PROTOCOL TITLE:

Response: *Modulating dietary protein to alter circulating and gut metabolites*

PROTOCOL VERSION/AMENDMENT # AND DATE

Response: *IRB2019-00415_MOD006; 1/23/20*

PRINCIPAL INVESTIGATOR:

Response: *David C. Montrose, Ph.D.*

DATE:

Response:

1.0 Objectives

1.1 Describe the purpose, specific aims, or objectives of this research. Specifically, explain why it is important to do the study.

Response: This study will test whether altering protein content in diet will change the levels of small molecules in blood and feces, with a major focus on amino acids. Additionally, we will test whether these dietary alterations affect gut bacteria. Many of the nutrients that individuals take in through diet can impact human health. More specifically, our group and others have shown that dietary amino acids enhance tumor growth through their use of these small molecules as a fuel source. We believe that reducing overall protein consumption will benefit cancer patients by reducing the amount of amino acids that tumors can use to support their growth. Before testing this, a study in healthy volunteers needs to be carried out to determine whether altering the amount of protein in their diet, impacts the levels of amino acids (and related metabolites) in blood or feces. By demonstrating that we can alter these small molecules in healthy individuals, it will provide us with information on the dietary formulation and duration of consumption required to achieve the biological effect we are seeking in future studies with cancer patients. To this end, we plan to carry out a study to determine whether modulating the amount of protein in specially formulated diets alters small molecules (e.g. amino acids) found in the blood and feces, as well as the types of bacteria found in feces, in healthy volunteers. Positive data from this study will provide the rationale for testing whether modulating dietary protein in cancer patients is beneficial.

1.2 State the hypothesis to be tested, if applicable.
NOTE: A hypothesis is a specific, testable prediction about what you expect to happen in your study that corresponds with your above listed objectives.

Response: *We hypothesize that modulating dietary protein content will alter blood and fecal metabolites and cause a shift in the types of bacteria found in the gut.*

2.0 Scientific/Safety Endpoints

2.1 Describe the scientific endpoint(s), the main result or occurrence under study. NOTE: Scientific endpoints are outcomes defined before the study begins to determine whether the objectives of the study have been met and to draw conclusions from the data. Include primary and secondary endpoints. Some example endpoints are: reduction of symptoms, improvement in quality of life, or survival. Your response should not be a date.

Response:
To determine whether reducing protein intake alters:
Circulating metabolites as determined by targeted metabolite profiling of blood.
Fecal metabolome as determined by targeted metabolite profiling.
Fecal microbiota as determined by 16S rRNA and metatranscriptomic analyses.

3.0 Background

3.1 Provide the scientific or scholarly background, rationale, and significance of the research based on the existing literature and how it will contribute/fill in gaps to existing knowledge.

Response: *It is known that tumors have an affinity for taking up amino acids from circulation or nearby tissues to use as a fuel source, to enhance their growth. Work in rodents by our group and others, has shown that when the levels of amino acids are reduced in diet, tumor growth is slowed and tumors are more susceptible to anti-cancer therapies. There are currently limited dietary recommendations for cancer patients, which represents an urgent and unmet need. It is likely that reducing dietary protein will be beneficial, however this has not been tested. In advance of carrying out a study in cancer patients a study in healthy volunteers needs to be carried out to determine whether altering the amount of dietary protein impacts the levels of amino acids (or other metabolites) in blood or the intestine. By demonstrating that we can induce alterations in healthy individuals, it will provide us with the information on the dietary formulation and duration of consumption required to achieve the biological effect we are seeking in cancer patients, for future studies.*

3.2 Include complete citations or references:

Response: *Maddocks et al., Nature, 2012*
Labuschagne et al., Cell Reports, 2014
Maddocks et al., Nature, 2017
Montrose et al., unpublished

4.0 Study Design

4.1 Describe and explain the study design (e.g. case-control, cross-sectional, ethnographic, experimental, interventional, longitudinal, and observational). Indicate if there is randomization, blinding, control group, etc. If randomizing, explain how this will be achieved.

Response: *The proposed study is experimental. Individuals will be provided a 'baseline' diet for 1 week then switched to a high protein diet for 2 weeks then to a low protein diet for 2 weeks. An additional 2 days will be allotted at the end of each feeding period to allow for sufficient time to collect samples) potentially adding an additional week to the entire study (See Figure 1 below). If subjects are able to provide samples immediately after completing a given feeding period, they will immediately be advanced to the next stage of the study. Therefore, the study will last from 5 to 6 weeks depending on the efficiency of sample collections. Samples will be collected after the acclimation period and after completion of the high protein diet period and after completion of the low protein period (see Figure 1 below). Therefore, individuals will serve as their own controls.*

5.0 Local Number of Subjects

5.1 Indicate the total number of subjects who will be enrolled or records that will be reviewed through Stony Brook.

Response: *We will enroll 14 subjects with the prediction that 10 will complete the study. Given that the subjects are healthy volunteers, medical records will not be searched but instead subjects' health status will be evaluated through a brief health questionnaire (provided as an attachment to this protocol application).*

5.2 If this study is only being conducted through Stony Brook, provide statistical justification (i.e. power analysis) for the number of subjects provided in 5.1 above. If qualitative research, so state, and provide general justification for the total number of subjects proposed.

Response: The statistical analysis for the primary aim of the study will compare circulating amino acid levels in subjects after consuming high or low protein diets for 2 weeks. We will

enroll 14 subjects with the prediction that 10 will complete the study. A sample size of 10 subjects will allow 80% power to detect minimum differences of 0.996 standard deviations in plasma amino acid levels at 5% significance with the use of paired t-tests.

5.3 If applicable, indicate your screen failure rate, i.e., how many subjects you expect to screen to reach your target sample.

Response: We will have 2 layers of screening during recruitment. The initial screening will be Qualtrics-based and done remotely upon a potential subject viewing an advertisement and accessed from a QR code on the paper announcements or as a link in the email announcements. This initial screen will exclude potential participants if they don't meet the basic eligibility criteria (screening survey attached to this protocol). This will be done anonymously if they don't pass the survey. We predict that about 40 people will take the screening survey and 50% of the individuals who take this initial survey will not be eligible. The second screen will occur in person and consist of individuals completing a health questionnaire and being provided a detailed description of study design and requirements to participate in the study. The detailed description will include a review of the two choices of menus/ingredients for each phase of the study, followed by written agreement by the subject to eat the foods provided. Of the 20 people remaining who will undergo the in-person screening, we predict that approximately 20% of those individuals will be excluded, resulting in our goal of enrolling 14 people to result in 10 people having completed the entire study.

5.4 Justify the feasibility of recruiting the proposed number of eligible subjects within the anticipated recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?
Response: In light of the fact that we will be targeting SBU students for recruitment, there is a pool of approximately 25,000 individuals. Therefore we believe that reaching our goal of enrolling 14 will be feasible.

6.0 Inclusion and Exclusion Criteria

NOTE: If your study is more than minimal risk, you must also upload a copy of your inclusion/exclusion checklist (with space for specific subject values) to be completed at time of enrollment of each subject.

6.1 Describe, in bullet points, the criteria that define who will be included in this study:

Response: **Age of 18-30 years*
**BMI of 18-29.9*
**Pregnancy*

6.2 Describe, in bullet points, the criteria that define who will be excluded from this study:

Response: **Those with a history of significant illness including diabetes, renal, liver or cardiovascular disease, malnutrition, GI disease (including IBS, IBD, chronic constipation or diarrhea), mental illness (i.e. depression, bipolar disorder)*
**Those adhering to a vegetarian or vegan diet.*
**Those having taken antibiotics within 1 month of starting the study*

6.3 Describe how individuals will be screened for eligibility. Upload all relevant screening documents with your submission (screening protocol, script, questionnaire). Identify who will certify that subjects meet eligibility requirements.

Response: *We will have 2 layers of screening during recruitment. The initial screening which will be Qualtrics-based and done remotely (accessed from a QR code on the paper announcements or as a link in the email announcements) and will immediately exclude potential participants if they don't meet the basic eligibility criteria (screening survey attached to this protocol). This will be done anonymously if they don't pass the survey. If they do pass the survey, individuals will submit their name, email address and phone number so that they can be scheduled for a follow up in-person screening. The second screen will occur in person. The in-person interview will be conducted by a dietetic intern from the Dietetic Internship of the Division of Nutrition at Stony Brook University in room 206 of the Earth and Space Science (ESS) building at Stony Brook University (private office) or in room 3-064C in the HSC building on the East side of the Stony Brook University campus (conference room located in the office suite of the Division of Nutrition). During this interview, interested participants will be provided greater detail of the study design (i.e. told that they must only consume the meals they are provided and must pick up their daily meals each day for the period of the study) to determine whether they are still willing to participate after receiving this information. They will also be asked to complete a health questionnaire (health questionnaire attached to this protocol). Based on inclusion and exclusion criteria the dietetic intern will enroll subjects and obtain consent. Dietetic interns will then teach the subjects how to complete the Qualtrics-based food log and conduct the indirect calorimetry study.*

6.4 Indicate whether you are specifically recruiting or targeting any of the following special populations in your study using the checkboxes below. (You will be asked for additional information in Section 7 if you check any of these boxes)

Response: N/A

☐ Adults unable to consent
☐ Minors (under 18 years old)
☐ Pregnant women
☐ Prisoners

6.5 Indicate if you will include minorities (American Indians, Alaskan Native, Asian, Native Hawaiian, Pacific Islander, Black [not of Hispanic origin] and Hispanic) as Federal mandates require that you include minorities unless you can justify their exclusion

Response:

☒ Yes
☐ No, Justify:

6.6 Indicate whether you will include non-English speaking individuals in your study. Provide justification if you will specifically exclude non-English speaking individuals. Review http://research.stonybrook.edu/human-subjects-standard-operating-procedures/policy-non-english-speakers-research-subjects for SBU policy on inclusion of non-English speakers. Upload any translated materials (consent, questionnaires, etc).

Response: *Since our subject population will be undergraduate, graduate or medical/dental students, therefore all participants will have a working knowledge of English.*

7.0 Vulnerable Populations

7.1 For research that involves pregnant women, review, complete and upload Supplemental Form A: Pregnant Women, Fetuses, Non-Viable Neonates , or Neonates of Uncertain Viability.

☐ Confirmed
☒ N/A: This research does not involve pregnant women.

7.2 For research that involves neonates of uncertain viability or non-viable neonates, review, complete and upload Supplemental Form A: Pregnant Women, Fetuses, Non-Viable Neonates, or Neonates of Uncertain Viability.

☐ Confirmed
☒ N/A: This research does not involve non-viable neonates or neonates of uncertain viability.

7.3 For research that involves prisoners, review, complete and upload Supplemental Form H: Prisoners

☐ Confirmed

☒ N/A: This research does not involve prisoners.

7.4 For research that involves minors (under 18 years), review, complete and upload Supplemental Form F: Minors

☐ Confirmed

☒ N/A: This research does not involve persons who have not attained the legal age for consent to treatments or procedures ("children").

7.5 For research that involves adults who cannot consent for themselves, you will be asked additional information in Section 25 ("Informed Consent")

☐ Confirmed

☒ N/A: This research does not involve this population

7.6 Consider if other specifically targeted populations such as students, employees of a specific firm, or educationally or economically disadvantaged persons are vulnerable. Provide information regarding their safeguards and protections, including safeguards to eliminate coercion or undue influence.

Safeguards include:

☒ N/A

8.0 Eligibility Screening

8.1 Describe screening procedures for determining subjects' eligibility. Screening refers to determining if prospective participants meet inclusion and exclusion criteria.

📎 Include all relevant screening documents with your submission (e.g. screening protocol, script, questionnaires) as attachments.

Response: *We will have 2 layers of screening during recruitment. The initial screening which will be Qualtrics-based and done remotely (accessed from a QR code on the paper announcements or as a link in the email announcements) and will immediately exclude potential participants if they don't meet the basic eligibility criteria (screening survey attached to this protocol). This will be done anonymously if they don't pass the survey. If they do pass the survey, individuals will submit their name, email address and phone number so that they can be scheduled for a follow up in-person screening. The second screen will occur in person. The in-person interview will be conducted by a dietetic intern from the Dietetic Internship of the Division of Nutrition at Stony Brook University in room*

206 of the Earth and Space Science (ESS) building at Stony Brook University (private office). During this interview, interested participants will be provided greater detail of the study design (i.e. told that they must only consume the meals they are provided and must pick up their daily meals each day for the period of the study) to determine whether they are still willing to participate after receiving this information. They will also be asked to complete a health questionnaire (health questionnaire attached to this protocol). They will be asked if they are pregnant or are planning to become pregnant. Based on inclusion and exclusion criteria the dietetic intern will enroll subjects and obtain consent.

☐ N/A: There is no screening as part of this protocol.

9.0 Recruitment Methods

☐ N/A: This is a records review only, and subjects will not be recruited. NOTE: If you select this option, please make sure that all records review procedures and inclusion/exclusion screening are adequately described in other sections, including date range for records that will be reviewed.

9.1 Describe source of subjects: When, where, and how potential subjects will be recruited.
NOTE: Recruitment refers to how you are identifying potential participants and introducing them to the study. These may include, but are not limited to: ResearchMatch.org, physician referral, Office of Clinical Trials database, West Campus departmental pools, reviewing medical charts, Research Participant Groups/help groups, advertising companies, call centers, in person announcements / presentations

Response: The study will be advertised on the weekly 'Campus Announcements' email blast, emails sent via student group listservs (e.g. medical students and student clubs) posted in academic buildings, residence halls, cafeterias and locations of student activity.

9.2 Describe how you will protect the privacy interests of prospective subjects during the recruitment process.
NOTE: Privacy refers to an individual's right to control access to him or herself. This is NOT asking about confidentiality of data.

Response: Recruitment flyers and email blasts will be distributed via public venues and inclusive list-serve groups.

9.3 Identify/describe any materials that will be used to screen/recruit subjects and upload copies of these documents with the application. They may include, but are not

limited to Telephone scripts for calling, flyers, Questionnaires, Posters, Letters or written material to be sent or emailed, pamphlets, posted advertisements, email invitations.

Response: The study will be advertised as text in the weekly email announcements (text attached) and as a paper flyer posted around campus (flyer attached). An initial screening questionnaire will be Qualtrics-based and completed remotely (accessed from a QR code on the paper announcements or as a link in the email announcements) and will immediately exclude potential participants if they don't meet the basic eligibility criteria (screening survey attached). A health questionnaire will be provided at the in-person interview to assess basic health status and medications (questionnaire attached).

10.0 Research Procedures

Provide a detailed description of all research procedures or activities being performed on the research subjects. **This should serve as a blueprint for your study and include enough detail so that another investigator could pick up your protocol and replicate the research.** For studies that have multiple or complex visits or procedures, consider the addition of a schedule of events table in in your response. Be sure to include:

- Procedures being performed to monitor subjects for safety or to minimize risks.
- All drugs and devices used in the research and the purpose of their use, and their regulatory status

Response: After providing consent, subjects will undergo an indirect calorimetry test, which involves breathing through a disposable, single-use mouth piece into a machine that estimates calorie needs based on oxygen consumed and CO2 produced. Then subjects will be instructed on how to keep a 3-day food record and complete the Qualtrics survey designed for this purpose within 2 weeks of consenting. Dietetic interns will calculate nutrient content of the participants' typical diet, based on the 3-day, Qualtrics-based food/beverage log, using Food Processor software. Meals for the acclimation period will be created to mimic the participants' typical diet (daily calories and percent of calories from carbohydrate, protein and fat), as well as to meet their calorie needs per the indirect calorimeter test, for the first 7-9 days of the study (Figure 1). High protein diets (2g/kg/day) will then be devised and provided for the next 14-16 days. Following this period, low protein diets (o.8g/kg/day) will be devised and provided for the next 14-16 days (Figure 1). Fat content will be modulated to keep diets isocaloric while keeping carbohydrate content stable. Participants will choose between two 3-day cycle menus for each feeding phase. Meals will be prepared by Megan Bennett, RD, Chef in a metabolic kitchen housed in SBU's Business Incubator at Calverton with the help of SBU dietetic interns. Meals will be prepared in batches, packaged and frozen at the incubator, then transported in a cooler with thermometers by members of the Nutrition Division to the Food Service Department at SBU Hospital. Meals will be kept frozen in freezers until the day they are provided to participants. Each day of the feeding period, dietetic interns will take all meals for all participants for that day from the freezers and place them in a cooler with a thermometer to bring them to a campus dining area. Logs of temperatures of coolers will be maintained daily, with temperatures checked every hour.

In order to provide meals, participants will come to room 3-064C in the HSC building on the East side of the Stony Brook University campus (conference room located in the office suite of the Division of Nutrition) ~~a semi-private area on the 2nd floor of the Student Activity Center~~ between 8:30-11AM to eat breakfast onsite and pick up other meals and snacks for the rest of the day, from a dietetic intern. Participants will be provided a closed envelope containing a form that will list their participant ID# and age in which they'll sign in and indicate if they've begun any new medications or been diagnosed with a disease since starting the study (form attached). Additionally, every Monday and Thursday, participants will be weighed using a digital scale in a private area within the Division of Nutrition Suite~~on the 2nd floor of the Student Activity Center~~, in order to track changes in body weight while on the study. If weight changes greater than 2% of baseline, calorie content of the meals will be modified to avoid further weight changes. Food for the rest of the day will be provided in an insulated bag containing frozen cold packs and participants will be instructed to keep lunch and dinner in the insulated bag or a refrigerator until eaten. They will be instructed to heat the respective meals in a microwave until hot, prior to consumption. They will be instructed to take a picture of each meal before and after eating and upload the pictures via a Qualtrics-based survey, in order for the study team to estimate how much food has been eaten at each meal. Although participants will be instructed to only eat the food and beverages provided for the study, they will log any consumption of food or beverages not provided for the study via this same Qualtrics-based survey. Consumption of water, black coffee and tea will not be logged.

During days 7-9, 24-26 and 41-43 (Figure 1) participants will provide a stool sample to be placed into a sterile container (provided by the study team) then placed into an opaque brown paper bag and brought to the Division of Nutrition Suite ~~student health services~~ within 1 hour, where it will be kept cold in a cooler until ~~refrigerated until picked up by the study team~~ ~~to be~~ stored at -80ºC. On the same day as providing~~At the same time~~ the stool sample ~~is delivered~~, blood will be drawn by a phlebotomist at Student Health Services to generate plasma. An order for the blood draw will be placed by a physician at Student Health Services. The physician will not have any interaction with the study subject. After the blood draw, the phlebotomist at Student Health Services will call and email a member of the Montrose Lab to report the sample collection and samples will kept at 4º C until picked up. ~~Stool and p~~Plasma samples will be picked up by a member of the Montrose Lab within 1 hour of being contacted. Plasma will be distributed into 2mL screw cap tubes in 4 equal aliquots and ~~stool sample will be cut in half and stored in two 50mL tubes and all samples will be~~ placed into a -80ºC freezer until analysis. If a participant provides samples in the earlier part of the collection period (e.g. day 7) they may proceed immediately to the next phase of the study.

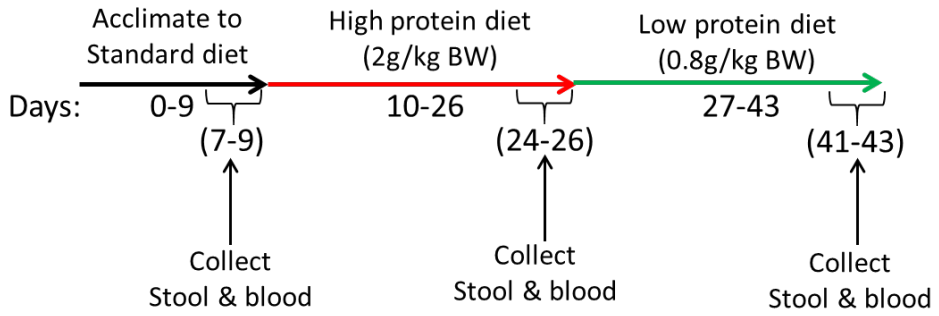

Figure 1. Experimental design.

10.1 Describe what data, including long-term follow-up, will be collected.
NOTE: For studies with multiple data collection points or long-term follow up, consider the addition of a schedule or table in your response.

Response: Primary endpoints:
Circulating metabolite levels in blood
Fecal microbiota profiling by 16S rRNA analysis & metatranscriptomics
Fecal metabolite levels

List, and upload, any instruments or measurement tools used to collect data (e.g. survey, scripts, questionnaire, interview guide, validated instrument, data collection form).

Response: These documents have been uploaded.

10.2 Describe any source records that will be used to collect data about subjects (e.g. school records, electronic medical records) and include the date range for records that will be accessed..

Response: N/A

10.3 Indicate whether or not the results for individual subjects, such as results of investigational diagnostic tests, genetic tests, or incidental findings will be shared with subjects or others (e.g., the subject's primary care physician) and if so, describe how these will be shared.

Response: N/A

10.4 Indicate whether or not generalized study results will be shared with subjects or others, and if so, describe how these will be shared.

Response: N/A

11.0 Study Timelines

11.1 Describe the anticipated duration of the study needed to enroll all study subjects.

Response: We anticipate that we will enroll all participants within a 3 month period and before the end of Fall semester.

11.2 Describe the duration of an individual subject's participation in the study. Include length of study visits, and overall study follow-up time.

Response: The maximum length of time a participant will be on the study is 6 weeks (see Figure 1 above). Subjects will commit approximately 30 minutes each morning to eat breakfast and collect the rest of the meals for the day. They will commit approximately 30-60 minutes on 3 occasions to deliver stool sample sand have blood drawn. After completing the study, no follow up will be needed.

11.3 Describe the estimated duration for the investigators to complete this study (i.e. all data is collected and all analyses have been completed).

Response: We anticipate that primary endpoints will be generated within 1 year of initiating subjects on the study.

12.0 Research Setting

12.1 Describe all facilities/sites/locations where you will be screening and conducting research procedures. Include a description of the security and privacy of the facilities (e.g. locked facility, limited access, privacy barriers). Facility, department, and type of room are relevant. Do not abbreviate facility names.

Example: "A classroom setting in the Department of Psychology equipped with a computer with relevant survey administration software," "The angiogram suite at Stony Brook University Hospital, a fully accredited tertiary care institution within New York State with badge access,"

Response: The in-person interview will be conducted in room 206 (private office) of the Earth and Space Science (ESS) building at Stony Brook University. Meals will be provided in a semi-private reserved area on the 2nd floor of the Student Activity Center. Stool samples will be delivered and blood samples taken at Student Health Services, a location already established to ensure confidentiality.

12.2 For research procedures being conducted, for this study, external to SBU and its affiliates (e.g., in schools, out-of-state, internationally, etc.) describe:

- *Site-specific regulations or customs affecting the research*
- *The composition and involvement of any community advisory board*
- *Local scientific and ethical review structure outside the organization.*

- *Local issues affecting the research and rights of research subjects.*

NOTE: This question is not referring to multi-center research. If this research is being conducted internationally, Supplemental Form C must be completed and uploaded.

Response:

☒ N/A: This study is not conducted outside of SBU or its affiliates.

13.0 Resources and Qualifications

13.1 The Principal Investigator (PI) must confirm, in consultation with Chair and Dean as applicable, that adequate resources are present to conduct and complete the study compliantly and safely. Specifically:

☐ **NO** ☒**YES** *The proposed subject population(s) are available in sufficient numbers to meet the study requirements*

☐ **NO** ☒**YES** *Sufficient funds are available to conduct and complete the study compliantly and safely*

☐ **NO** ☒**YES** *The PI and study team have sufficient time to conduct and complete the study compliantly and safely*

☐ **NO** ☒**YES** *The PI has determined that the named study team is qualified to conduct the research compliantly and to monitor the safety and welfare of the enrolled research subjects effectively.*

☐ **NO** ☒**YES** *The PI ensures that the study team is fully aware of his/her involvement in this study and the details of the study protocol*

☐ **NO** ☒**YES** *The PI ensures that the study teams will only be involved in research procedures for which they have been trained, and are currently certified and/or licensed, if required..*

13.2 Describe the availability of medical or psychological resources that subjects might need as a result of anticipated consequences of the human research, if applicable. (e.g, "on-call availability of a counselor or psychologist for a study that screens subjects for depression").

Response: We don't anticipate any adverse events given the nature of this study, but subjects will have access to any medical care that is needed through Student Health Services.

13.3 Describe your process to ensure that all study team members are updated on the progress of the research and the regulatory requirements (including enrolled subjects, unanticipated problems etc.)

Response: The study team will meet weekly to review the progress of recruitment, enrollment and sample collection. The PI will update the rest of the study team when data related to primary endpoints are generated.

14.0 Other Approvals

14. List approvals that will be obtained prior to commencing the research (e.g., University Hospital sign-offs per the UH Application, Cancer Center Scientific review, school, external site, funding agency, laboratory, Radiation Safety, IBC, SCRO, IACUC, RDRC).

Response:

☒ N/A: This study does not require any other approvals.

15.0 Provisions to Protect the Privacy Interests of Subjects

15.1 Describe how you will protect subjects' privacy interests during the course of this research and any steps you will take to make the subject feel at ease.
NOTE: Privacy refers to an individual's desire/right to control access to or to place limits on whom they interact with or whom they provide personal information. Privacy applies to the person. Confidentiality refers to how data collected about individuals for the research will be protected by the researcher from release. Confidentiality applies to the data.
Examples of appropriate responses include: "participant only meets with a study coordinator in a private office setting where no one can overhear", or "the participant is reminded that they are free to refuse to answer any questions that they do not feel comfortable answering."

Response: In person screening will take place in a private office in which no one can overhear the discussion taking place. If they do not feel comfortable answering the health questionnaire they will be reminded that they do not have to. Meals will be provided in a semi-private area so participants don't feel uncomfortable receiving meals in an atypical manner. Blood will be drawn in a private room in a confidential manner.

16.0 Data Management and Analysis

16.1 Describe the data analysis plan, including any statistical procedures. This section applies to both quantitative and qualitative analysis.

Response: For our primary endpoint of measuring circulating amino acid levels, data will be transformed to reflect log2 fold change values at each of the 2 time points following administration of the experimental diets, compared to the levels at baseline. Paired t-tests will be used to determine whether significant changes occur.

16.2 If applicable, provide a power analysis.
NOTE: This may not apply to certain types of studies, including chart/records reviews, survey studies, or observational studies. This question is asked to elicit whether the investigator has an adequate sample size to achieve the study objectives and justify a conclusion.

Response: The statistical analysis for the primary aim of the study will compare circulating amino acid levels in subjects after consuming high or low protein diets for 2 weeks. We will enroll 14 subjects with the prediction that 10 will complete the study. A sample size of 10 subjects will allow 80% power to detect minimum differences of 0.996 standard deviations in plasma amino acid levels at 5% significance with the use of paired t-tests.

17.0 Confidentiality

A. Confidentiality/Security of Study Data

Describe the local procedures for maintenance of security and confidentiality of **study data and any records that will be reviewed for data collection.**

17.1 Where and how will all data and records be stored? Include information about: password protection, encryption, physical controls, authorization of access, certificates of confidentiality, and separation of identifiers and data, as applicable. Include physical (e.g. paper) and electronic files.

Response: Qualtrics uses "Transport Layer Security" encryption for all transmitted data, and is hosted by Stony Brook University Division of Information Technology for the purpose of conducting research. Subjects will enter their ID number on every survey and this will be data to be downloaded and organized by subject ID in an Excel spreadsheet. Electronic documents will be stored on a password-protected share drive and access to the drive will be limited to members of the study team. Paper records will be stored in a locked filing cabinet in the office suite of the Division of Nutrition. All documents will be de-identified and coded except for a master electronic document containing the key to the codes, which will be stored on a password-protected drive by the PI.

17.2 How long will the data be stored?

Response: Data will be stored indefinitely to allow for endpoints to be assessed at a later time point.

17.3 Who will have access to the data?

Response: Members of the study team listed in "myResearch" proposal

17.4 Who is responsible for receipt or transmission of the data?

Response: Members of the study team listed on "myResearch" proposal

17.5 How will the data be transported/transmitted?

Response: Participant-generated data (i.e. responses to screening questions) will be submitted via Qualtrics. Data input or analysis carried out by members of the study team will be done on SBU tagged computers and files saved to the password-protected drive.

B. Confidentiality of Study Specimens

Describe the local procedures for maintenance of confidentiality of study specimens.

☐ **N/A:** No specimens will be collected or analyzed in this research.
(Skip to Section 18.0)

17.6 Where and how will all specimens be stored? Include information about: physical controls, authorization of access, and labeling of specimens, as applicable.

Response: Tubes containing subject specimens will be de-identified by replacing the subjects' name with a numbered code. The tubes will be stored in a -80º C freezer located in room 9-166 in the BST.

17.7 How long will the specimens be stored?

Response: Indefinitely

17.8 Who will have access to the specimens?

Response: Members of the study team listed on "myResearch" proposal

17.9 Who is responsible for receipt or transmission of the specimens?

Response: Members of the study team listed on "myResearch" proposal

17.10 How will the specimens be transported?

Response: Specimens will be picked up from Student health services and transported to the Montrose lab by members of the study team listed on "myResearch" proposal.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

☒ **N/A:** This study is not enrolling subjects OR is limited to records review procedures only OR is a minimal risk study

18.1 Describe the plan to evaluate the data periodically regarding both harms and benefits to determine whether subjects remain safe. The plan might include establishing a data safety monitoring committee and a plan for reporting data monitoring committee findings to the IRB and the sponsor.

Response: N/A

18.2 Describe what data are reviewed, including safety data, untoward events, and efficacy data.

Response: N/A

18.3 Describe any primary or secondary safety endpoints.

Response: N/A

18.4 Describe how the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants).

Response: N/A

18.5 Describe the frequency of safety data collection, including when safety data collection starts.

Response: N/A

18.6 Describe who will review the safety data.

Response: N/A

18.7 Describe the frequency or periodicity of review of cumulative safety data.

Response: N/A

18.8 Describe the statistical tests for analyzing the safety data to determine whether harm is occurring.

Response: N/A

18.9 Describe any conditions that trigger an immediate suspension of the research.

Response: N/A

19.0 Withdrawal of Subjects

☐ N/A: This study is not enrolling subjects. This section does not apply.

19.1 Describe anticipated circumstances under which subjects may be withdrawn from the research without their consent.

Response: Subjects may be withdrawn from the study if they begin taking antibiotics or are diagnosed with a significant illness of the gastrointestinal tract while on the study. Additionally, if subjects fail to pick up their meals or eat the provided meals for more than 2 days in a row, they will be removed from the study.

19.2 Describe any procedures for orderly termination.
NOTE: Examples may include return of study drug, exit interview with clinician. Include whether additional follow up is recommended for safety reasons for physical or emotional health.

Response: The subject will be contacted and informed of their removal from the study. No follow up will be needed.

19.3 Describe procedures that will be followed when subjects withdraw from the research, including retention of already collected data, and partial withdrawal from procedures with continued data collection, as applicable.

Response: If a subject is removed from the study before the second specimen collection, the specimens and dietary data from that individual will not be used for analysis. Already collected specimens will be destroyed. If a subject is removed from the study following the second

specimen collection but before the last collection, the samples from the first 2 collections and dietary data collection up to withdrawal will still be analyzed.

19.4 Describe what will happen to data already collected.

Response: Data from specimen analyses will not be generated until all subjects have completed the study. If a subject is removed from the study following the second specimen collection but before the last collection, the samples from the first 2 collections will still be analyzed and dietary data collected up until that point will be analyzed.

20.0 Risks to Subjects

20.1 In your opinion, what is the overall risk (physical and nonphysical) to research subjects in this study (minimal, greater than minimal or unknown)

Response: Minimal

20.2 Describe if any subjects are withdrawn from therapeutic procedures or drugs (e.g., washout periods) prior to, or during, their participation in the study.

Response: N/A

20.3 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related to their participation in the research. Consider physical, psychological, social, legal, and economic risks. Include a description of the probability, magnitude, duration, and reversibility of the risks.

NOTE: Breach of confidentiality is always a risk for identifiable subject data.

Response: The requirement to collect meals each day will be a minimal but temporary inconvenience. Subjects will experience mild discomfort upon having blood drawn due to the insertion of the needle and the potential to develop bruising in that area (as described on the consent form). Such effects will be temporary and are not expected to have a significant impact on the individual. There is no risk or discomfort in performing the indirect calorimetry test.

20.4 Describe procedures performed to minimize the probability or magnitude of risks, including procedures being performed to monitor subjects for safety.

Response: The minimal risks described above cannot be avoided due to the nature of the study but are not expected to have any major impact on the subject.

20.5 If applicable, indicate which procedures may have risks to the subjects that are currently unforeseeable.

Response: We do not anticipate that this will occur.

20.6 Indicate which research procedures, if any, may have risks to an embryo or fetus should the subject be or become pregnant.

Response: N/A

☒ **N/A**

20.7 If you responded to 20.6 that there are such risks, how will you minimize the risk of a pregnancy occurring during the course of the study? (Select all that apply)

☐ Counseling on birth control and /or abstinence
☐ Pregnancy test during the study
☐ Pregnancy test prior to initiation of the study
☐ Other _____
☒ **N/A**

20.8 If applicable, describe possible risks to others who are not subjects.

Response: N/A

21.0 Potential Benefits to Subjects

21.1 Describe the potential benefits that individual subjects may experience by taking part in the research. Include the probability, magnitude, and duration of the potential benefits.

Response: No benefit

21.2 Indicate if there is no direct benefit.
NOTE: Compensation cannot be stated as a benefit.

Response: No direct benefit

Indicate if there is a potential benefit to others, future science or society.

Response: The ability to demonstrate that reducing dietary protein lowers circulating amino acids levels in healthy volunteers will lay the foundation for testing this approach in cancer patients with the goal of enhancing cancer therapy and improving outcome.

22.0 Compensation for Research-Related Injury

☒ **N/A:** The research procedures for this study do not present risk of research related injury. This section does not apply.

22.1 If the research procedures carry a risk of research related injury, describe the available compensation to subjects in the event that such injury should occur.

Response: N/A

22.2 Provide a copy of contract language, if any, relevant to compensation for research related injury.
NOTE: If the contract is not yet approved at the time of this submission, submit the current version here. If the contract is later approved with different language regarding research related injury, you must modify your response here and submit an amendment to the IRB for review and approval.

Response: N/A

23.0 Economic Burden to Subjects

23.1 Describe any costs that subjects may be responsible for because of participation in the research.
NOTE: Some examples include transportation or parking.

Response: Subjects are not expected to have significant costs associated with participating in this study.

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

24.0 Compensation for Participation

☐ **N/A:** There is no compensation for participation. This section does not apply.

24.1 Describe the amount/nature and timing/scheduling of any compensation to subjects, including monetary, course credit, or gift card compensation. Describe any prorated payments based on participation.

Response: Subjects will be given $100 every 2 weeks (up to 6 weeks or $300) while participating in the study. Additionally, they will be provided with all meals and snacks over a 6 week period, which represents an approximate value of $1,000.

24.2 Justify the amount and scheduling of payments described above to ensure that they are reasonable and commensurate with the expected contributions of the participant. If multiple visits are involved payments should be prorated.
Note: If using West Campus Departmental pools, participation in studies may be offered for credit in class but students MUST be given other options for fulfilling the research component that are comparable in terms of time, effort, and education benefit. Please list alternative activities

Response: The compensation being provided to the participants is significant enough to compensate them for their time and any inconvenience they may experience. The payment schedule helps to ensure that subjects will complete the study. Moreover, there is significant value of the food being provided to them.

25.0 Informed Consent

25.1 Will you be obtaining consent from subjects?

☒ ***Yes*** *(If yes, Provide responses to each question in this Section, and upload your consent documents where indicated in the electronic submission system)*

☐ ***No*** *(If no, Skip to next section)*

25.2 Describe how the capacity to consent will be assessed for all subjects. Review for guidance http://research.stonybrook.edu/human-subjects-standard-operating-procedures/determining-potential-adult-subjects-ability-consent:

Response: Subjects are being recruited from the pool of Stony Brook undergraduate and graduate student population, and need to complete a web-based Qualtrics survey to be considered and consented. By navigating completion of this survey and having student status, subjects will be demonstrating the ability to consent for this study.

25.3 Describe the consent process that will be conducted to ensure that subject is fully informed regarding study details and subject rights. Include where the consent process will take place, with consideration of the need to protect the subject's right to privacy.

Response: Subjects will be consented by trained dietetic interns of the Nutrition Division of SBU. Interns will be trained on how to consent by supervisors of the internship program who are also research team members and experienced researchers. The consenting process will take place in a private office (described above) with the door closed to ensure privacy. The consent form will be read to the potential subject and a detailed description of the study will be made so that the individual is fully aware of the requirements and their role in the study.

25.4 Describe how you will ensure that subjects are provided with sufficient time to consider taking part in the research study. Detail if there is there any time period expected between informing the prospective subject and obtaining the consent.
NOTE: It is respectful to the prospective subject to ensure that sufficient time is given to have their questions answered and to consider their participation

Response: Individuals will make an appointment for the final screening and consent process. Potential subjects will be given the time to have all their questions addressed, and the study fully explained. Subjects will be asked if they need additional time to consider consenting, and if they express the need for more time, they will be given a second appointment within 1 week.

25.5 Describe the process to ensure ongoing consent, defined as a subject's willingness to continue participation for the duration of the research study.

Response: When participants arrive to eat breakfast and pick up their meals each day, they will be asked whether they are comfortable continuing on the study.

Non-English Speaking Subjects

☐ *N/A: This study will not enroll Non-English speaking subjects.*

25.6 Indicate which language(s) other than English are likely to be spoken/understood by your prospective study population or their legally authorized representatives.

Response: Given that the target population is made of students, they will have a working knowledge of English.

25.7 If subjects who do not speak English will be enrolled, describe the process to consent the subjects, as well as the process to be used to ensure their understanding of research procedures throughout the conduct of the study. Review SOP's section 17.8 for important policies in this regard: http://research.stonybrook.edu/human-subjects-standard-operating-procedures/policy-non-english-speakers-research-subjects for SBU policy on inclusion of non-English speakers.

Response: N/A

Adults Unable to Consent

☒ **N/A:** This study will not enroll adults unable to consent.

25.8 Justify why it is necessary to include adult subjects who are unable to consent.

Response: N/A

25.9 Describe how you will identify Legally Authorized Representatives (LAR) for the subjects that will be consistent with the NYS Family Health Care Decisions Act (FHCDA; see http://research.stonybrook.edu/human-subjects-standard-operating-procedures/definitions-2). Indicate why it is necessary to include subjects who are unable to consent.

Note: For research conducted outside of New York State, provide information that describes which individuals are authorized under applicable law to consent on behalf of a prospective subject to their participation in the research.

Response: N/A

25.10 Describe the process for obtaining assent from the adult subjects

Indicate whether assent will be obtained from all, some, or none of the subjects. If some, indicate which adults will be required to assent and which will not.

Response: N/A

If assent will not be obtained from some or all subjects, provide an explanation of why not.

Response: N/A

25.11 Describe whether assent of the adult subjects will be documented and the process to document assent.

Response: N/A

25.12 Describe how you will obtain consent from a subject to use their data if they later become capable of consent. How will competence be assessed and by whom?

Response: N/A

26.0 Waiver or Alteration of Consent Process

Complete this section if:

- Informed consent will not be obtained at all
- Informed consent will be obtained, but not documented, or
- consent will be obtained, but not all required information will be disclosed (e.g., in deception research)

☒ **N/A:** A waiver or alteration of consent is not being requested.

26.1 Review, complete, and upload SUPPLEMENTAL FORM G: Consent Waivers

☐ Confirmed

26.2 If the research involves a waiver of the consent process for planned emergency research, please contact the Office of Research Compliance for guidance regarding assistance in complying with federal regulations governing this activity (see: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=50.24)

27.0 Multi-Site Research (Multisite/Multicenter Only)

☒ **N/A:** This study is not an investigator-initiated, multi-site study. This section does not apply.

27.1 If this is a multi-site study where SBU is the lead site and/or the IRB of record, describe the processes to ensure communication among sites. Include:

- *All sites have the most current version of the IRB documents, including the protocol, consent document, and HIPAA authorization.*
- *All required approvals have been obtained at each site (including approval by the site's IRB of record).*
- *All modifications have been communicated to sites, and approved (including approval by the site's IRB of record) before the modification is implemented.*
- *All engaged participating sites will safeguard data as required by local information security policies.*
- *All local site investigators conduct the study appropriately.*
- *All non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy.*

Response: N/A

27.2 Describe the method for communicating to engaged participating sites:

- *Problems*
- *Interim results*

- *Study closure*

Response: N/A

27.3 Indicate and statistically justify the total number of subjects that will be enrolled or records that will be reviewed ***across all sites****.*

Response: N/A

28.0 Banking Data or Specimens for Future Unspecified Use

☒ **N/A:** This study is not storing data or specimens for research outside the scope of the present protocol. This section does not apply.

IMPORTANT: If you are proposing to bank specimens for future use, you may be subject to licensure requirements under the NYS Department of Health, and must be covered under the SBU license. See SOPs at http://research.stonybrook.edu/human-subjects-standard-operating-procedures/data-tissue-registries-banks

28.1 If data will be banked for research outside of the scope of the present protocol, describe where the data will be stored, how long they will be stored, how will they be accessed, and who will have access to the data
NOTE: Your response here must be consistent with the information provided to subjects in your Consent Documents

Response: N/A

28.2 If specimens will be banked (stored) for research outside of the scope of the present protocol, describe where the specimens will be stored, how long they will be stored, identifiers that will be associated with each specimen, how will they be accessed, and who will have access to the specimens

NOTE: Your response here must be consistent with the information provided to subjects in your
Consent Documents

Response: N/A

28.3 Describe the procedures to release banked data and/or specimens for future uses, including: the process to request a release, approvals required for release, who can obtain data or specimens, and the data to be provided with specimens.

Response: N/A

29.0 Drugs and Devices

☒ **N/A:** This study does not involve drugs or devices. This section does not apply.

29.1 Does this study involve use of radiopharmaceuticals? ☐ Yes ☒ No

29.2 For investigational devices (including marketed devices being used off label), Provide the following information below:
Where will the device(s) be stored? Note that the storage area must be within an area under the PI's control
Describe the security of the storage unit/facility
Provide full detail regarding how the dispensing of the device(s) will be controlled (accountability of removal/return of used devices, and disposition of remaining devices at the conclusion of the investigation) and documented (accounting records/logs)

Response: N/A

29.3 For investigational drugs (including marketed drugs being used off label), will the services of the Investigational Drug Pharmacy be used for storage, dispensing, accounting the drug (required for research conducted at UH, HSC, Cancer Center, and Ambulatory Surgery Center)?

☐ **Yes**

☐ **No** →PI Provide the following information below:

- *Where will the drugs/biologics be stored? Note that the storage area must be within an area under the PI's control*
- *Describe the security of the storage unit/facility:*
- *Provide full detail regarding dispensing of the drugs(s), how labeled, controlled (accountability, disposition of unused drug at the conclusion of the investigation) and documented (accounting records/logs):*

Response: N/A

30.0 Sharing of Results with Subjects

30.1 Describe whether results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) will be

shared with subjects or others (e.g., the subject's primary care physicians) and if so, describe how it will be shared.

Response: Results will not be shared with subjects.