Pilot Haskap Metabolite Timing Project

Dr. Mary Miles

NCT: 2025-2029-EXPEDITED

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

Study Title: Characterizing Gut Microbiota-dependent Metabolites of Haskap Consumption

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IRB Protocol Number: 2025-2029-EXPEDITED

Why are we doing this study?

You are being asked to participate in a research study to learn more about how gut bacteria impact health-promoting metabolites of Haskap berries. Haskap berries are a nutrient rich berry that can be grown locally in Montana. Nutrients, such as antioxidant and polyphenols, in these berries have been linked to health-promoting benefits such as improved function of blood vessels and reduced risk of metabolic diseases, like diabetes. The bacteria in the intestinal tract (gut) interact with these nutrients, influencing how they are digested and absorbed into the body. One way this occurs is by breaking down nutrients from food into metabolites, or small compounds derived from nutrients in food. The complex relationship between the bacteria in the intestinal tract and berry consumption drives the ability for health-promoting nutrients from Haskap berries to reach the blood stream and have a positive impact on the health of the consumer. Investigating the identity (what compound it is) and timing of health-promoting nutrients from Haskap berry consumption reaching the blood stream after gut microbiome digestion may help us obtain a better understanding of the effectiveness of Haskap berries to improve health.

What is the purpose of this study?

The purpose of this study is to determine when gut microbiome derived metabolites of Haskap berries enter the blood stream after consumption. This information will be used to inform blood sample collection timing for future studies. The second purpose is to identify these metabolites as Haskap metabolites that have undergone digestion by gut bacteria. Specifically, we are asking the following questions:

1) How long does it take after consumption of Haskap berries for metabolites to enter the blood stream?
2) How does gut bacteria digestion change the chemical structure of these metabolites?

If we learn how the gut microbiome digestion of Haskap berries impacts the ability for health-promoting metabolites to reach the blood stream, then we can use that information to do more research to improve the health of people in future studies.

Why am I being asked to participate in this 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 18-30 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 have taken antibiotics in the last 90-days, if you take any dietary supplements, if you have adverse reactions to blood draws (like consistent fainting), if you are unwilling to follow a prescribed no polyphenol diet (diet lacking foods that contain polyphenols) for 10 days, if you are taking medications to lower cholesterol, lipids, and/or inflammation, smoke cigarettes, have an allergy to berries, or if you are pregnant or breast feeding, have diabetes, or if you have other health concerns or conditions that may interfere with your participation in the study. **The first step in the study will be to screen you to see if you meet the criteria for inclusion. If you do, then you may be enrolled in 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.

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 and weight to confirm body mass index. You will remain clothed during these measurements; however, you will be asked to remove extra clothing such as sweaters and shoes.

Research Study Protocol. If you are eligible and agree to participate in this study, you will be asked to come to the Nutrition Research Laboratory for 3 consecutive days to complete the procedures of the investigation

DAY 1: Day 1 will take a total of 12 hours and consist of 4 blood sample collections. Your presence at the Nutrition Research Lab is not required for all 12 hours, but relative proximity to the lab is encouraged.

- In the early morning, you will be asked to come to the lab having fasted for 12 hours overnight. A fasting blood sample will be collected (baseline). Then you will consume a beverage containing Haskap berries. Three hours after the baseline blood collection, another blood sample will be collected. Six hours after the baseline blood collection, another blood sample will be collected. Lastly, 12 hours later after the baseline blood collection, another blood sample will be collected.
- **Health screening questionnaire.** Complete a health history questionnaire that asks questions about your health, particularly regarding heart disease.
- **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.

DAY 2: Day 2 will take a total of 12 hours and consist of 3 blood sample collections. Your presence at the Nutrition Research Lab is not required for all 12 hours, but relative proximity to the lab is encouraged.

- At the same time of morning as your baseline, another blood sample will be collected (24 hours after baseline). Six hours later, another blood sample will be collected (30 hours after baseline).

Lastly, 6 hours later (36 hours after the baseline blood sample) another blood sample will be collected.

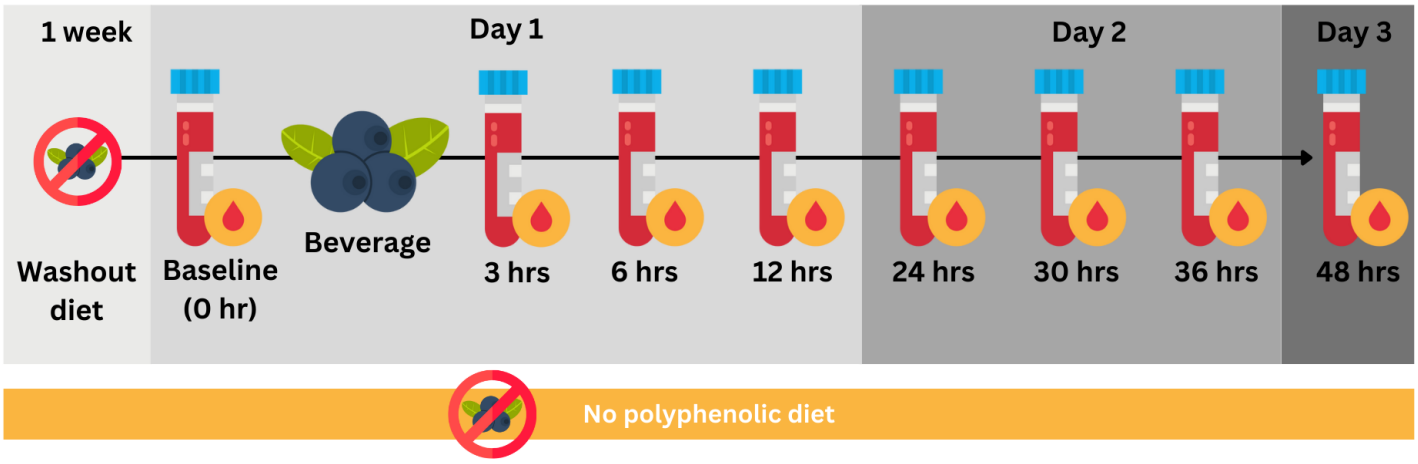
DAY 3: Day 3 will take approximately 15 minutes.

- At the same time of morning as your baseline, another blood sample will be collected (48 hours after baseline).

Dietary Guidelines: You will be asked to avoid eating foods that contain some of the nutrients found in the Haskap berries beginning 7 days prior to Day 1 and until the end of sample collection on Day 3. A specific list of foods to avoid will be provided. Only one cup (8 oz.) of coffee or tea will be allowed during the 7 days prior and 3 days of data collection. You will also be asked to record all foods consumed the day before the first blood sample collection and on the following two days (day 1 and day 2).

Blood collection. Blood will be drawn from a forearm vein a total of 8 times using standard procedures. The blood collected will be measured for the amount of certain metabolites present.

Completion of the study will take 3 days. This is a visual overview of the 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) Approximately 6 mL of blood (1.5 teaspoons) will be removed at each of the 8 blood sample collections across the 3-day study. 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) The 12-hour fast for the blood draw may cause hunger or fatigue but due to the short-term nature, risk is minimal.
3) Beverage may cause gastrointestinal disturbance (flatulence or other discomfort).
4) If you experience adverse effects from blood collection or dietary changes, please see a physician.

Benefits: No other benefits are promised to you.

Use of blood 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. Specifically, we may test these samples for other biomarkers and metabolites to investigate other impacts of Haskap berry consumption. 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. They may be shared with collaborating research groups and/or institutions. 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 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 investigate other metabolites observed in blood 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 $50 upon completion of the study ($5 per blood sample collected + $10 for completing the screening visit). 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. Data collected on paper will be kept locked in a file cabinet in the Nutrition Research Laboratory. A copy of this data will be kept in OneDrive, only accessible by research personnel. Only research personnel will have access to paper and online coded data. 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 and data collected will be deleted from the study records and/or destroyed, and you will not be contacted again. Paper data kept in a locked file cabinet and online data (OneDrive) will be kept for at least 3 years after the completion of 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: Characterizing Gut Microbiota-dependent Metabolites of Haskap Consumption

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: _____

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