Impact of the Gut Microbiome on Health Impacts of Haskap Berries

Dr. Mary Miles

NCT #: 2024-1417-FCR

November 5, 2024

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

Study Title: Impact of the Gut Microbiome on Health Impacts of Haskap Berries

Investigators: Mary Miles, Ph.D., Meghan Spears, Morgan Chamberlin
Department of Food Systems, Nutrition, & Kinesiology
Montana State University, Bozeman, MT

Why are we doing this study?

You are being asked to participate in a research study to learn more about how the gut bacteria influence health impacts from consuming Haskap berry juice. Haskap berries are very high in several compounds that function as antioxidants, which have many health promoting effects. For example, antioxidants lower inflammation, which is known to promote many diseases such as heart disease or type 2 diabetes. The antioxidants in foods interact with the bacteria in the gut and influence how well antioxidants are absorbed into the body. Humans have billions of bacteria in their intestinal tract (gut), and there are around 1,000 different species of bacteria that might be found in the gut. Most people have at least 120 of those different species in their gut and we have found that most people have 300 to 400 different species. The unique combination of bacterial species in a person's gut is called the gut microbiome. Some bacterial species break down the antioxidants in food and make it easier to absorb them. Investigating how the makeup of a person's gut microbiome influences the health benefits of high antioxidant berries may help us obtain a better understanding of how to most effectively use Haskap berries to decrease risk of diseases.

What is the purpose of this study?

The purpose of this study is to determine how certain food items may affect gut bacteria, molecules (metabolites) produced during chemical reactions in the body, inflammation, and the metabolic health of individuals. A second purpose of this study is to test how changes in the gut microbiome influence how people respond to dietary changes. Specifically, we are asking the following questions:

1) What is the impact of Haskap on the gut microbiome, metabolome, and health biomarkers in a cohort of adults with both low and high risk of metabolic syndrome?
2) How does gut microbiome composition and production of bioactive metabolites from Haskap impacts serum metabolites, health, and inflammation biomarkers in a cohort of adults with both low and high risk of metabolic syndrome?
3) How do Haskap varieties and growing practices increase production of health-promoting compounds?
4) The data collected in this investigation may also be used to ask additional questions not yet identified. For example, we may ask questions regarding the interaction and relationship amongst variables. We may ask questions regarding other variables in addition to the gut microbiome influence health biomarkers of the impacts of Haskap berries. These additional questions are called secondary analyses. Please note that no genetic analysis will be conducted and racial and ethnic differences among participants will not be used in any secondary analyses.

If we learn how food influences the bacteria in our gut 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.

Why am I being asked to participate in this study?

You are being asked to be in this study because you are 35-65 years old and may fit one of the following two groups: 1) having 0 metabolic syndrome criteria, or 2) having a waist circumference that fits the criterion for metabolic syndrome and at least one other criterion for metabolic syndrome (elevated blood pressure, elevated fasting glucose or triglycerides (a type of fat), or having a low level of high density lipoprotein (a blood lipid fraction that helps to lower risk of cardiovascular disease). **The first step in the study will be to screen you to see if you meet the criteria for one of these two groups. If you do, then you may be enrolled in the study.**

You cannot participate in this study if you have a food allergy that may include Haskap berries, lemon juice, or food colorings, or have a body mass index (measured based on height and weight) under 18 or over 40 kg/m^2. You cannot participate if you are pregnant, smoke cigarettes, or take cholesterol lowering, anti-inflammatory, blood pressure, weight loss, or other drugs that may interfere with the measures of the study, have type 1 or type 2 diabetes, or have other health conditions that may influence metabolic or inflammation measures in the study. You cannot participate in the study if you have taken antibiotics withing 90 days or supplements including probiotics/prebiotics or "superfood" supplements within 30 days of starting the study. You may also be excluded from the study if you are planning a weight loss diet or a change in exercise regimen or you follow a special diet including vegan, vegetarian, low carbohydrate, or ketogenic. Lastly, you may be excluded from the study if you regularly consume more than five servings of fruits and vegetables per day or are unwilling to reduce caffeine intake to one 8 oz serving per day for the duration of the study.

If you are eligible to be in the study after we complete the screening and choose to participate in the study, then you will be enrolled in the study to have measures taken before and after you consume either juice or a color and flavor matched beverage for eight weeks. Whether you receive the juice or the color and flavor matched beverage will be randomly assigned. Neither you nor the investigators will know which beverage you are consuming.

What will I do if I take part in this study?

Participation is voluntary. If you agree to participate then you will be asked to do the following things:

SCREENING VISIT. This visit will take 45-60 minutes and take place in the Nutrition Research Laboratory and will include the following activities:

Informed consent (this document). Researchers will go through the informed consent document with you, explain details of the study, and encourage you to ask any questions. You will be given a copy of this document for your own records. If you want to participate in the study, then you will give written informed consent to participate in the study by signing this document.

Body size measurements. Researchers will measure your height, weight, and circumference of your waist. You will remain clothed during these measurements; however, you will be asked to remove extra clothing such as sweaters and shoes.

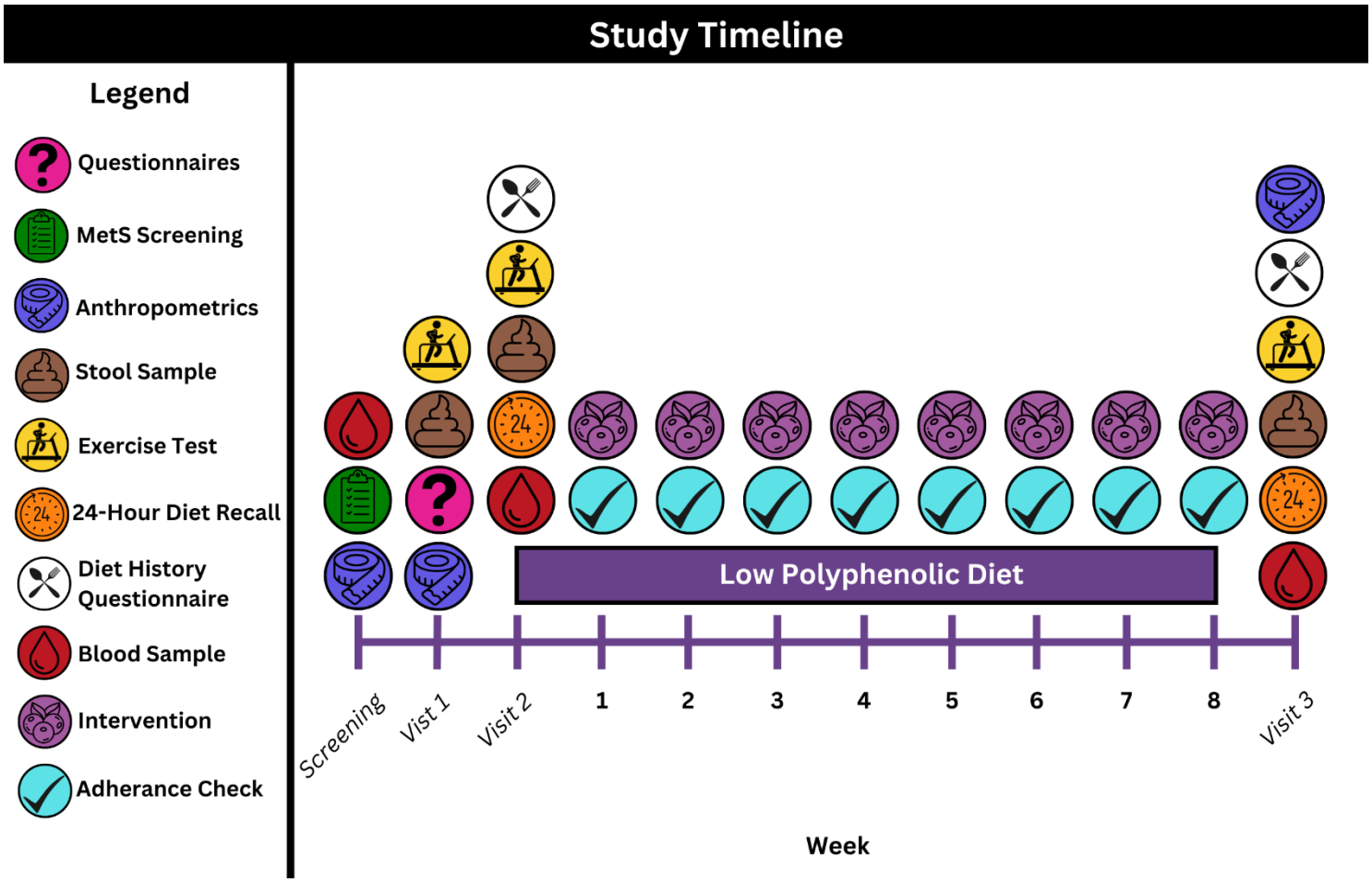
Blood collection. Blood will be drawn from a forearm vein one time using standard procedures. The blood collected will be measured for chemistry measurements that include glucose, triglycerides, and high-density lipoprotein.

These measurements will be used to determine if you fit the criteria for one of the two groups for the study. If you do not fit the criteria for one of the two groups, then your participation in the study will be done. If you fit the criteria and choose to continue with the study, then we will schedule an enrollment visit (Visit 1). Instructions and supplies will be given for **self-collection of a stool sample** for you to bring with you to Visit 1. A stool sample will be used to analyze the microbes in your gut. The analysis involves measurement of the genetic material of the bacteria. This does not give us any information about your genetic material, only that of the bacteria in your gut. You will be given

a self-collection kit that includes printed instructions to collect your stool sample. Specimen will be collected the day or evening before you come back to the lab for the enrollment visit.

Research Study Protocol. If you are eligible and agree to participate in this study, you will be asked to attend three visits to the Nutrition Research Laboratory to complete the procedures of the investigation. Visit 1 will take about 60 minutes and will include an exercise test and questionnaires. The second (Visit 2) and third (Visit 3) visits will take approximately 1.5-2 hours each and will each include an exercise test and 1 blood draw, collecting about 5 teaspoons (3 tubes) of blood each time. There are eight weeks between visits 2 and 3 during which time additional brief appointments to pick up juice for the study will be required. During the 8-week period, you will be asked to consume juice provided to you every day in the morning and evening (approximately 12 hours apart). You will be asked to avoid eating foods that contain some of the nutrients found in the Haskap berries during the 8-week intervention. You will also be asked to record whether you consumed the research juice that day and to identify any foods from the list of foods to avoid during the intervention that you may have eaten that day.

Completion of the study will take about 10 weeks (about 2 and a half months), depending on the visit scheduling. Total time spent in the Nutrition Research Laboratory is approximately 5 hours. This is a visual overview of the study:



VISIT 1: This visit will take 60 minutes and include the following activities:

1) **Stool sample drop-off.** Instructions and supplies will be given to you at the screening visit for **self-collection of a stool sample** that you will bring with you to Visit 1.
2) **Informed consent.** Due to the length of the study, we will revisit the informed consent (this form) at the beginning to discuss study activities and to answer any questions you might have. Read and provide written informed consent to continue the study.
3) **Health screening questionnaire.** Complete a health history questionnaire that asks questions about your health and the health of your family, particularly regarding heart disease.
4) **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.
5) **Race and ethnicity questionnaire**. Complete a 2-question survey that asks questions about your ethnicity and race. This survey is optional. Your information will not be used for analysis of race and ethnic differences.
6) **Sleep questionnaire**. Complete a 19-question survey that asks questions about your sleep quality and disturbances over a 1-month time interval.
7) **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.
8) **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.
9) **Submaximal exercise test.** During the first visit, this test will be done to determine which intensity of exercise allows you to burn the most fat. Test results will determine which intensity you will exercise at for the following exercise testing at Visits 2 and 3. The test involves a series of stages that get increasingly more difficult every three minutes by increasing treadmill incline for approximately 15-30 minutes total. You will wear a heart rate monitor and breathe through a mouthpiece that is connected to an analyzer. This allows researchers to measure how much you are breathing and the amounts of oxygen and fat you use during the test. Firstly, you will be asked to walk on a treadmill for 5 minutes at a self-selected comfortable speed at 0% grade to warm up before the test. After the warm-up, you will be asked to continue walking on a treadmill, and as you move to more difficult stages, intensity is increased by increasing grade. The researchers will end the test when fat utilization has reached maximum rates and begun to drop. You may end the test at any time.

Instructions and supplies will be given for **self-collection of a stool sample** for you to bring with you to Visit 2 and Visit 3.

VISITS 2 AND 3 (separated by 8-week dietary intervention): You will need to refrain from eating, exercising, and consuming alcohol for 12 hours prior to each visit. You should drink plenty of water so that you are hydrated throughout the study. Each visit will take approximately 90 - 120 minutes and include the following activities:

1) **Stool sample drop-off.** Instructions and supplies will be given to you at Visit 1 and at your final juice pick up for **self-collection of a stool sample** that you will bring with you to Visits 2 and 3, respectively.
2) **Informed consent.** Due to the length of the study, we will revisit the informed consent (this form) at the beginning to discuss study activities and to answer any questions you might have. Read and provide written informed consent to continue the study.

3) **Resting blood pressure measurement**. Two measures of blood pressure will be taken with a standard blood pressure cuff.
4) **Blood collection.** Standard procedures for collection of blood from a forearm vein will be used to collect blood. Blood samples will allow us to measure the following values: glucose and HbA1c (a measure that reflects your average glucose level for the previous 90 days), lipid panel, inflammatory markers, and metabolites (a wide variety of small molecules relating to all of the biochemical processes in your body) and other biomarkers related to metabolic health.
5) **Exercise test**. You will be asked to do 30 minutes of an uphill walk on a treadmill at the intensity determined during Visit 1 on a treadmill. You will wear a heart rate monitor and breathe through a mouthpiece that is connected to an analyzer for the duration of the test.
6) **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 (Visit 2) and the last month of the study (Visit 3) to estimate the types and amounts of nutrients that you typically consume in your diet. This questionnaire takes 1-2 hours to complete.
7) **24-hour diet recall obtained.** An online 24-hour diet recall will outline the types and quantities of foods and beverages consumed the day prior.

8-WEEK DIETARY INTERVENTION:

1) **Juice pick-ups from the Nutrition Research Laboratory.** Juice will be given to you in two-week increments. The first batch will be given to you at the completion of Visit 2, and you will need to pick up juice from the laboratory before weeks 2, 4, and 6 during the 8-week intervention.
2) **Juice consumption.** The determined dose (mL/day) will be divided into two doses to be consumed 12 hours apart (morning and evening dose). Juice dosage will be determined based on relative body weight (kg) and will be person dependent.
3) **Daily logs to verify consumption of research meal.** A paper log sheet will be given to participants on a twice daily basis and asked to respond "yes" or "no" to indicate whether you consumed the research juice that day. While we hope you will drink all the juice, we understand you may not be able to do that, and we ask you to report when you do.
4) **Adherence to low polyphenolic diet**. You will be provided with a comprehensive list of foods that you need to avoid eating during the 8-week intervention because they contain compounds also found in Haskap berries. This includes foods such as red or purple berries and fruits or juices. You will be asked to limit your caffeine intake from coffee and tea consumption to one 8 oz serving per day.
5) **Diet Adherence questionnaire**. Participants will be asked to respond to questions regarding their adherence to the diet guidelines provided. If you consume a food or beverage from the prohibited food list provided, record the amount and type consumed on this form. **You are not disqualified from the study if this occurs.** Please continue to take your supplements and follow the diet guidelines. This will be sent as an additional question in your daily text or email verification.

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) Approximately 23 milliliters (about 0.8 ounces) or 5 teaspoons of blood will be removed one time at each during three of the study visits (screening and visits 2 and 3). A trained phlebotomist or clinician will be drawing the blood. You may experience momentary pain when a needle goes into your arm. 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 or hematoma, or significant external blood loss is less than 1 in 1,000. Some people may feel lightheadedness, nausea, or perhaps faint.

2) This diet may cause gastrointestinal disturbance (flatulence or other discomfort).
3) There is a total of three exercise tests, a sub-maximal exercise test and two separate 30-minute exercise bouts. The sub-maximal exercise test and two 30-minutes exercise bouts could make you feel tired in the last stages, possibly leaving you a little bit fatigued. You may experience muscle soreness and general discomfort. This sub-maximal test is commonly used in clinical procedures for clinical diagnostics and disease evaluation. People rarely have adverse side effects, but some have occurred before. The mortality rate of this test is approximately 1 in 10,000 tests, and serious complications such as abnormal heart rhythm or chest pain for prolonged periods of time are present in about 4 out of every 10,000 tests. You can stop at any time.
4) Bacteria from the stool samples can cause illness if ingested. This risk is no different than when you normally have bowel movements. Washing your hands thoroughly with soap and hot water after collection of the sample will minimize this risk.

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

Use of blood and stool samples for future studies: The samples collected from you as part of this study may be valuable for future studies that are not yet planned. For example, we may learn new things in this study that spark new research questions that may be answered by analyzing the samples for things not planned in the current study. Or we may realize that this study will allow us to ask different questions than those identified in this document. This will not include your DNA for genetic analysis because your DNA is not being collected in the present study. If these opportunities arise, we would like to do more research using the samples collected in this study. This will not involve any extra procedures beyond those described for this study. The samples that will be stored for future analysis will be coded, and there will be no way to connect the samples with your identity.
The samples will be owned and controlled by the principal investigator of this study, Dr. Mary Miles, and they will be stored in a freezer in her laboratory. Information linking your name to the coded samples will be destroyed at the end of the current study. You have the right to refuse consent to having your samples stored for future studies. This will not prevent you from participating in the current study. If you consent to use of your samples in future studies, then you will not be able to withdraw your consent once the information linking your name to your coded samples is destroyed because we will have no way of knowing which samples are yours at that time. The samples may be stored until Dr. Miles leaves Montana State University. Dr. Miles will be the only researcher with authority to allow use of the samples if other investigators request access to the samples for future studies. You will not receive any information on data from your samples in future studies because there will be no way to link you to the samples. While it is not known what the future uses of these samples will be, some examples of future uses might be to measure new health biomarkers in the blood and urine or functional potential of the microbes from stool samples.

Compensation: You will receive a $10 Amazon gift card if you complete the screening visit but are not eligible for the study. If you are enrolled in the study, you will receive up to $200 upon completion of the study ($50 for visit 1, and $75 each for visits 2 and 3). You may withdraw from the study at any time. If you choose not to complete the study, then the amount of money paid to you will be prorated depending on how much of the study 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 (to Mary Miles at mmiles@montana.edu), over the phone (to Mary Miles at 406-994-6678), 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 USDA Agricultural and Food Research Initiative.

Please ask any questions: You are encouraged by the researcher to ask any and all questions you may have, as well as address 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 the data. This data will be kept locked in a file cabinet in the Nutrition Research Laboratory. This information could be published in scientific journals and public data repositories, but your identity will remain confidential. If you withdraw from the study at any time, all your information will be deleted from the study records, and you will not be contacted again. There are no penalties for withdrawing from the study.

Statement of Compensation: In the event your participation in this research supported by USDA AFRI results in injury to you, referral(s) to appropriate health care (Student Health Services, Bozeman Deaconess Hospital, your health care provider, or calling 911) will be available. Further information may be obtained by calling Mary Miles at 406-994-6678, or emailing her at mmiles@montana.edu.

Other question regarding this study: 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

Study Title: Impact of the Gut Microbiome on Health Impacts of Haskap Berries

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 withdraw from the study then. I have received a copy of this consent form for my own records.

SCREEENING VISIT
Signed: _____ Date: _____

Investigator: _____ Date: _____

VISIT 1
Signed: _____ Date: _____

Investigator: _____ Date: _____

VISIT 2
Signed: _____ Date: _____

Investigator: _____ Date: _____

VISIT 3
Signed: _____ Date: _____

Investigator: _____ Date: _____

Do you give us permission to use your blood or tissue for future research?

Please indicate if you agree to let us use your blood or tissue samples for future research. You do not have to give permission to use your blood or tissue samples for future research to participate in other parts of this study. Please ask questions if you do not understand why we are asking for your permission to use your samples for future research.

I agree to allow use of my blood or tissue sample for future research. *Please check Yes or No.*

☐ Yes – Please sign: _____ Date: _____
☐ No