the incision. A pressure dressing will then be applied, and the subject will be advised to keep the pressure dressing on for approximately two hours.
10. The subject will be provided written wound care instructions, and these will be reviewed verbally as well.

Post Procedure and Discharge:

1. Provide subject with written wound care instructions and ensure they understand the instructions.
2. Call subject within 72 hours after the most recent bx to follow up for any problems or concerns.

Emergency Procedures and Adverse Event Reporting:

If bleeding persists, apply additional pressure to the site where muscle was biopsied. If necessary, apply ice pack to area to assist with hemostasis. Refer to study protocol for reporting procedures.

Documentation:

The study staff will complete the Muscle Biopsy Procedure note for each bx performed. Notes will be filed in the CRF.

11.2 Stable Isotope Infusion SOP

Policy: The following information is written to serve as a resource and guideline for the stable isotope infusion procedure. This SOP will ensure that the procedure is performed in a consistent manner that protects participant safety.

Purpose: The stable isotope infusion procedure is conducted to measure in-vivo substrate kinetics (amino acid, glucose, fatty acids, etc) (1).

Responsibility and Accountability:

Principal Investigator: Will oversee, direct, and be responsible for assuring adherence to the entire procedure.

Study Physician: Responsible for overall medical supervision of the procedure. Will sign order(s) for related procedures/supplies, including infusions.

Study/staff nurse: Will provide immediate supervision throughout the procedure. Responsible for administering correct compound in correct dose to correct subject, as ordered (2).

Pharmacist: Will prepare infusions *per* physician's order.

Definitions:

Stable isotope – a non-radioactive variant (heavier by electron mass) of a naturally-occurring substance, e.g. glucose, amino acids, etc.

Materials:

1. Equipment: Calibration records for infusion pumps used will be maintained according to institutional policy.
2. All infusions will be prepared by a licensed pharmacist.

Pre-Procedure:

1. Verify subject has already completed the consent and screening process.

2. Nurse documents subject's vital signs, verifies that subject has fasted as required by protocol, and has met any other protocol-specific requirements and notes this in the record.
3. Nurse inserts blood sampling catheter and draws baseline sample and records these events in the record.
4. Nurse inserts infusion line catheter.
5. All syringes containing isotopes will be labeled by the institutional pharmacy for subject name/ID, isotope, dose, date and initials.
6. A licensed medical person must perform the setup of infusions.
7. The study physician will be notified of the date and time for each procedure and will be available by pager or cell phone throughout the procedure.
8. A nurse will be responsible for maintenance of all catheters and be present during the procedure.

Procedure:

1. The subject will be advised to rest in a recumbent and/or supine position for the procedure.
2. Blood and muscle samples will be obtained according to the study-specific CRF. Blood samples will be obtained by licensed medical personnel or specifically trained and credentialed technical staff. Muscle biopsies will be obtained according to SOP by UAMS physicians.
3. Subjects will be monitored for adverse reactions to the infusion and blood and muscle sampling by study personnel and nurse. Any adverse reactions will be reported to the study physician immediately. If a reaction is suspected to be attributed to the infusion, it will be stopped and a sample preserved for later quality testing.
4. Subjects will be asked to comment on their general well-being during the procedure.

Post Procedure and Discharge:

1. Immediately following the completion of the procedure, each subject will be offered a snack and will be encouraged to consume it.

Emergency Procedures and Adverse Event Reporting:

All stable isotope infusion procedures will be performed as described in this SOP to minimize the risk of an unanticipated event. These procedures will be performed in the CRC at UAMS Central hospital.

Emergencies will be handled according to institutional guidelines. Adverse events will be reported by the PI to all relevant regulatory bodies (IRB, sponsor) pertaining to the specific protocol. Incident reports will be filed according to individual institutional guidelines.

Documentation: The study nurse will sign the study flowsheet, which will be reviewed by the PI at the completion of the study. Documentation for outpatients will be according to institutional policy.

Sources/References: All drug products for any study shall be prepared in accordance with Arkansas Law (Regulation 07-02-0001; Drug Products and Descriptions; Standards for Compounding and Dispensing Sterile Products).

1. Wolfe RR and Chinkes DL. Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis. Wiley, New York, New York, 2004, 274 pages.

2. Deglin JH and Vallerand AH. Davis's Drug Guide for Nurses. 9th ed. F.A.Davis, Philadelphia, PA, 2005, xx-xxii.