INFORMED CONSENT FORM

The Effect of Macronutrient and Caloric Intake on Interstitial Fluid Concentrations

OUR STATEMENT

We are asking you to be in a research study, in which the purpose of this consent form is to give you the information you will need to help you decide whether or not to be in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent."

PURPOSE

The purpose of this study is to run a discovery cohort of 10 subjects to see if we can detect macronutrient biomarkers. If the quantity of certain macronutrients can be predicted using biological data after people eat, this finding will be used to develop methods of continuously monitoring the food we eat on a daily basis. This is to make personal daily tracking of macronutrients and calories more accurate and much easier. There are 10 people that are expected to be enrolled in this study.

BENEFITS

There is no direct benefit to participating in this study.

COMPENSATION

Upon completion of participating in this research study, you will receive a $100 Amazon gift card.

PROCEDURES

The study will take place on three separate days that are at least 1 week apart from each other. The study will be completed in our office at 20 Blue Hill Plaza Pearl River, NY and will require a 12 hour overnight fast before each visit, and **will take 4-5 hours each visit**. Water is allowed during the fast.

1. **Part 1**: After your 12 hour fast, you will come into the office and be informed on the process that will take place by either Myles or Ashley (the PI or Co-PI). You will also have your weight and BMI measured. You will be given a pregnancy test to make sure that you are not pregnant if you are a woman, as pregnancy will be excluded in women of childbearing age. You will be given your first meal, which is an Ensure drink that is either strawberry, chocolate, or vanilla, and the 5 samples of interstitial fluid (a fluid right below the skin) will be drawn at different time points. You will be allowed to choose the flavor that you wish to drink. The samples will be drawn using a needle syringe, and will be drawn from a blister that is created on your thigh. The blister will be roughly 8 mm in diameter. It will be created using a suction vacuum machine that lifts a very thin top layer

of your skin and allows the interstitial fluid to flow underneath to create the blister. The vacuum will be used for roughly 40-45 minutes to create each blister. In total,10 blisters will be created and 5 samples of interstitial fluid will be drawn from the blisters in part 1. Part 1 will take roughly 4-5 hours.

The PI and Co-PI have determined that the suction device poses a non-significant risk and is safe to use in this research study. This system has been in use by major researchers within the United States, as well as abroad, and has been found to be a simple, yet effective, system to operate with high repeatability and reliability.

Obtaining interstitial fluid samples:

*** The following will be done by the PI or Co-PI**

1. You will lie down, and your thigh will be cleaned with alcohol to prevent infection.
2. A plate that has a hole will be placed around your thigh using a strap that is adjusted for comfort.
3. A vacuum machine will be placed above the plate, allowing a light suction to begin creating the blister.
4. The vacuum pressure will start extremely low, and as we validate your comfort, we will slowly increase the vacuum pressure. At this point, you may feel an itching sensation.
5. The suction will occur for 40-45 minutes so that the blister is formed.
6. We will quickly remove the vacuum and plate from your thigh, and using a sterilized syringe (0.5 inches), quickly puncture the blister to collect the blister fluid.
7. This method will be repeated 5 times on each of the visits (will be obtained 30 minutes before drinking the ensure, and then 60, 120, 180, and 240 minutes following).

After participants have the 5 samples drawn from the blisters and they are provided a blister Band-Aid, Ashley Zahabian or Myles Ingram will advise each participant to keep the blister Band-Aid on for at least 24 hours. They will also be given 3 extra blister Band-Aids for replacement. They will also be advised not to spend time in the sun unless the Band-Aid is covering the blister area.

Part 2: Part 2 will be taken at least 1 week after part 1. After your 12 hour fast, you will come into the office to take your second meal. This will be an exact replication of the part 1 step, however, there will be an increase in the size of the Ensure drink. In part 2, the 5 samples will be drawn again using the same methods. Part 2 should take roughly **4-5 hours**.

Part 3: Part 3 will be taken at least 1 week after part 2. After your 12 hour fast, you will come into our office at 20 Blue Hill Plaza Pearl River, NY, to take your third and last meal. This will be an exact replication of the part 2 step, however, there will be a slight increase in the size of the Ensure drink again. In part 3, the last 5 samples will be drawn again using the same methods. Part 3 should take roughly **4-5 hours**.

Each sample taken at each time period will be labeled with identifier code so that personal information is not attached to the data, and will be stored in a personal freezer at -80 degrees Celsius at 20 Blue Hill Plaza Pearl River, NY.

At any time, if there are any questions or concerns, Ashley or Myles will be available to provide oversight throughout the duration of the study. Additionally, entertainment (a laptop with Netflix) will be provided throughout the study. Once all parts are complete, we will provide you with compensation. Compensation cannot be provided to participants who do not complete the research study.

Failure to comply with the fasting regimen or falling out of the inclusion criteria at any point in the study will be reason for us to terminate your participation in this study regardless of your consent.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

1. Between ages 21-40.
2. Healthy individual - will be defined as having no known chronic health problem (any disease/illness that requires chronic treatment) at the time of the study.
3. No major surgeries within the last 12 months (defined as any invasive operative procedure in which tissue and/or an organ is cut, e.g. a body cavity is entered, organs are removed, or normal anatomy is altered).
4. BMI between 18.5% - 24.9%.
5. At least 110 lbs.

Exclusion Criteria:

1. Having a chronic disease that requires prescription medication treatment. This includes but is not limited to hypertension, diabetes, hyperlipidemia, hypothyroidism, hyperthyroidism, hyponatremia, rheumatoid arthritis, chronic pain, anemia, cancer or cancer treatment.
2. Having a major surgery in the past 12 months – defined as invasive operative procedures in which tissue and or an organ is cut, e.g. a body cavity is entered, organs are removed, or normal anatomy is altered).
3. Having a BMI < 18.5 or > 24.9%
4. Smoking cigarettes or e-cigarettes, use of nicotine patches or gum, or use of smokeless tobacco during the past 12 months.
5. Use of opioids, benzodiazepines, marijuana, or illegal drugs in the past 12 months.
6. Below 110 lbs.
7. Pregnant (determined by a urine pregnancy test)

USE OF DATA

Using data from the samples that are drawn and the data from the foods that were ingested, we will run statistical analysis to determine whether there are any molecules in the fluid sample that

correlate with the meal. The data will be confidential, Ashley and Myles will de-identify all participant data, and no personal information will be sent out to any labs when analyzing the samples. Additionally, names and personal information will not be included in the analysis or dataset.

Questions before and after the study will be asked of the participant, with the most sensitive questions relating to health complications in the past or present, or any health complications that run in the family. You may refuse to answer any question or refuse to eat any of the provided snacks, however, this will be counted as voluntary withdrawal from the study. You may do this by calling the PI at (678) 777-0616.

New findings, developed during the course of the research, which may relate to participant's willingness to continue in the research, will be provided to the participant.

RISKS, STRESS, OR DISCOMFORT

The suction vacuum machine that we will be using has the potential to leave marks of discoloration on the skin and temporary scarring. We will provide a blister Band-Aid and Neosporin to help the would heal properly without any scarring.

There is also a risk of discomfort from the suction that the vacuum provides in order to create the blister. Ashley or Myles will be beside you the entire duration of the study. Should there be any discomfort, either Ashley or Myles will immediately stop the suction and vacuum pressure to release any discomfort.

With any use of needles, there is a risk of infection. The syringes and needles will be packaged in sterile conditions, and skin will be cleansed with isopropyl alcohol swabs prior to use. After completion, the subject's thigh will be inspected to make sure that no reaction has occurred, and the skin will again be cleansed with an alcohol swab to prevent bacteria and infection. A blister Band-Aid with Neosporin will be provided.

There is also a risk of dizziness/low blood sugar/dehydration from fasting. We will conduct experiments in the morning to minimize the disruption to your eating habits. Experiments will also take place on non-consecutive days to further lessen the impact of fasting.

There is also the risk of a breach of confidentiality. Throughout the recruitment process, you will be required to email the team's email address, which is password protected. Nobody outside of the research team will be in contact with the participant or have any personal information on the participant. Additionally, any datasets attached to generated data will not have your personal information. All electronic data will be stored on password-protected single-user machines in our office at 20 Blue Hill Plaza Pearl River, NY, and the researcher's password protected computer, and will only be accessible to researchers of this study.

VOLUNTARY PARTICIPATION

Participation in this research is voluntary. You may refuse to participate or withdraw from participation at any time without jeopardizing anything other than the $50 Amazon gift card. The investigator or research staff may withdraw you at his/her professional discretion as well for any reason.

ALTERNATIVE PROCEDURES
The alternative to this study is to not participate.

PRIVATE INFORMATION
Any information derived from this research that personally identifies you will not be voluntarily released or disclosed without your separate consent, except as specifically required by law. The data extracted from the samples, will be confidential (your name will not be linked to the data and will be linked to a unique number ID instead), and will only be accessed by the research team.

CONTACT INFORMATION AND VOLUNTARY WITHDRAWAL
If at any time you have questions or concerns regarding the research or your participation, please reach out to the PI (principal investigator) via email at ingrammyles8@gmail.com. You should also contact the investigator if you have any complaints or would like to voluntarily withdraw from this research project. If at any time you have comments regarding the conduct of this research, please call the investigator (Myles) at (678) 777-0616 or the co-investigator (Ashley) at (201) 638-6216.

If you experience a research related injury, please contact Ashley Zahabian at (201) 638-6216 or by email at azahabian@gmail.com. You can also contact Myles Ingram at (678) 777-0616 or by email at ingrammyles8@gmail.com. If you require any medical evaluation or treatment, Ashley or Myles will advise and help you contact your primary care physician.

PARTICIPANT'S STATEMENT
I have read the above purpose of the study, and understand my role in participating in the research. I volunteer to take part in this research. I have had a chance to ask questions, and should more questions arise, I will ask the individuals listed above. I understand that I may refuse to participate or withdraw from participation at any time without jeopardizing anything other than the $50 Amazon gift card, which is the participation reward. I also understand that Ashley or Myles may withdraw me at his/her professional discretion when deemed necessary.

I certify that I am equal to or older than 21 and under the age of 40, and freely give my consent to participate in this study. I will receive a copy of this document for my records.

Subject's signature/consent:
Date:

Name:

OUR STATEMENT

I have discussed the proposed research with this participant and in my opinion, the participant understands the benefits, risks, and alternatives (including non-participation) and is capable of freely consenting to participate in this research.

Signature/Member of the Research Team:
Date:
Print Name:

If you have any questions regarding your rights as a participant in the study, you may contact Solutions IRB (the body that oversees our protection of study participants) at **(855) 226-4472** or participants@solutionsirb.com.