OFFICE OF RESEARCH
UtahStateUniversity

Approval of the Convened IRB

From: Melanie Domenech Rodriguez, IRB Chair

Nicole Vouvalis, HRP Director

To: ***Stephen van Vliet***

Date: ***March 17, 2022***

Protocol #: ***12499***

Title: ***Effects of Different Sources of Beef on Inflammatory and Metabolic Profiles of Consumers***

Your proposal has been reviewed and approved by the Institutional Review Board (based on the Belmont Report and Department of Health and Human Services (DHHS) regulations for the protection of human research subjects, 45 CFR Part 46, as amended to include provisions of the Federal Policy for the Protection of Human Subjects, January 21, 2019).

This approval applies only to the proposal currently on file for the period of one year. You will be asked to submit information regarding the status of the project approximately **one month prior to the anniversary of the date of original approval**. It is your responsibility to submit renewal paperwork at least one week prior to the IRB's **February meeting** by clicking "Renew" and completing the required steps. Kuali Protocols will send a notification informing you to complete these steps, but you can also locate IRB meeting dates for the semester on the IRB's website, at https://research.usu.edu/irb/timelines/. As part of the IRB's quality assurance procedures, this research may also be randomly selected for post-approval monitoring. If so, you will receive a request for completion of a Monitoring Report Form during the month of the anniversary date of original approval. Protocols reviewed and approved by the Convened IRB are subject to continuing review every year.

Any change affecting human subjects, including extension of the expiration date, must be approved by the IRB **prior** to implementation by submitting an Amendment request. Injuries or any unanticipated problems involving risk to subjects or to others must be reported immediately to the Director or Chair of the Institutional Review Board. If Non-USU Personnel will complete work on this project, they may not begin until an External Researcher Agreement or Reliance Agreement has been fully executed by USU and the appropriate Non-USU entity, regardless of the protocol approval status here at USU.

Prior to enrolling human subjects, properly executed informed consent must be obtained from each subject or from an authorized representative, and documentation of informed consent must be kept on file for at least three years after the project ends. Each subject must be furnished with a copy of the informed consent document for their personal records.

Upon receipt of this memo, you may begin your research. If you have questions, please call the IRB office at (435) 797-1821 or email to irb@usu.edu.

The IRB wishes you success with your research.

TITLE: Effects of Different Sources of Beef on Inflammatory and Metabolic Profiles of Consumers

PRINICIPAL INVESTIGATOR:

Stephan van Vliet, PhD
Department of Nutrition, Dietetics and Food Sciences
Utah State University
stephan.vanvliet@usu.edu
T: 435-797-5369 C: 217-778-5001

TABLE OF CONTENTS

1. BACKGROUND AND SIGNIFICANCE 2
2. STUDY OBJECTIVES 2
3. DESIGN AND PROCEDURES 2
3.1 Study duration 2
3.2 Participants 2
3.3 Study Schedule 3
4. SUBJECT RECRUITMENT AND COMPENSATION 6
4.1 Recruitment 6
4.2 Compensation 7
5. RISK/BENEFIT ASSESSMENT 7
6. COSTS TO THE SUBJECT 7
7. ANALYTICAL PROCEDURES AND STATISTICAL CONSIDERATIONS 7
8. DATA STORAGE AND CONFIDENTIALITY 8
9. DATA SAFETY AND MONITORING 9
10. LITERATURE 9

1. BACKGROUND AND SIGNIFICANCE

An increasing number of people is interested in consuming either grass-fed beef or plant-based beef alternatives, such as the Impossible Burger™ or Beyond Meat™, believing that these choices are beneficial for consumer health; however, little to no work has been performed to study these beliefs and claims. The *purpose of this study* is to evaluate effects of plant-based meat, grass-fed beef, and grain-fed beef on postprandial plasma inflammatory and metabolite profiles in middle aged adults (30-60 y old). This work will offer new insight into manipulating the acute, post-meal inflammatory state by consumer food choices. It will also provide more insight how food-derived metabolites impact systemic metabolites in the human body through metabolomics analysis. This study will utilize a randomized cross-over design and every participant will consume each type of meat on three separate occasions separated by a minimum of three days.

2. STUDY OBJECTIVES

- To determine the effects of consuming 9 oz of the Impossible Burger™, grass-fed beef or grain-fed beef on 300 min postprandial **plasma inflammatory markers (IL-6, TNF-α, C-reactive protein, and VCAM-1).**

- To determine effects on **plasma metabolites (vitamin and mineral derivatives, phenolics, amino acids etc.)** collected through regular urine sampling during the 300 min postprandial phase (at 60 min, 180 min, and 300 min).

- To determine the effects on **subjective satiety** at 30, 60, 90, and 120 min and every hour thereafter following consumption.

3. DESIGN AND PROCEDURES

3.1 Study duration

The study timeline for this protocol is a minimum of 3-4 weeks depending on participant and study staff availability.

3.2 Participants

We will include up to 40 middle aged adults (30-60 years) in this study. Inclusion/exclusion criteria are listed below.

***Inclusion criteria*:**

- Age ≥30 and ≤60 years;
- BMI ≥25 and ≤35 kg/m^2;
- Weight stable in last 3 months (Loss or gain <4%);
- Hemoglobin A1C (HbA1C) ≤6.4%;
- Fasting plasma glucose concentration <126 mg/dl;
- For the safety of the participant and proper consent of the procedures, subjects must be able to speak and understand English to participate in this study

***Exclusion criteria*:**

- Use of medications that are known to affect the study outcome measures (e.g., NSAIDs, corticosteroids) or increase the risk of study procedures (e.g., anticoagulants) that cannot be temporarily discontinued for this study;
- Consuming >14 drinks per week;
- Use of cigarettes (or other tobacco products) in last 3 months;
- COVID vaccine within the last two weeks;
- Engaged in high-level competitive exercise (e.g., triathlon, marathon, powerlifting);
- Any inflammatory diseases (e.g. autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease, arthritis);
- Diagnoses of active malignancy, congestive heart failure, diabetes mellitus and/or chronic obstructive pulmonary disease;
- Use of antibiotics in last 60 days;
- Pregnant or planning to in the next month*;
- Lactating women;
- Soy allergy;
- Persons who are unable or unwilling to follow the study protocol or who, for any reason, the research team considers not an appropriate candidate for this study, including non-compliance with screening appointments or study visits.

* Participants that are pregnant or planning on becoming pregnant in the next month are excluded. The justification is the documented alterations in metabolism that occur during pregnancy (Lain and Catalano, 2007), which would impact our metabolomics analysis in this study.

3.3 Study Schedule

Participants will be asked to complete one screening/consent visit and three testing visits throughout their participation in the study. All visits will take place in clinical research space located at the Center for Human Nutrition Studies (CHNS). The research team will accommodate the availability of the research participants, including weekend days if needed. The table below describes these activities.

Visit	Timepoint	Activities	Location	Duration
Phone Screen	Week 1	- Pre-screening questions to determine eligibility.	Virtual	15 min
Consent	Week 1	- Consent (online or in person)	Virtual or CHNS	1 hour
Screening	Week 1	- Height/weight - Blood draw - Screening questionnaires	CHNS	1 hour
Metabolic visit (3 visits separated by 3-7 days)	Week 2-4	- IV Placement - Eat study provided meal - Postmeal phase (300 min) - Regular urine and blood collection	CHNS	7 hours each

Phone Screen (Week 1. Duration: 15 min): Interested participants will be pre-screened by telephone, using a scripted list of questions (see attached) to identify individuals that may be eligible for study entry. Suitable candidates will then be provided a REDCap survey link to complete additional screening questions that asks them further details about their health, dietary habits, and sleep habits (see inclusion/exclusion criteria). Only the minimum PHI will be collected to determine eligibility. Responses will be reviewed by a study team member (all study team members will have completed the necessary CITI training) and potentially eligible candidates will be contacted to schedule a consent/screening visit. The pre-screen is put in place to confirm that the participant meets most of the inclusion/exclusion criteria before the subject is scheduled for a consent meeting. The phone screen is thus performed to limit the amount of ineligible research participants that are consented/screened that could easily be excluded by means of a phone screening. This would also provide interested participants with the opportunity to ask additional questions about the study and to determine if they are interested in moving forward with a consent/screening visit. This will limit both subject and research staff time burden.

Consent Visit (Week 1. Duration: 1 hour): This visit can be done virtually through Zoom or occur in person in the Center for Human Nutrition Studies (CHNS) at Utah State University. Potential subjects will attend a consent session conducted by a trained member of the research staff to present the details of the study. Interested participants will complete the informed consent process privately with study staff. We will allow up to 60 minutes for the subjects to read the consent and ask questions. If a subject wants additional time to think about the study, this will be allowed. The study team will contact the subject three days after the initial consent visit ask if he or she has come to a decision. No study procedures will take place before written consent is obtained. To minimize subject burden, participants will be instructed during the phone screen that they have to opportunity to combine the consent visit with their screening visit (see below). If participants wish to combine visits, they are instructed that they will have to perform this visit in the morning after an overnight, 12-hour fast.

Screening Visit (Week 1. Duration: 1 hour): This visit will take place at the Center for Human Nutrition Studies (CHNS) and will last approximately 1 hour. We ask participants to come in during the morning after an overnight, 12-hour fast. During this visit the following procedures will be performed:

- **Blood Draw:** A trained phlebotomist (the PI) will draw a fasted blood sample for routine blood work (see table 2 below) to ensure the participant qualifies for the study. Using a small needle, 12 ml blood will be collected from a vein in the participant's forearm or hand. Blood samples will be sent to LabCorp to be analyzed for HbA1C, Glucose, Basic Metabolic Panel (BMP), and Lipid panel. The blood of women of childbearing potential will be subject to a pregnancy test (hCG) as part of this screening blood draw. This will also occur through LabCorp.

- **Body mass and height.** Height (cm) will be measured without shoes. Body mass (kg) will be measured without shoes, coats, or sweaters. BMI will be calculated as body mass (kg) / height $(m)^2$.

- **Blood pressure:** A blood pressure measurement will be taken using an automated upper arm blood pressure monitor (Omron BP5250). A total of three measurements will be taken and the average value of those will be collected for participant characterization.

- **Questionnaires:** Participant will be given a three-day food and sleep log to fill out for three days prior to each of the metabolic visits described below. The participant will also be given a food frequency questionnaire once at the beginning of the study, which will ask them about how often they consume animal products and if they are any of the following: "grass-fed, organic, pasture-raised, or bought on a farmers market". This will give us an indication of the habitual consumption of pasture-raised animal products of the participants. The food frequency questionnaire that will be used is a modified version of the Diet History Questionnaire III (NIH) with a 5-point ordinal scale as adapted from Baudry et al. (2015) (see attachment). The questionnaire reviews food and beverages typically consumed over the past year and takes approximately 30 minutes to complete.

Metabolic visits (Weeks 2-4. Number of Visits: 3. Duration: 7 hours each): These three visits will take approximately 7-hours each and will take place at The Center for Human Nutrition Studies (CHNS). Visits will be at least 3 days apart. Participants are required to fast for 12 hours prior to reporting to the laboratory. During this, participants will complete the following:

- **Meat consumption:** Using a randomized cross-over design, participants will ingest 250 g (9 oz) of an Impossible Burger™ burger (plant), a grain-fed beef burger (grain-fed), a grass-fed beef burger (grass-fed). The meat will be stored as 9 oz. (250 g) patties in designated food freezers (-40 °C) in the NDFS kitchen and thawed overnight in a refrigerator (4 °C) prior to the subject's arrival the next morning. The meat will be cooked on a griddle in the CHNS metabolic kitchen on each side until an instant-read meat thermometer registers an internal temperature of 160 °F (71 °C). The patties will then be immediately served to the participant. Participants are provided with a standardized amount of water (1.5 liters) to drink during the 300 min post-meal phase. Participants will be asked to eat and drink the water at a comfortable rate but to try and finish within 30 min. No other food is allowed to be consumed during the ensuing 300 min postprandial phase. No other liquids are allowed to be consumed during that time as this would interfere with our protocol (see urine collection below). Subjects are also expected to remain seated in a chair during this time and can work/read/or watch entertainment on their own electronic device. Participants will be allowed to get up and use the rest-room; however, we will ask participants to remain sedentary otherwise.

- **Blood Draws:** An intravenous catheter (IV) will be temporarily placed into a forearm or hand vein by a trained member of the study team (the PI) to collect repeated blood samples. The catheter will remain viable by a slow drip of saline. We will collect four samples total (10 mL each; 40 mL or 2 ½ tablespoons) on each of the three metabolic visits. One sample will be collected right before participants eat and three more samples will be collected at 60, 180 min and 300 min after they consume the meal. These draws will be taken in a private room in clinical facilities, in a reclining leather chair. Participants will be provided with their

clinical lab results that will be obtained from LabCorp. Results from bloodwork will be shared with participants via encrypted email using a USU email account.

- **Urine Collection:** A baseline urine sample will be collected prior to food consumption. Participants are asked to completely empty their bladder prior to food consumption. After food consumption, all urine will be collected in a urine jug (a 3000 mL urine collection bottle) during the 300-minute postprandial phase to study metabolites in the urine. All urine collections will be done by the participant themselves in private restroom facilities.

- **Satiety Scale:** Participants are asked to mark a satiety scale (Holt et al., 1995) at 30, 60, 90, 120 min, and every hour thereafter until 300 min to assess subjective satiety after meat consumption.

Dietary and activity control: We will ask participants to maintain their habitual diet, physical activity, and sleep levels. We will ask participants to fill in 3-day dietary and sleep logs for the three days prior to their metabolic visit (see attachments). We will also ask them to refrain from alcohol and strenuous physical activity (running, weightlifting etc.) for 2 days prior to each metabolic visit. The dietary and sleep logs will give us insight if lifestyle factors leading up to each visit were similar and are likely to promote compliance to ensure robust biomarker results (inflammatory cytokines and metabolomics). Participants are asked to replicate their 3-day foods logs and sleep schedule as much as possible prior to each visit. Participants are also asked to not consume red meat or soy (main ingredient of plant burger) the day before each visit. This is done to minimize any prior presence of common red meat and soy-derived metabolites in the urine.

Table 2. Schedule of sample collection

Lab/Blood Work	Screening	Met Visit 1	Met Visit 2	Met Visit 3
Pregnancy test (hCG)	**X**			
Insulin	**X**			
Glucose	**X**			
BMP	**X**			
Lipid panel	**X**			
HbA1C	**X**			
Blood inflammatory markers		**X**	**X**	**X**
Urinary/plasma metabolomics		**X**	**X**	**X**

4. SUBJECT RECRUITMENT AND COMPENSATION

4.1 Recruitment

Subjects will be recruited by posting flyers in designated areas around USU's campus, through advertisements on social media. In the advertisement, potential subjects for this study will be directed to contact the study team by email or phone to obtain more information about the study, if interested. Participants who are interested will be provided with a copy of the informed consent by email. When the participant expresses interest in being in the study, the study team member will confirm by telephone that the participant meets most of the inclusion/exclusion criteria (ones that can be determined without

the need for a blood draw) before scheduling him/her for a consent meeting. Subjects will attend a one-on-one information session that will be conducted by trained member to present the details of the study. We anticipate that we will need to telephone screen, approximately 150 people and that approximately 45 will be consented. We will enroll up to 30 participants to meet our goal of having at least 24 participants complete the study (assuming a 20 % drop-out rate).

4.2 Compensation

Participants will receive compensation for completing each study procedure for a maximum of $475, which will be paid at the end of the study. If a participant drops out of the study before completing the entire study, they will be paid according to the degree of completion. Participants will receive $25 for the screening blood draw visit and $150 for each of the metabolic visits.

5. RISK/BENEFIT ASSESSMENT

This study provides no potential benefit to the participant other than the satisfaction of having contributed to the potential future benefit of others. Participants will be provided with their lab clinical results, which may be of interest to them. Society may benefit by having more information about the health effects of different types of meat, which may help inform consumer food choices. Participation in this study may lead to increased risk for the following:

- **Blood draws:** Blood drawing can result in bruising, mild discomfort, and rarely, an infection where the needle enters the skin. There also is a possibility of lightheadedness and fainting. Sterile techniques and the use of trained phlebotomists will minimize these risks.

6. COSTS TO THE SUBJECT

There will be no costs to participants for any of the treatments or testing done as part of this research study. Immediate necessary care is available if an individual is injured because of participation in a research project. However, there is no provision for free medical care or for monetary compensation for such injury. During this study, hospitalizations or additional care beyond the scope of this study will be the responsibility of patients and/or their insurance company.

7. ANALYTICAL PROCEDURES AND STATISTICAL CONSIDERATIONS

- **Inflammation:** Inflammatory biomarkers (IL-6, VCAM-1, TNF-alpha and C-reactive protein [CRP]) will be measured using ELISA kits at Dr. van Vliet's laboratory at Utah State University. Plasma samples will be stored at -80ºC until cytokine analyses are performed.

- **Metabolomics:** Mass spectrometry techniques will be used for untargeted and/or targeted assessment of blood and/or urinary metabolites. This analysis will give insight into the appearance of food-derived metabolites in the human body and how they may impact inflammatory responses after eating. This analysis will be performed using de-identified samples by Metabolon Inc (a company dedicated to metabolomics analysis) and/or the Plant for Human Health Institute. Samples will be provided to these core services as de-identified samples. No other data will be shared with these core services. Plasma and urine samples will be stored at -80ºC until metabolomics analyses are performed.

- **Dietary logs:** The Diet History Questionnaire III (NIH) and 3-day food records will be analyzed by dietetic staff within Dr. van Vliet's laboratory at Utah State University.

- **Statistical analysis:** Change scores (post-dietary intervention – baseline) will be created for all inflammatory biomarkers and metabolites. Metabolic intermediate data will be reduced into Metabolic Factors using Principal Component Analysis (PCA) of the change scores. Factor scores will be computed for each individual for both diets. Change scores and factor (change) scores for both diets will be compared using paired t-tests. $P< 0.05$ will be considered statistically significant and analyses will be performed in SAS 9.4 (SAS, Cary, NC).

- **Power Calculations:** Using untargeted metabolomics for our power calculations of the number of participants we need to recruit, we assume a P-value ≤ 0.05, Q-value ≤ 0.3, and standard deviation of 0.3 based on previous work (Pimentel et al., 2018). Since there are no entirely comparable previous studies available to draw from, the effect size and number of truly different metabolites are meant to give general estimates. 600 metabolites are typically identified (Nieman et al., 2015) and conservative estimation is that 20-40 metabolites will be significantly different with a mean of 0.2624 (30% difference). An n=24 per group is expected to provide true discovery rates ranging from 89-93 % assuming differences in 80-120 metabolites. Assuming a drop-out of 20%, the final number of participants we will recruit is N=30. This, we consider adequate without having to engage more participants than necessary to address the research aims.

8. DATA STORAGE AND CONFIDENTIALITY

Clinical study data (including screening data, food logs, questionnaires, satiety responses, bloodwork) will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a participant coded data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium initiated at Vanderbilt University. REDCap servers are hosted in the College of Education & Human Services at Utah State University, where data will be stored and processed. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team. This iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap is flexible enough to be used for a variety of types of research and provides an intuitive user interface for database design and data entry. Coded clinical data will be stored indefinitely on REDCap and/or USU's Box. The enrollment log, with participant names/addresses that can be linked back to participant codes, which is kept on the Van Vliet Lab Shared Drive on USU's box will be destroyed 3 years after completing the study. It is anticipated that all manuscripts will be published at that time and have been sent to interested study participants. Online activity always carry with them a risk of breach. Participants will be made aware of these potential threats and risks to their confidentiality.

Biospecimens will be stored in a -80ºC freezer at the Center for Human Nutrition Studies for the duration of sample collection. All samples will be stored using coded labels. Blood samples collected during screening will be sent to Labcorp for routine bloodwork. Once all biospecimens have been collected from all participants, all biospecimens will be transported to a -80ºC freezer in the Nutrition and Food

Science building for long-term storage (maximum of 20 years). Any participant coded analytical data (including inflammatory biomarkers, metabolomics data, and analyzed food log data) will be kept on the Van Vliet Lab Drive at USU's Box Drive to be accessed only by Dr. Stephan van Vliet and his study staff. Study records that identify subjects will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, they will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Utah State University. For records disclosed outside of USU, they will be assigned a code number. The key to the code will be kept on the Van Vliet Lab Drive at USU's Box Drive. For participants that choose to withdraw from the study after biospecimens have been collected, they will have three years to instruct the research team on whether they'd like the unused identifiable blood and urine samples destroyed or if they would like the samples to be used for other research. After the three year time period, information samples will be de-identified and it will not be possible to know whose is whose.

9. DATA SAFETY AND MONITORING

All study members will have completed CITI- and other institutional training pertinent to the study. Any unanticipated problems will be reported to the USU IRB by the PI as per institutional policy.

10. LITERATURE

Baudry, J., Méjean, C., Allès, B., Péneau, S., Touvier, M., Hercberg, S., Lairon, D., Galan, P., and Kesse-Guyot, E. (2015). Contribution of Organic Food to the Diet in a Large Sample of French Adults (the NutriNet-Santé Cohort Study). *Nutrients* 7**,** 8615-8632.

Holt, S., Brand Miller, J., Petocz, P., and Farmakalidis, E. (1995). A satiety index of common foods. *European journal of clinical nutrition* 49**,** 675-690.

Lain, K.Y., and Catalano, P.M. (2007). Metabolic changes in pregnancy. *Clin Obstet Gynecol* 50**,** 938-948.

Nieman, D.C., Gillitt, N.D., Sha, W., Meaney, M.P., John, C., Pappan, K.L., and Kinchen, J.M. (2015). Metabolomics-Based Analysis of Banana and Pear Ingestion on Exercise Performance and Recovery. *Journal of Proteome Research* 14**,** 5367-5377. doi: 5310.1021/acs.jproteome.5365b00909.

Pimentel, G., Burton, K.J., Von Ah, U., Butikofer, U., Pralong, F.P., Vionnet, N., Portmann, R., and Vergeres, G. (2018). Metabolic Footprinting of Fermented Milk Consumption in Serum of Healthy Men. *J Nutr* 148**,** 851-860.