University of Arkansas for Medical Sciences (UAMS) Clinical Protocol

Study Title: "Ounce-equivalents" in the Protein Foods Group: Benefits of Quality Protein

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1.0 Background and Rationale

Over the past 35 years the United States Department of Agriculture (USDA) Dietary Guidelines for Americans (DGAs) has sought to translate recommendations on nutrient requirements (i.e., Recommended Dietary Allowances, RDAs) from the Food and Nutrition Board of the Institute of Medicine (IOM) into practical nutritional advice for the American public. In addition to the RDAs, the DGAs are intended to incorporate additional scientific evidence as it arises into the recommendations for a healthy diet.

The lack of appropriate focus on protein nutrition is a major shortcoming of the DGAs. Not only is the amount of protein not a major focus, absolutely no mention is made of protein quality. Protein quality refers to the amount, profile, and true ileal digestibility of the essential amino acids (EAAs) in the protein [1]. The concept of protein quality is not new, as the Protein Digestible Corrected Amino Acid Score was published by the Food and Agriculture Organization of the World Health Organization in 1993. This scoring system was supplanted by the same organization in 2013 by the Digestible Indispensable Amino Acid Score (DIAAS) [1]. In general, animal proteins have much higher DIAASs than plant proteins, often by as much as two fold. Account has not been taken of DIAAS scores, or even the general concept of the importance of the amount and profile of EAAs in individual proteins, in formulation of MyPlate [2] or the scientific report of the DGAs Committee [3]. This is despite the fact that in the IOM report stating the RDA for protein it is specified that this refers to "high quality protein, a classification that does not apply to most plant proteins [4]. The current version of MyPlate continues to disregard the importance of protein quality in the selection of dietary food sources of protein [2]. Under the section in MyPlate titled "Go lean with protein", it is recommended to "choose from a variety of meat, poultry, seafood, bean and peas, eggs, soy foods like tofu, nuts and seeds". To help the consumer meet protein needs while achieving the goal of varied protein food sources, the DGAs Committee published "ounce equivalents" in the protein foods group. It is stated among other equivalents cited, that 1 ounce (oz.) of meat is equivalent to 1 tablespoon (Tbsp.) of peanut butter and 1/4 cup (0.5 ounces) of cooked kidney beans [2]. But are they really equivalent? For example, one ounce cooked beef contains 7.8 g of protein and 57 kcal; 1/4 cup cooked kidney beans contains 3.8 g of protein and 56 kcal; and 1 Tbsp. of

reduced fat peanut butter contains 4.66 g of protein and 94 kcal [5]. The inequities of the "ounce equivalents "are even more glaring when protein quality as quantified by the respective DIAASs of animal and plant protein sources is considered. For the examples provided, the DIAAS of beef protein is 117, for kidney bean protein is 59, and for peanut protein is 44 [5]. When account is taken of EAA composition and bioavailability of the protein in each food source according to the DIAAS, it requires consumption of 5 oz. of beef to obtain at least 100% of the daily requirements of all EEAs, whereas it requires 18 cups of cooked beans and 50 Tbsp. of reduced-fat peanut butter. In caloric equivalents, these amounts of food correspond to 10 kcal/kg/day for beef, 33 kcal/kg/day for kidney beans, and 38 kcal/kg/day for reduced-fat peanut butter. The equivalencies have been expressed in the same units as the recommended daily caloric intake defined in the Dietary Reference Intakes as 35 kcal/kg/day [4]. These calculations clearly indicate that the "ounce equivalents" of protein foods in MyPlate are not equivalent in any parameter that might be used to assess nutritional benefit, and demonstrate the bias against animal proteins in the Dietary Guidelines.

The misrepresentation of the equivalencies of various food sources of protein in MyPlate raises the question of the process by which this occurred, and how can the process be influenced to more accurately reflect that high quality of animal proteins? A new DGAs Committee is formed every five years to consider modification of existing recommendations in accord with new information obtained since the last Consultation of the Committee. The Committee not only researches the literature itself for new advances in nutrition research, but holds public hearings in which concerned individuals can make presentations. Publication of new data directly addressing aspects of the DGAs is the most effective approach to sway the Committee to alter a previously-held position.

Developing convincing data to correct the MyPlate "ounce equivalents" of protein foods is an achievable goal. Data from the USDA nutrient data base regarding caloric values and protein content of different protein food sources are helpful in making the argument that the "ounce equivalents" should be adjusted, as are the calculated DIAASs. However, those values are calculated and do not directly reflect functional responses to the "ounce equivalents". Further, those calculations could have been made before the current DGAs were issued, but if those calculations were made they did not sway the Committee. The most convincing data would be

direct measurement of the physiological responses to "ounce-equivalents" of protein food sources. Dietary protein intake serves many physiological roles, but the most prominent is the maintenance or gain of body protein. This is accomplished by stimulation of protein synthesis, the inhibition of protein breakdown, or a combination thereof. A net gain in protein balance (i.e., synthesis minus breakdown) defines an anabolic response, as opposed to a catabolic response caused by the rate of protein breakdown exceeding the rate of protein synthesis. An anabolic response usually refers to gain of muscle protein, but actually involves the entire body. Thus, the functional response to consumption of a given amount of a protein food source is best assessed by quantifying the rates of protein synthesis and breakdown at the whole body level as well as at the muscle level in order to calculate the anabolic response. We propose to make these measurements in response to intake of "equivalent "(according to MyPlate) amounts of pork, mixed nuts and tofu. Demonstration that the functional responses to these varied sources of protein coincide with the predictions from the USDA nutrient data base and calculation of the DIAAS will provide needed support to redefine "ounce equivalents" of protein food sources according to those data bases for all animal and plant sources of protein.

1.1 Summary of Previous Related Studies

A.) Essential amino acids (EAAs) are responsible for the anabolic response to protein consumption [6-8].

B.) Stimulation of muscle protein synthesis in response to EAA is dose-dependent and can be detected with as little as 3 g of free EAA intake.

C.) Importance of protein breakdown in determining the net anabolic response to protein intake [9].

2.0 Hypothesis

1. Ingestion of 2 ounces of cooked lean pork will induce a greater anabolic response, i.e., increase in the difference between protein synthesis and breakdown, than consumption of either 1 ounce of mixed nuts or 4 ounces of tofu.

3.0 Study Design and Procedures

We will study a total of up to 24 healthy male and females between 18 and 40 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 FSR over the 4 hours following the test meal (either pork, mixed nuts or tofu).

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, weight, and vital signs will be measured. A blood sample will be drawn for complete blood count [CBC]. A physical exam will be performed at this visit or at visit 2 or 3 at the discretion of the study physician. 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. 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. Randomization will be performed as mentioned below. Subjects will be provided with complete, ready-to-eat meals that they will consume for 3 consecutive days prior to their stable isotope study visit (visit 3). They will be loaned a digital camera to document their compliance for these meals. Visit 3 will be scheduled. See Appendices for menus of the 3 days of meals. Food volume will be tailored for age/sex/body weight so that subjects do not gain or lose weight during the diet run-in period. A UAMS dietician will prepare the specific menus for each

subject, and UAMS Clinical Nutrition staff will prepare and package the meals in the IOA research kitchen located on the 3rd floor of the IOA.

Visit 3: Subjects will return to the IOA having fasted overnight from 10:00 P.M. They will also return their loaner camera. They will be asked about any adverse events since last visit. Study staff will ascertain that subjects complied with the meal requirements prior to proceeding with the stable isotope infusion study. If not performed earlier, the physical exam will be done at this visit.

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 frequent 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 test meal will be served directly after the second muscle biopsy procedure. Subjects will consume 1 serving of one of the test foods: cooked pork loin, mixed nuts or tofu.

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)	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 test meal
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 Subject Compliance

If a subject fails to eat at least 80% of the run-in meals, or fails to completely eat the test meal, they will be dropped from the study.

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. If they quit the study during visit 3, they will receive prorated pay of $25 per hour.

Visit	Amount
1	$10
2	$15
3	$25 per hour up to $300

3.4 Randomization

We will use permuted block randomization, with random block sizes of 2 or 4, to assign subjects to one of three possible treatment groups. The permuted block design will ensure that group sizes are equal after each block is filled. In addition, the randomization procedure will be stratified by gender to guard against group imbalance. A randomization procedure will be implemented using sealed envelopes, each one being labeled with stratification level (gender) and a label number. The label numbers will be sequential within each of the groups. As a participant is identified and enrolled on study, the study coordinator will ascertain gender, which will determine the set of randomization envelopes to be used. The study coordinator will choose the lowest numbered envelope, which will reveal group assignment. In the event of incomplete subjects that significantly unbalance the groups, subsequent subjects will be randomized into remaining unfilled groups.

A: subjects will consume 2 ounces of cooked pork loin one time during the stable isotope study visit.

B: subjects will consume 1 ounce of mixed nuts one time during the stable isotope study visit.

C: subjects will consume 4 ounces of tofu one time during the stable isotope study visit.

3.5 Blinding

This is a single-blinded study. The PI and any other data analysts are blinded to test meal. After all study visits have been performed and all data entered into a database, the blind will be broken.

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 from subjects via the consent form, muscle samples will be kept indefinitely for approved use

in the PI's lab for metabolic or metabolomic analyses. Samples that have not been approved for future use by the subject will be destroyed only after all data has been analyzed and reported to the sponsors. 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, 2) the study staff will place IRB-approved flyers around the Little Rock community, 3) study staff will engage Research Match.org, and 4) study staff will engage ARResearch program through the UAMS TRI. 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. This study will enroll up to 30 subjects

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4.1 Inclusion Criteria

- Ages 18-40 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 do not or will not eat animal proteins
- Subjects allergic to pork, tree or peanuts, or soybeans
- Subjects who cannot refrain from consuming protein or amino acid supplements during their participation in this study

- 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
- 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 concentrations. The total amount of blood taken will be approximately 96 mL. Subjects will have no noticeable effects.

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 Test meals:

Subjects who are allergic to one of the test meal components will not be permitted to participate. Their allergies will be ascertained during the initial phone call or at visit 1.

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 Recordkeeping 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.

7.0 Data Analysis

7.1 Statistical Analysis plan

The purpose of this study is to estimate and compare the effect of five protein sources 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 which protein sources 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=24 subjects (8 per protein source) will be required for this study. 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-

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

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%.

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. The publications will not contain any identifiable information that could be linked to a participant.

10.0 References

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2. https://ndb.nal.usda.gov
3. http://health.gov/dietaryguidelines/2015
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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).

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