SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH AT MONTANA STATE UNIVERSITY

Study Title: Impact of Beef on Metabolites and Inflammation

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Summary:
You are being asked to volunteer as a participant in a research study of how food may influence your body's response to a meal. This response will include the measurement of many different markers in the blood. Many different substances will be measured that relate to inflammation, health, and metabolic markers in your blood. These substances are produced by your immune system and chemical reactions in your body (metabolites) or the microorganisms such as bacteria that are in your digestive system. One of the many things we will measure in the blood are called "bile acids". Bile acids are compounds made from cholesterol and amino acids (from proteins) that help with breaking down fat in your intestine during digestion.

The types and quantities of carbohydrate, fat, protein, fiber, and antioxidants in foods can influence the amount of bile acids that enter your gut during digestion. We are specifically interested in how different foods affect bile acids, inflammation, metabolism, and other health measures.

Purpose:
The purpose of this study is to determine how a beef steak meal may affect bile acids in the gut, inflammation, and the metabolic health of healthy individuals. Specifically, we are asking the following questions:

1) How do specific foods affect bile acids in the blood?
2) How do specific foods changes inflammation, metabolism, and other health measures?

If we learn how food affects different health related markers in the blood and how that may influence our health, then we can use that information to do more research to improve the health of people in a future study.

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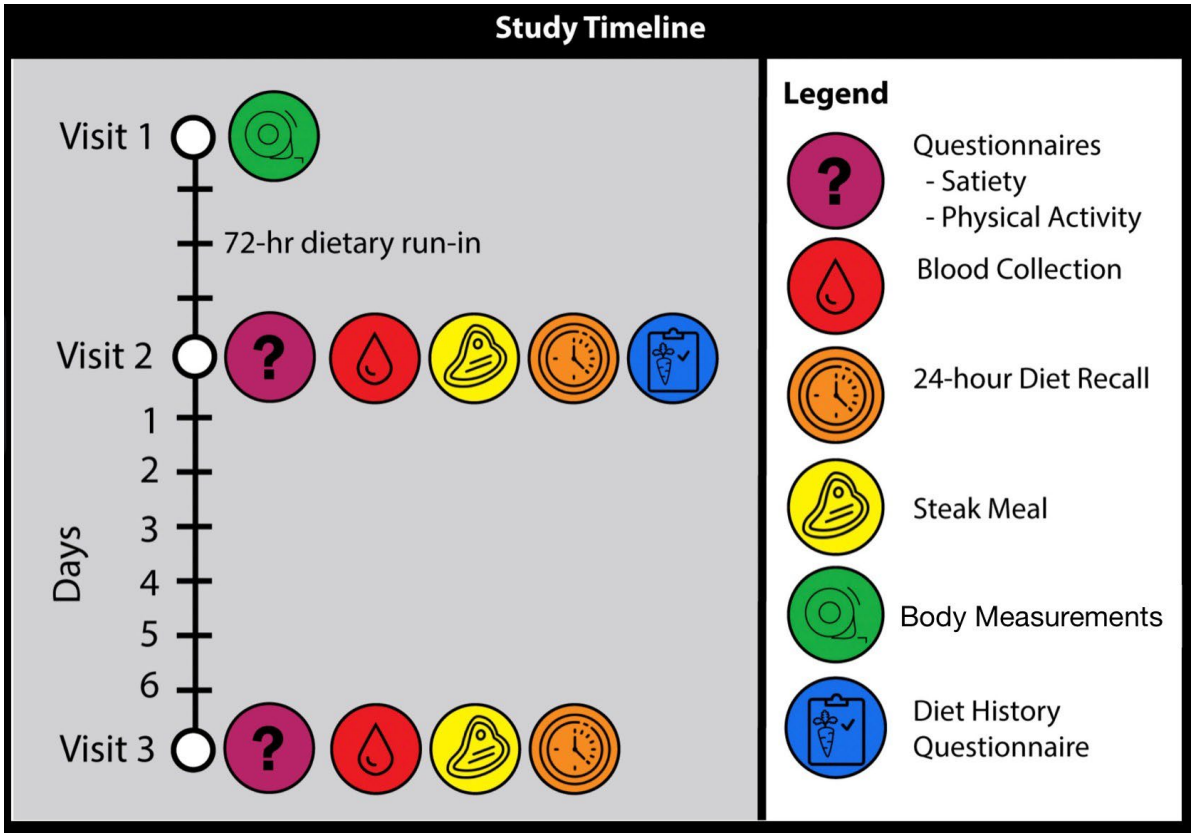
Participants in the study:
You have been asked to participate in this study because you meet the following criteria: 18-45 years of age, with a body mass index between 18-27 kg/m^2. You may not be a participant if you do not meet eligibility requirements of our screening questionnaire because of health history, symptoms, issues, or risks. You are not eligible to participate if you are taking medications to lower cholesterol, lipids, and/or inflammation, smoke cigarettes, have a gallbladder condition or have had your gallbladder removed, have an allergy to red meat, or if you are pregnant or lactating, have diabetes, or if you have other health concerns or conditions that may interfere with your participation in the study.

Procedures:
Participation is voluntary. If you agree to participate in this study, you will be asked to complete three visits to the Nutrition Research Laboratory to complete the procedures of the investigation. An initial visit (Visit 1) will take approximately 45-60 minutes. The second (Visit 2) and third (Visit 3) visits will take approximately 5 hours each and will each include a total of 9 blood draws through a sampling catheter, with each blood draw collecting about 2-3 teaspoons of blood. Blood will be drawn before a provided steak meal then every thirty minutes for 4 hours after meal consumption. There are a minimum of 72-hours between visits 1 and 2, and 7 days between visits 2 and 3. During this minimum 72- hour and 7-day period, you will be asked to follow provided dietary and physical activity instructions.

Completion of the study will take approximately three weeks, depending on scheduling of the visits. Total time spent in the Nutrition Research Laboratory is approximately 11 hours. A visual overview of the study is provided below.



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Visit 1: This visit will take 45-60 minutes and include the following activities:

1) **Informed consent.** Read and provide written informed consent (this form). We also will provide you with a copy of this form and discuss it with you prior to proceeding with any additional activities in visit 1.
2) **Health screening questionnaire.** Complete a health history questionnaire that asks questions about your health and the health of your family, particularly regarding heart disease.
3) **Physical activity questionnaire.** Complete a 3-question survey that asks questions about the types and amount of physical activity that you typically perform during the week.
4) **Body size measurements.** The researcher will take baseline measurements of height, and circumferences of your waist. You will remain clothed during these measurements; however, you will be asked to remove extra clothing items such as coats or sweatshirts. Thin, light weight clothing is preferred.
5) **Measurement of muscle and fat tissue using bioelectrical impedance analysis.** This test simply involves standing on a scale with your feet and hands on sensors for a few seconds. A very low electrical current that you cannot feel and that is not dangerous is transmitted and received across the sensors. The technology is commonly used in a variety of settings such as gyms and health screenings, but the instrument that we will use is more sophisticated in being able to estimate muscle and fat tissue in different regions of your body, including the abdominal cavity that is particularly important for predicting risk of diabetes.

Visit 2 and 3 (separated by a minimum 7 days): You will need to refrain from eating, exercising, tobacco use, and consuming alcohol for 12 hours prior to each visit. You should drink plenty of water so that you are well hydrated throughout the study. Each visit will take approximately 5 hours and include the following activities:

1) **Resting blood pressure measurement**. Two measures of blood pressure will be taken with a standard blood pressure cuff.
2) **Blood collection.** Standard procedures for the collection of blood from a forearm vein will be used to collect blood. A trained phlebotomist or physician will clean your skin with an iodine-based solution or alcohol and place a catheter needle in a vein in the front part of your elbow, (most common), forearm, or hand. The catheter needle (20 gauge or 18 gauge) is slightly larger than what is used for donations (16 gauge). A small amount of a sterile solution (saline) will be put into the catheter to keep the line open for the collection of nine total blood samples. Blood samples will allow us to measure the following values: glucose, insulin, lipid panel, inflammatory markers, and bile acids.
3) **Steak meal test.** After the line has been in your arm for 15 minutes, we will begin the test by collecting a fasting blood sample, and then having you eat a 6-ounce beef steak meal, cooked to 158°F. Blood collection will then occur through the catheter every 30 minutes for the next four hours. We will keep the arm with the catheter warm during the test and ask you to squeeze a foam ball in your hand a few times a minute to help keep blood flowing to your arm. If blood collection is not possible through the catheter toward the end of the test, then we will ask you whether you want us to complete the test by

drawing blood with a needle stick using the standard blood collection method. You can stop at any time during this test. You will have limited ability to use a laptop computer, cell phone, or read during this test. When the test is completed, the catheter will be taken out of our arm. We will have you keep pressure on the site where the catheter was inserted for 5-10 minutes.

4) **Diet History Questionnaire.** An online questionnaire that will ask you about the frequency and quantity of foods consumed in your diet. This questionnaire will go through food groups and different times of year prior to the study to estimate the types and amounts of nutrients that you typically consume in your diet. This questionnaire takes 1-2 hours to complete, but you will be able to complete this at the same time you are conducting the medium fat meal test. This questionnaire will be given only at visit 2.
5) **24-hour diet recall.** An online 24-hour diet recall will outline the types and quantities of foods and beverages consumed the day prior.
6) **24-hour physical activity recall.** An online 24-hour diet recall will outline the types and frequencies of exercise done the day prior.

Physician review of your data: After you have completed the study, you will receive the results of your blood tests in a written report. A physician, Dr. Sarah Bronsky, will review your data and provide feedback in the written report. If there are concerns, Dr. Bronsky will highlight them in the report and recommend in the report that you follow-up with your personal physician to discuss the concerns. Dr. Bronsky may be contacted to discuss the concerns, but the discussion will be separate from this research study.

Risks: There are side effects and risks involved from having blood drawn or doing certain activities. These side effects are often called risks, and for this project, the risks are:

1) An intravenous needle/plastic catheter will be placed in your arm for the removal of blood samples and infusion of fluids. This will be left in for approximately 5 hours. Approximately 12 milliliters or 2-3 teaspoons of blood will be removed on 9 occasions during the Steak Meal test. You can expect to experience some pain at the moment the needle/needle containing the plastic catheter goes into your arm. In addition to this momentary pain, there will be minor discomfort of having the catheter taped to your skin. In about 10% of cases, a small amount of bleeding under the skin will produce a bruise (hematoma). The risk of temporary clotting of the vein is about 1%, while the risk of infection of hematoma or significant external blood loss is less than 1 in 1,000. Some people may feel lightheadedness, nausea, or perhaps faint.

Biospecimens: Your samples will be used for analysis on amino acids, lipids, bile acids, and hundreds of other metabolites. The analysis of your metabolites and inflammation from your samples may take up to 2 years to complete and are not stored or banked for other analyses.

Benefits: You may gain some benefits by participating in this study, such as body composition, blood pressure, fasting blood glucose, blood lipid panels, and a diet history report. No other benefits are promised to you.

Compensation: You will receive up to $150 in the form of a gift card upon completion of your testing, $10 for the first visit, $70 each for visits 2 and 3. Participants may withdraw from the

study at any time. If they choose not to complete a condition, then the amount of money paid will be prorated depending on how much of the condition is completed.

Freedom of Consent: You have the right to withdraw from participating in the study at any time with a no questions asked policy. You may withdraw in writing, over the phone (to Mary Miles at 994-6678), or in person. If you withdraw, you will not lose any benefits you incurred up to the time of withdrawal. Your participation in this study is completely voluntary.

Funding: This study is funded by the Organic Advisory Educational Committee.

Please ask any questions: You are encouraged by the researcher to ask any and all questions you may have, as well as addressing any concerns about the study. The researcher will answer your questions as fully and as accurately as possible. Your peace of mind and comfort in the study is of utmost importance to the researchers.

Confidentiality: All data and information received from you for this study will be kept completely confidential. You will be given a subject identification number that will be used to describe all data. This data will be kept locked in a file cabinet in the Nutrition Research Laboratory. This information could be published in scientific and/or medical journals, but your identity will remain confidential. If you withdraw from the study at any time, all of your information will be deleted from the study records, and you will not be contacted again regarding the study. There are absolutely no penalties for withdrawing.

In the event of injury due to participation in this study, medical treatments such as first aid and help getting to adequate health care providers (such as transport to Bozeman Deaconess Hospital) will be provided, however, there is no compensation for any of this provided by Montana State University. You can access further information involving this policy and treatment by contacting Mary Miles at 994-6678, or emailing her at mmiles@montana.edu.

Any other questions you may have regarding your rights as a participant may be answered by the chairman of the Human Subjects Committee, Mark Quinn. He can be reached at 406-994-4707 or mquinn@montana.edu.

STATEMENT OF AUTHORIZATION

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AUTHORIZATION: I have read the above and understand the discomforts, inconveniences, and risk of this study. I, _____(name of subject), agree to participate in this research. I also agree that my health information can be collected and used by the researchers and staff for the research study described in this consent form. I understand that I may later refuse participation and that I may withdraw from the study at that time. I have received a copy of this consent form for my own records.

Signed: _____ Date: _____

Investigator: _____ Date: _____

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