**INFORMED CONSENT**

**Title of project**

Skeletal muscle protein synthetic response to amino acid and dipeptides

**Key Information**

The purpose of this study is to understand the effects of amino acid peptides ingestion on muscle growth and breakdown at rest and after resistance exercise. Participating in this study will involve one pre-testing session (3 h) and two testing sessions (6.5 h each). During testing sessions, you'll be randomized to either 1) consume a protein drink only; or 2) consume a protein drink and perform a bout of resistance exercise. Blood and muscle samples will periodically be taken throughout each testing session. Benefits related to this research include gaining information about your body composition (Lean mass, body fat and bone density) and learning how you respond to acute exercise and nutritional stimuli.

**Introduction**

This form contains information about this study and it is necessary that you understand its contents prior to enrolling in this study. Please ask any questions you may have about the research; we are happy to explain anything in greater detail. You will be provided with a copy of this form to take with you. For this study, we are interested in how people respond to individual amino acids and dipeptides, with or without resistance exercise.

Please contact members of the research team with any questions you may have about the study:

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If you wish to report a research related injury, please contact Dr. Nicholas Burd at the listed phone number or e-mail as soon as possible. This should be done within 24 hours of discovering the injury.

This study is expected to last for the next year, but your involvement will only last about 2 weeks. You will be invited to the lab on three occasions consisting of: one screening, two experimental days.

**Pre-Test Procedures**

Prior to the intervention, you will visit the laboratory at Louise Freer Hall, UIUC for one screening (time commitment: ~1.5 h). During, we will determine if you are eligible for this research study. If it is determined that you are not eligible for the study, your paperwork and data will be destroyed. The researchers will give you a detailed explanation of each procedure during the screening. However, the following information will give you an overview:

1. *Medical history, physical activity readiness, & physical activity habits.* You will be asked to complete a medical history and physical activity readiness questionnaire. Additionally, you will complete a questionnaire that asks about your current physical activity habits. We will also review your answers to ensure you are eligible and that it is safe for you to participate in this study.

2. *Blood pressure.* We are only enrolling participants with healthy blood pressure for this study.

3. *Body weight and height.* With these measures we will calculate your BMI.

4. *Body composition.* We will use a DXA scan (Dual-energy X-ray Absorptiometry) to determine your fat mass, bone mass, and muscle mass. For this scan, you have to remove all metal from your body such as jewelry or watches. You will be required to lie on your back on a padded table. This test lasts about 15 minutes.

   *Women:* If you are pregnant we will have to exclude you from participating to avoid unnecessary radiation exposure.

5. *Maximal Strength Testing.* A ten repetition maximum (10-RM) will be determined for the leg extension exercise, which will be used to calculate their workload for experimental days. 10-RM will be defined as the point at which the participant can no longer increase weight of resistance while maintaining proper form for ten consecutive repetitions. Handgrip strength on both hands will also be tested using a small machine that you will squeeze as hard as you can in order for us to measure your grip strength.

Afterwards, the research team will randomize you into one of two groups: a) exercise; or b) non-exercise. If assigned to the exercise group, you will perform the exercise outlined below. Participants in the non-exercise group will not perform the resistance exercise session.

**Experimental Days**

You are asked to participate in two trial days, lasting approximately 6.5 h each. Detailed explanations of each procedure is provided below. During the screening and testing day, researchers will explain every procedure in detail.

1. *Preparations.*
   *Exercise:* You are expected to abstain from alcohol consumption and strenuous physical exercise for the 72h (3 days) leading up to each experimental trial day. “Strenuous” activities include weight lifting (upper and lower body), cycling, running, and competitive sports (soccer, basketball, etc.). On the morning of the experiment, we ask that you arrive to Freer Hall by public transport or car (cycling, walking is unacceptable) without breakfast/caffeine (coffee, tea, etc.).

   *Diet:* For the 48 h (two days) leading up to each experimental trial, you will be asked to record all consumed food and beverages through an online portal, Automated Self-Administered Recall System (ASA 24), developed by the National Cancer Institute. We ask that you repeat the same dietary habits for the 48 h leading up to the second trial. Confidential, non-identifiable, login information will be provided in order to access the dietary recording website. The evening prior to test days, you are asked to eat a provided meal no later than 10:00 pm. After that time, we ask that you remain fasted (no food, regular amount of water is OK) through the experimental day.

2. *Blood Collection.* On the test day, a small catheter (flexible plastic tube) will be inserted into a hand vein to allow for blood sampling. The catheters will be inserted with the assistance of a small needle. The needle will not remain in your hand, but only the flexible plastic tube. The discomfort felt during this procedure passes quickly and feels

similar to donating blood. To minimize discomfort, a numbing cream will be used prior to the catheter placement. Once the needle is removed you should not feel the catheter.

During the test day, blood will be drawn periodically from the catheter in the hand vein. Before drawing the blood samples, your hand will be placed into a heated blanket warmed to 60°C. A total of ~3 oz. of blood will be drawn over the entire test. For comparison, when you donate blood, ~17 oz. is drawn in a minute.

After each sample is taken the catheter is "flushed" with a sterile saline solution in order to prevent blood from clotting in the catheter. This is a salt solution similar in composition to your own blood and it will not affect you.

3. *Stable Isotope Infusion.* A second catheter will be placed in a vein in your opposite arm for an infusion (long slow injection) of two amino acids. The amino acids will be dissolved in saline (a sterile salt solution similar to your blood). Our bodies use amino acids to make new proteins. By labeling an amino acid, we can see where these amino acids go and the rate at which protein is built and broken down. One amino acid will be labeled with a stable isotope of carbon—the other with a stable isotope of nitrogen. An isotope is slightly heavier form of this element. Since the isotope is stable (i.e., non-radioactive), it poses no health risk to you due to radioactive exposure. A certain fraction of all of the carbon—and nitrogen—within your body is already in the same form as that of the stable isotopes used. Hence, the infusion of the stable isotope-labeled amino acids will simply result in a slight increase in the amount of stable isotope within your body; we refer to this as "enriching" the amount of stable isotope within your body.

   This enrichment will not remain high, however, and will be back to pre-infusion levels within a few days. The infusions are prepared under sterile conditions and are filtered through a very selective filter prior to entering your body. This solution does not contain anything that will adversely affect your health.

4. *Muscle Samples.* During the test day we will take a small piece of muscle tissue (muscle biopsy) at four different time points by using a sterile hollow needle. For each biopsy, Dr. Burd will clean an area over your thigh muscle and inject a small amount of numbing solution (lidocaine) into and under the skin. He will then make a small incision (~4-5 mm) in the skin in order to insert a sterile needle into your thigh. Dr. Burd will quickly remove a very small piece of muscle (~50-100 mg; which is about the size of a corn kernel) and remove the needle from your leg. During sample collection (~30 sec), you may feel the deep pressure in your thigh and on some occasions it may feel painful. However, the discomfort subsides very quickly. Following the biopsies, the incisions will be closed with sterile bandages.

   After leaving the laboratory, you are encouraged to perform light exercise (cycling, jogging) and daily activities. However, you should refrain from excessive muscle use for the remainder of the day. Namely, weightlifting sessions that involve deep squatting motions should be avoided for that day. Once the numbing agent wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". The tightness in the muscle usually improves within 2 days. You will be provided with care instructions to take with you.

5. *Resistance Exercise.* Before eating, you will perform resistance exercise. For this you will perform a warm-up on the leg extension, then you will perform 3 sets of 10 repetitions of the leg extension exercise. A trained member of the research team will be present during your exercise bout.

6. *Protein Beverage.* You will consume one of two amino acid- or dipeptide-based nutrient solutions immediately following the exercise bout. Specifically, amino acids are the individual building blocks of dietary proteins, and dipeptides molecules consist of two amino acids –both of which occur in all food products containing protein. Your second experimental day, you will consume the other protein choice. Order of consumption between experimental trial days will be randomized.

**Risks and Benefits**

*Benefits.* There are no direct benefits from participating in this study, but after completion you will be given information about your body composition (height, weight, body fat, etc.), habitual dietary intake, and maximal strength. Additionally,

we can provided information about your ability to metabolize protein upon request. You may wish to have this information evaluated by a health professional. However, your participation in this study will help us understand how our muscles respond to the protein that we eat in a meal. Understanding this process will ultimately help provide information for designing nutrition programs to prevent or reverse the loss of muscle mass. The maintenance of muscle mass is important for your metabolism as well as for removing fat and sugar from the blood.

*Risks.* You are not expected to be in great risk from participation in this study. The potential risks involved in participating in this study are described below.

1. *Potential risks with the muscle biopsy procedure*
   The muscle biopsies are routinely used in research and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in temporary bruising (1 in 30) lasting up to 5 days. Small lump may form under the site of the incision (~1 in 500), but this normally disappears within a few weeks by massaging the lump with your thumb. As with any incision there is also a slight risk of infection (~1 in 2,200). However, this risk is virtually eliminated through proper care. In very rare occasions there can be damage to a superficial sensory nerve, which will result in temporary numbness in the area (~1 in 1,500) lasting up to 3 months. There is also an extremely remote chance that you will be allergic to the numbing agent; the chance of lidocaine allergy is currently unknown. Your muscle may feel sore for 1 or 2 days following the procedure, as if you have performed difficult exercise. However, this is normal and will pass. While there is also a theoretical risk of damage to a small motor nerve (this is used to allow your muscle to move) of your thigh muscle, this has never been seen in past experiences.

   To minimize the risk of skin infection and facilitate proper healing, you will be provided with explicit written instructions ("Biopsy Care Kit") that details the proper care of the wound.

2. *Potential risks involved with catheterization*
   The insertion of catheters for blood sampling is a common medical practice and involves few risks if proper precautions are taken. The catheters are inserted under completely sterile conditions. However, there is a theoretical risk of infection. There is also a chance of internal bleeding if adequate pressure is not maintained upon removal of the catheter. This may cause some minor discomfort and could result in bruising/skin discoloration, which could last for up to a few weeks. In very rare occasions, trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication with catheter placement.

3. *Potential risks involved with stable isotope infusion*
   Despite all precautions, there is a theoretical risk (less than 1 in 1,000,000) that you could have a rapid drop in blood pressure during the infusion due to some small bacterial contamination of the infusion solution (infusate). Such an effect would occur during your visit to the laboratory. This has never occurred in our experience.

4. *Potential risks involved with DXA scan*
   The level of radiation emitted during a DXA is very low, <1 µSv. This is very minimal exposure compared to the total background radiation level per year in North America, which is approximately 3.0 mSv/year).

5. *Potential risks involved with resistance exercise*
   There is a small risk of sustaining minor muscle, bone and/or tendon injury during exercise. In addition, you may experience a feeling of discomfort after the exercise bout due to intensified use of major muscle groups. There is a theoretical risk that heart irregularities or sudden death may occur during exercise. However, these events generally happen to people who already have heart conditions. If you have been diagnosed with any type of heart condition you cannot participate in this study. If, despite precautions, an emergency occurs during exercise; research staff is trained in first aid and CPR. Also, advanced life saving equipment (e.g. AED) will be immediately on hand to respond in any manner necessary; including calling 911 if deemed appropriate.

6. *Potential risks involved with protein beverage*

Beverages will be prepared with protein powder reconstituted in water. Leucine is an amino acid found naturally in dietary protein. The 2g amino acid or peptide beverage is well within a safe quantity for healthy adults. Individuals with metabolic disorders that indicate reduced capacity to metabolize protein and/or amino acids will be excluded from participation.

**Injury and Liability**

If you have any questions or problems with severe soreness, bleeding, or if the biopsy site becomes red or warm to the touch, please contact any of the researchers (found at the top of this form). If you sustain an injury as a result of participating in this research project that requires medical treatment you are strongly advised to get that treatment. However, the treatment you receive is not free of charge, and we have not set aside money to pay for related injuries. The University of Illinois does not provide medical insurance coverage for participants in this research study. Also, the University of Illinois does not provide compensation for any injury sustained as a result of participation in this research study, except as required by law. Signing this form does not waive any legal rights.

**Confidentiality**

*Will my study-related information be kept confidential?*

Yes, but not always. In general, we will not tell anyone any information about you. When this research is discussed or published, no one will know that you were in the study. However, laws and university rules might require us to tell certain people about you. For example, your records from this research may be seen or copied by the following people or groups:

- Representatives of the university committee and office that reviews and approves research studies, the Institutional Review Board (IRB) and Office for Protection of Research Subjects;
- Other representatives of the state and university responsible for ethical, regulatory, or financial oversight of research;
- Federal government regulatory agencies such as the Office of Human Research Protections in the Department of Health and Human Services

Some samples obtained during this study will be stored in the laboratory (maximum 15 years), and may be used for further research. These extra samples are used for determining isotopic enrichment analyses that need to be repeated, or conducting additional cellular/molecular analyses. Also, when publishing, reviewers often ask for additional measures and these samples could be used for this as well. Instead of contacting you later, you are asked to indicate whether you will permit these samples to be used in future research by selecting the appropriate option at the bottom of this form.

***Voluntariness & Compensation***

Your participation in this study is voluntary. You may withdraw your consent and discontinue participation in this study at any time without penalty or loss of benefits to which you are otherwise entitled. The investigators reserve the right to withdraw you from the study if they believe that circumstances have arisen that warrants doing so.

You will receive $200 upon full completion of the study. If participation in this study is ended early, you will be paid according to what was completed. Participation we be prorated $25 per biopsy. No compensation will be provided for the completion of the screening day.

*If you have any questions about your rights as a participant in this study or any concerns or complaints, please contact the University of Illinois Institutional Review Board at 217-333-2670 or via email at irb@illinois.edu*

**My signature indicates that I understand the information provided in this form and voluntarily agree to participate in this study and, on the date provided, received a copy of this informed consent. I certify that I am over 18 years of age.**

As stated above, we would like to keep your data, blood samples and biopsies for possible (related) future research. Occasionally, when publishing our work, we are asked to provide more information to clarify our findings, which can

require additional blood or tissue. If we must do so, your permission will not be asked for again. The samples will be kept for a maximum of 15 years. Please check the box of your choice.

I **do** authorize the researchers to use my data, blood samples and biopsies for future research ☐

I **do not** authorize the researchers to use my data, blood samples and biopsies for future research ☐

(signature of participant) Date

(print name of participant)

(name of investigator) Date