



Blueberry
Absorption
Metabolism

NC STATE UNIVERSITY
**Plants for
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INVITATION TO PARTICIPATE IN A RESEARCH STUDY

BAM (Blueberry Absorption Metabolism) Study: a study investigating the nutritional quality of fresh and processed berries



You have been invited for a research study. Before you decide if you want to participate, it is important for you to understand why the research is being done and what it will involve. There are two parts to this information sheet:

Part 1- tells you the purpose of the study and what will happen to you if you participate

Part 2 - gives you more detailed information about the study procedures.

Please take time to read the following information (Attachment 1, version 1) carefully and discuss it with us if you wish any clarification or more information.

Thank you for taking time to read this!

Monique Carvalho de Santana
BAM Study Coordinator
North Carolina State University
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Phone: (704) 250-5451



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PART 1: Purpose of the study and what will happen to you if you decide to participate

What is the purpose of the study?

It is becoming increasingly clear that eating plenty of fruits and vegetables is beneficial to our health. In particular, blueberries contain substances which are shown to provide benefits to human health, which may help protect against chronic illnesses such as cardiovascular disease, and lowering the risk of developing type 2 diabetes. However, little is known about the difference in absorption of these berry-derived substances by the body when eaten. The aim of this study is to evaluate what happens to berry components in the body after they are consumed. This information will be useful for future studies looking into how fruits and vegetables may better benefit human health.

How will the study investigators determine what happens to blueberries after they are consumed?

We aim to trace the route that berry compounds take through the body, by analyzing the blood and urine of study volunteers. We will ask participants to consume a portion of two varieties of blueberries (approximately 1 cup or 5.3 oz., see picture below for portion size), a berry-rich protein bar or a nutrient-matched berry control over 4 separate occasions followed by two 1 hour follow-up visits per occasion. The blueberry-rich protein bar or control drink will provide equivalent calories to the 1 cup of berries). You will be fed each of the four berry products in a random order, and we are unable to provide an order preference. You will also be asked to consume a dairy product, specifically whey protein beverage together with the berries to match the macronutrients provided across the feeding products



Why have I been invited to participate in the study?

You have been invited to participate in this study because you have already met the basic criteria to be included in the study.

IMPORTANT!

Do I have to participate?

It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason. An expression of interest does not commit you to participation.

What happens next?

Step 1: We'll contact you!

The study coordinator will contact you after you receive this information sheet by telephone or email to answer any questions you may have. If you're **happy to proceed**, we'll arrange a consent visit at the Plants for Human Health Institute, part of the North Carolina State University, established on the North Carolina Research Campus in downtown Kannapolis. Don't worry if you're unsure how to find us, we'll send you a map.

Step 2: Come to the consent visit

Before you start the screening process and begin the study itself we need you to give your consent to take part. We will ask you to visit the North Carolina Research Campus at a convenient time where the study coordinator will discuss the study with you. This is an opportunity for you to ask any questions you may have about the study. If you are happy to proceed we will then ask you to sign the consent form (Annex 1-Consent form).

Step 3: Come to a clinical screening session

The **clinical screening session** is another opportunity for you to ask us any questions and for us to take a blood sample from you to make sure that you are healthy and suitable to take part. Women will also be asked to collect a midstream-urine sample for a pregnancy test to confirm eligibility.

You will need to avoid eating any food for at least 10 hours before the clinical screening session but you can drink as much water as you like.



Step 4: Waiting for the results

The results of the blood test will be available within 48 hours after the blood collection. If the results are within the reference range for a healthy person (as determined by the medical doctor at the pathology laboratory), we will then call you to arrange a convenient time to come in for the first study day.

Sometimes blood results can fall outside the normal reference ranges. This does not always mean you are unwell, it may be perfectly normal for you. If your blood sample is outside the normal reference range, and the medical doctor evaluating the blood results feels you are not suitably healthy to meet our inclusion criteria, you will not be eligible to participate. If this occurs you may want to contact your family doctor to see if they advise further blood tests.

Step 5: The study - What will I have to do?

Preparing for the study

For **7 days** before each feeding day we will ask you to consume a diet that is **low in** berries and foods which are similar to berries. We will of course give you both advice and instructions on how to do this and identify alternative foods (nutritionally equivalent) that can

be consumed freely (Annex 2- Food Exclusion and Alternatives List). We will provide you with a list of alternative foods to consume so you shouldn't find alternative foods as berries and red and purple fruits and vegetables make up only a small portion of the average US diet (for example, berries, jams, red fruit juices and red wine). We will also ask you to record all the food you eat each day during the 2 days prior to the first blueberry product feeding day, and this will be repeated for the other 3 feeding visits. A template will be provided for you to fill in this information (food record). We have included an example of a food intake record (Annex 3-Food intake record) with this information pack and a full version will be given to you before you start the study, at the clinical screening session.

On the two days before the feeding visit, we will ask you to collect your total urine void sample into two containers (one per 24-hour collection). We have included a copy of the instructions on how to best collect this sample (Annex 4-Urine collection instructions), how to store it and how to transport it with you to the Human Research Core at the North Carolina Research Campus, in Kannapolis, NC

The evening before the feeding day at the Human Research Core, we will also ask you not to eat at least 10 hours before your visit and have nothing to drink except water during this time.

What will happen on the day of the study?

All the study days will occur on weekdays. At the start of the first study day of each study period in the Human Research Core, we will collect your urine sample from you, ask you a few basic health questions and answer any questions you may have. Over the course of the visit a total of 5 blood samples will be taken equaling about 3/4 teaspoon of blood each time with either an individual needlestick venipuncture or via cannulation at your discretion.

We will then ask you to consume your assigned food. After this, other blood samples will be collected at 1 hour, 3 hours, 6 hours and 9 hours after consumption of the berry product. You will also be asked to collect your total amount of voided urine from 0h to 9h after consuming the intervention food. You will be given standardized "berry-free" meals (breakfast, lunch, dinner, and snacks) during your visit.

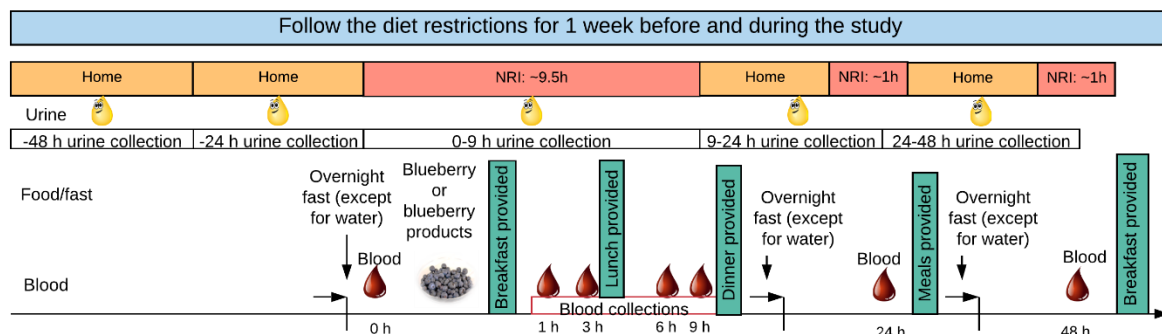
You will be asked to choose your standardized meals from a list of foods provided (breakfast, lunch, dinner and snacks) based on your preference for: white bread*/ bagel*/white roll* with cheese, cream cheese, honey, butter, salad dressing, ham, turkey, chicken, and or roast beef, chickpeas, plain breakfast biscuit, hard boiled eggs, chips, white rice, macaroni and cheese, noodles, cucumber, baby carrots, peas, canned or dried pineapple, canned or dried peaches, crackers (no wholemeal, dark chocolate, red fruits), butter cookies; and beverages: water (mineral, flavored or sparkling), milk (1% or 2% reduced fat, skim, lactose-free, or rice milk), diet soda, artificial fruit drink. During your full day at the clinic and the following day you will be asked to abstain from consuming coffee or tea which may result in the development of a headache which can be caused from caffeine withdrawal. Caffeinated diet beverages will be provided should you wish to consume them as an alternative to coffee or tea.

*Available as plain or gluten-free

The following night and 2 days after the blueberry product feeding day, we will request that you collect all your urine samples (all voided urine) from the time you leave the clinic until the time of the next visit into one container (urine collection from 9h-to-24h after intervention), and from the time of this second visit until the third visit, in the following day

(urine collection from 24h-to-48h after intervention). You will have to return to the Human Research Core in the morning of the first day after the blueberry product feeding day to provide us with the 9h to 24h urine sample and have one blood sample collected. We will ask you to repeat this procedure on the second day after the feeding day, return again to provide the 24h-to-48h urine sample and give a final blood sample. After that, you will have completed the first study period of the study and no more samples will be collected for about 2-3 weeks (depending on your schedule/availability), after which you will return to fulfil the next study period (blueberries, blueberry-rich protein bar or nutrient matched control beverage). This process is repeated for a total of 4 times.

A summary of when and what samples will be collected per intervention visit follows:



Find more detailed step-by-step instructions of what will happen during the study days in the two last pages of this document (if anything is unclear above).

What is the composition of the protein bar and the control beverage?

The protein bars contain 27.5 g protein, blueberry powder (equivalent to 1 serving of blueberries), 20 g fat, 41.25 g carbohydrates, and 440 total calories/113 g. The protein bars do not contain any peanuts, soybeans or soy, eggs, fish, crustaceans, tree nuts, wheat, gluten, or sulfites.

How much blood and other samples will I provide?

The total blood taken during the entire study is around 25% of the amount generally provided at a blood donor session. In total, you will provide about 0.29 oz. (or 1/2 tablespoon) sample for screening and 28 samples of 0.13 oz. (about 3/4 teaspoon) samples for the study.

Participants will also be asked to collect a midstream urine at the screening session for assessment of the kidney and liver health, and, in the case of females, also for a pregnancy test. All participants are required to collect their total urine voids for two days prior to the study, on the intervention days, and for the two days following each intervention.

How long will the visits take?

The berry feeding visits will take around 9.5 hours, followed by 2 follow-up visits of 1 hour each. Magazines, daily newspapers, computers and internet are available in the Human Research Core. It is expected that 1 hour will be needed for each of the follow up visits.

How long will the study take?

The study will take 4 study periods, each consisting of of one 9.5 hour visits and 2 one-hour visits (a total of 54.5 hours commitment) over the period of 3 months.

What are the possible benefits of taking part?

The study will not benefit you directly, but the information we get from this study will be invaluable to answering the following questions: what happens to berries when they are eaten?; can fruits and vegetables be naturally bred and screened to be more nutritious?; and can the benefits of eating fruits and vegetables be improved at the present consumption level by using screening and processing technologies?

Expenses and Compensation

If you live within 20 miles from the North Carolina Research Campus and want to be reimbursed for travel expenses for study visits (not for the consent visit), we will provide you the forms through which you can ask for travel reimbursement directly with the North Carolina State University administrative office on the North Carolina Research Campus. In case you take a train or bus, we ask you to save any receipt/ticket so you can be reimbursed. In the extreme case where there is an emergency and you need to take a taxi rather than cancelling your visit, keep the taxi receipt and we will reimburse you. Free parking is available to study participants.

You will also receive a \$312 maximum compensation (in addition to travel reimbursement) calculated in proportion to the number of collections or food records you provide, which is broken down as follows: a 24-hour urine collection is \$5/collection, a blood sample is \$7/sample, the completion of food intake report is \$4/four days. This will be paid upon completion of the study or post dropout. Please be aware that inconvenience and travel payments are liable to tax, and North Carolina State University is not responsible for informing the Government Revenue Office.

What if I am a visa holder?

Compensation for participants who are H-1B visa holders and sponsored by NC State University is dependent upon the presentation of the below documents, or the participant can opt of receiving compensation to participate in this study:

- Copy of passport photo page
- Copy of entry stamp in passport
- Copy of I-797 approval notice with I-94 (Can be printed from: <https://i94.cbp.dhs.gov/I94/#/home>)
- Confirmation from sponsoring institution's Office of International Services indicating they are aware of the visitor's activity with NCSU and the payment s/he is receiving (University best practice).

However, H-1B visa holders who are sponsored by another Institution need to get the permission of their host Institution before participation and the compensation would be processed directly to the sponsoring Institution.

North Carolina State University does not authorize compensation to individuals under J-1 or F-1 visas. In this way, participation of J-1 and F-1 visa holders is voluntary.

Travel reimbursement can be provided for everyone, and visa holders need to present some documents depending on his/her visa status (See appendix D in the Consent Form).

Are there any side effects or risks of taking part in the study?

There are no expected adverse effects as a result of ingesting the blueberries or protein bars or shakes, as the allergic potency of the chosen products are low and a lack of allergy to

berries, salicylates, dairy products, specifically whey protein, and chosen diet foods were previously acknowledged during study screening. As a precaution however, we have decided to exclude individuals in our study that have known food allergies to any berries. Furthermore, presently licensed berry supplements (manufactured using berry extracts) on the market often contain a single dose of berries far exceeding the levels fed in the present study.

Here we define a supplement or health supplement as any dietary ingredient which is consumed in a format which is extracted from a food or plant. For example, vitamins, minerals, amino acids, herbs or botanicals, or even components or extracts from plants or animals such as extracts from fruits or omega-3-fatty acids from fish are generally considered supplements. These are most often consumed as a powder, pill or beverage, like an energy shot or drink. You may consume vitamins or minerals or amino acids or protein drinks or bars consumed as a meal replacement as long as it is typical to your usual diet and you consume this regularly. In the case where these are consumed accidentally, you are asked to simply write this in your food intake diary and try to avoid it in the future, or if it's within one week of the study visit, to repeat it on subsequent visits for consistency.

If your diet is generally low in fruits and vegetables, the increase in fiber associated with berry consumption may result in gastric discomfort (mild bloating or gas).

It is normal that you may feel some discomfort whilst a blood sample is taken with a needle. After the samples have been taken, there is a slight risk of bruising associated with taking blood samples. To minimize these risks, only fully trained and experienced phlebotomists will be responsible for collection of blood samples.

What if there is a problem?

All adverse events will be recorded and dealt with according to standard ethical practice. Detailed information on this is given in Part 2 on the following page.

PART 2: Details of how the study will be conducted

What will happen if I don't want to continue participating in the study?

You are free to withdraw from the study at any stage if you wish and you will be reimbursed in accordance with what procedures you have completed. Remember, your participation is entirely voluntary and you are free to withdraw without penalty or consequences or bias in future treatments or other studies. If you do withdraw from the study and have had some samples collected already, we will ask you if it is fine for us to keep the samples for analysis. Any samples collected will be coded to ensure your confidentiality.

What if there is a problem?

Complaints

If you have a concern about any aspect of this study, you should speak to the study coordinator (Monique Carvalho de Santana, at (704) 250-5451 or, blueberrystudy@ncsu.edu, who will do her best to answer any questions you may have. If you remain unhappy and wish to complain formally, you can contact the NC State IRB office at irb-director@ncsu.edu or via phone at 919-515-8754.

Harm

It is very unlikely that harm will come to you as a result of eating the blueberries or blueberry-rich protein bar as these ingredients are present as raw or processed products in typical American diets. In addition, the Human Research Core at the North Carolina Research Campus is equipped to handle and resolve any medical problems. To minimize risk, only trained phlebotomists will collect blood. Infection, excess bleeding, clotting, and/or fainting after blood collection via single needlestick venipuncture or via cannulation are also possible, although unlikely. The choice of cannulation will also be offered to you on study visit day-1 to minimize pain or possible trauma due to repeated needle sticks (i.e., at 0h, 1h, 3h, 6h and 9h after berry feeding). Even though the cannula is flushed with saline between successive blood draws, there is a possibility it could become blocked in which case remaining samples would be collected via single needlestick venipuncture, with your consent. The amount of blood being withdrawn will not influence your ability to participate in normal daily activities.

Will my participation in the study be kept confidential?

All personal information supplied during the study will be treated as strictly confidential and will be anonymized to protect your identity. All samples and data we obtain from you will be coded with a random 3-digit number. Any documents that link your name to this code will be stored securely in locked filing cabinets in the office of the Principal Investigator, which are only accessible by the study staff and the sponsoring organization (North Carolina State University) for auditing purposes. All coded data will also be stored in separate secure filing cabinets and coded samples stored in secured freezers in the Human Research Core. All samples and data will be stored for no longer than 10 years from their collection date and will be destroyed after this time using standard procedures.

What happens to the samples that you give?

The urine and blood samples that you give at the clinical screen will be analyzed for indicators of general health, which will be used to decide if you are eligible to participate in the study. These samples will be destroyed at the Core Lab Clinical testing facility. Should you decide to participate, the blood and urine samples collected for the study will be stored at the clinical team's research laboratory at the Plants for Human Health Institute of the North Carolina State University, located on the North Carolina Research Campus. This laboratory is only accessible by authorized personnel via an assigned badge to the main building, followed by a key to the laboratory. The samples will be stored in an anonymous form, with a code on them so it will not be possible to link the sample to you personally. The samples will be analyzed for berry components which are absorbed and excreted.

What will happen to the results of the research study?

The results of this research study will be published in scientific journals and presented at national and international scientific meetings. We are unable to pass on individual results but once the study is completed, we will send you a short letter discussing the general findings of the study.

What can cause the researcher to ask a participant to withdraw from the study?

Participants can be withdrawn from the study if they fail to comply with dietary restrictions, or fail to refrain from consuming dietary supplements that contain berry extracts for 1 month before and during the study. Also, those who find it difficult to stick to the instructions during the trial, and those experiencing substantial shifts in body weight during the study, or starting dietary regimes to facilitate this, may be withdrawn from the trial.

Who is organizing and funding the research?

This study is funded by the Foundation for Food and Agriculture Research (FFAR) in collaboration with the North Carolina State University-Plants for Human Health Institute. This study is managed and organized by researchers at the Plants for Human Health Institute of the North Carolina State University on the North Carolina Research Campus. The study is coordinated by Monique Carvalho de Santana and managed by Dr. Colin Kay, who is the Principal Investigator for this study and has 20 years of experience in berry research.

Who has reviewed the study?

The study has been reviewed and approved by the Institutional Review Board at the North Carolina State University.

Contact details for further information

Monique Carvalho de Santana at North Carolina State University

Email: blueberrystudy@ncsu.edu

Phone: (704) 250-5451



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BAM Study Timeline

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CAMPUS

Consent visit

If you are still eligible and interested in the study after reading this material, we will schedule a visit to the North Carolina Research Campus in Kannapolis, to discuss the study, answer any questions, and to have you sign a consent form for the study.



Clinical screening session

We ask you to arrive fasted (for at least 10h when only water can be consumed). You will have your blood collected, blood pressure, height, and weight measured, a health and wellness questionnaire asked, and, for women only: a urine pregnancy test done to verify if you are eligible to participate in the study. Breakfast will be provided. If you are eligible, we will contact you to arrange a convenient date for the first intervention day.



1 week before the study day (washout week)

You will be asked to avoid foods rich in berries, berries themselves or red and purple fruits and vegetables (Annex 2- Food Exclusion and Alternatives List) for the week before and during the study days, and to record a food diary for 2 days prior to the first study day.



Two days (48 hours) prior to the first study day

You will be asked to collect your total urine voids during the 48 hours before the first study day: one collection from 24h to 48h prior and another from 0h to 24h prior.



The evening before the first study day

You will be asked not to eat/drink anything except water for at least 10 hours prior to your first blood sample.



The first study day (the feeding day)

You will be asked to bring the urine collections from the previous 2 days to the clinic, and the food intake record. The researcher or clinical staff will measure your height, and weight, and ask you some health and wellness questions. You will have a blood sample collected in a fasted state, then receive the berries or berry products, followed by 4 more blood draws at 1h, 3h, 6h and 9h after you finished consuming the intervention food. We will also ask you to collect your urine in a given container from the moment you arrive until when you leave the clinic. We will provide you breakfast, lunch, dinner and snacks, and we will ask you to record food and drink intake.



The mornings of the following 2 days

You will be asked to collect any urine voided from the previous day and bring it to the clinic. You will also have blood samples collected from you in a fasted state. Breakfast, lunch, dinner and snacks will also be provided for these days. We will ask you to record food and drink intake during one of these days.



"Free-living" period

During the approximate 11 to 18 day periods between interventions (depending on your availability for participation in the next intervention), you will have a break, when you can eat regularly (without following the dietary exclusion list).



Repeat the steps inside the dotted lines for 3 more times, each separated by one "Free-living" period



STUDY FINISHED

BAM (Blueberry Absorption Metabolism) Study



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Preparation for the assessment day at the Human Research Core (at the North Carolina Research Campus)

Timing	Preparation for Study Period
Within 7d before feeding visit and during study days	<ul style="list-style-type: none"> Avoid foods similar to blueberries, berries themselves and red or purple fruits and vegetables. Check Annex 2-“Food Exclusion and Alternatives List” Avoid alcohol intake (<i>no more than 7 alcoholic drinks per week for women and 14 drinks per week for men</i>) Avoid caffeinated products [<i>a maximum of 2 medium cups (12-16 oz.) a day of tea and coffee, but no coffee or tea</i> on the day before the feeding visit and on study visit days 1 and 2] <p><i>If you accidentally consume restricted foods, simply write this in the food intake diary and try to avoid the restricted foods in the future, or if it is within one week of the study visit, to repeat eating these foods on subsequent visits for consistency.</i></p>
48 hours (two days) prior to feeding visit	<ul style="list-style-type: none"> Fill out a food intake record (template provided on Annex 3-Food intake record) Collect all the urine you generate in these days in provided collection containers (collection instructions on Annex 4-Urine collection instructions)
Evening before clinical assessment	<ul style="list-style-type: none"> Observe an overnight fast (for 10hr prior to visit, only drink water)
	Study days (there are 4 study periods, each lasting 3 days. The study periods are separated by a 18-25 days interval)
Study days (consecutive days) Location: 500 Laureate Way, Kannapolis, Suite 1315, NC 28081	<p>Day 1. Feeding visit (about 9 hours-visit)</p> <ol style="list-style-type: none"> 1. Researcher to take receipt of biological samples collected prior to attendance (i.e. urine from the previous 2, 24h collections) 2. Measuring resting blood pressure, height, and weight 3. General health assessed via questionnaire, as well as adverse event and medication use monitoring. Observance of pre-visit restrictions is assessed (e.g. overnight fast etc.) 4. 48h food intake record reviewed / collected 5. Baseline blood sample is collected in a fasted state 6. Blueberries, blueberry-rich protein bar or matched-control beverage provided and consumed 7. Breakfast is provided 8. Blood samples are collected at 4 time points (1h, 3h, 6h and 9h after eating the berries or berry products) 9. Urine is collected from your arrival at the clinic until your departure (0h to 9h after eating the berries or berry products) 10. Lunch, dinner and snacks are provided 11. Travel expense form is provided upon request 12. Food and drink intake are recorded throughout the day. <p>Days 2 and 3. Follow-up days (about 1 hour-visits)</p> <ol style="list-style-type: none"> 1. Researcher to take receipt of urine from previous 9-to-24h or 24-to-48hr 2. Blood sample is collected in a fasted state 3. Breakfast is provided

	<ol style="list-style-type: none"> 4. On day 2: lunch, dinner and snacks are provided as “take-home.” Food and drink intake are recorded throughout the day. 5. Travel expense form is provided upon request
11-18 days interval between each study period	<ul style="list-style-type: none"> • Consume any food you want; no restrictions are applicable during this time period.
End of the study	<ul style="list-style-type: none"> • Participation compensation is arranged in person; • Exit questionnaire (optional) is answered in person or via email



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Title of Study: Establishing optimal nutritional quality of blueberries: *a proof of concept study to improve the nutritional quality of the average diet using common plant breeding and processing practices* (eIRB # 19138)

Principal Investigator: Dr. Colin D. Kay, cdkay@ncsu.edu, 704-250-5451

Funding Source: Foundation for Food and Agriculture Research (FFAR)

Faculty Point of Contact: Cheri Granillo, cdgranil@ncsu.edu, 704-250-5492

What are some general things you should know about research studies?

You are invited to take part in a research study. Your participation in this study is voluntary. You have the right to be a part of this study, to choose not to participate, and to stop participating at any time without penalty. The purpose of this research study is to gain a better understanding of how berries are processed by the human body. We will do this by feeding you berries or products with berries in them and measuring how your body processes those berries through blood and urine samples.

You are not guaranteed any personal benefits from being in this study. Research studies also may pose risks to those who participate. You may want to participate in this research because you like eating blueberries or are curious about how your body processes food. You may not want to participate in this research because you might be uncomfortable or get a bruise when your blood is drawn for research purposes.

Specific details about the research are contained below. If you do not understand something in this form, or at any time, you have questions about your participation in this research, do not hesitate to contact the researcher named above or the NC State IRB office. The IRB office's contact information is listed at the end of this form.

What is the purpose of this study?

The purpose of the study is to evaluate how berries are utilized by the body, in healthy human volunteers.

Am I eligible to be a participant in this study?

In order to be a participant in this study, you must agree to be in the study and meet the eligibility criteria that are listed in Appendix A and reviewed with the researcher.

What will happen if you take part in the study?

If you agree to participate in this study, you will be asked to do all of the following:

Timing	Participant Selection
Consent visit (½ hour) Location: 600 Laureate Way, Suite 1315, Kannapolis, NC 28081	<ul style="list-style-type: none"> • Opportunity to ask questions about the study; • Review and sign the consent form • Have your height and weight measured; • Fill out a medical history form; • Fill out a medication use survey.
Clinical screening visit (1 hour) Location: 500 Laureate Way, Suite 1315, Kannapolis, NC 28081	<ul style="list-style-type: none"> • Come to the appointment having not had anything to eat or drink for 10 hours; • Your height, weight and blood pressure will be measured; • Completion of Medical History and medication use questionnaire; • We will draw your blood once; • We will ask you to give us a urine sample. We will use this sample to assess general health and pregnancy status (if applicable).
	Preparation for each Study Period
The 7d before feeding visit and during study days	<ul style="list-style-type: none"> • Avoid berries, foods rich in berries and red or purple fruits and vegetables. Check Annex 2-“Food Exclusion and Alternatives List” • Avoid alcohol intake (<i>no more than 7 alcoholic drinks per week for women and 14 drinks per week for men</i>) • Avoid caffeinated products [<i>a maximum of 2 medium cups (12-16 oz.) a day of tea and coffee, but no tea or coffee one day before the feeding visit and on study visit days 1 and 2]</i>] <p><i>If you accidentally consume restricted foods, simply write this in the food intake diary and try to avoid the restricted foods in the future, or if it is within one week of the study visit, to repeat eating these foods on subsequent visits for consistency.</i></p>
48 hours (two days) prior to feeding visit	<ul style="list-style-type: none"> • Write down everything you eat and drink on a food intake record each day (template provided on Annex 3- “Food Intake Record”) • Collect all the urine you generate each day into study provided collection containers (collection instructions on Annex 4- “Urine Collection Instructions”)
Evening before clinical assessment	<ul style="list-style-type: none"> • Observe an overnight fast (for 10 hours prior to your visit, only drink water and do not eat anything)
	Study days (there are 4 study periods, each lasting 3 days. The study periods are separated by a 18-25 day interval)
Location: 500 Laureate Way, Kannapolis, Suite 1315, NC 28081	Day 1 (9 hour long visit) <ol style="list-style-type: none"> 1. Bring your food intake records and urine samples to the visit 2. Your resting blood pressure, height, and weight will be measured

	<ol style="list-style-type: none"> 3. We will ask you to fill out a survey about your experiences while you've in the study, such as your health and if anything unexpected has happened. 4. We will take samples of blood and urine from you before you eat or drink anything. 5. We will ask you to add all your urine voids during this course of your 9 hour visit into a container we will provide to you. 6. You will be offered one of the following to consume: fresh berries, a berry protein bar, or a beverage. 7. You will then eat breakfast that we will give you. 8. After you've eaten, your blood will be taken 4 more times over the course of your 9 hour visit. 9. We will feed you lunch, dinner, and snacks. You will write down everything you eat and drink. 10. We will take the container where you were adding urine samples from you at the end of your 9 hour visit. 11. You will fill out a travel expense form before you leave if applicable. <p>Day 2 (about a 1 hour visit)</p> <ol style="list-style-type: none"> 12. Bring your food intake records and urine samples to the visit. 13. We will take a sample of blood from you before you eat or drink anything. 14. You will eat breakfast at the study site. You'll write down everything you eat and drink. 15. We will give you lunch, dinner, and snacks to take home and eat. 16. You will fill out a travel expense form before you leave. <p>Day 3 (about a 1 hour visit)</p> <ol style="list-style-type: none"> 1. Bring your food intake records and urine samples to the visit. 2. We will take a sample of blood from you before you eat or drink anything. 3. You will eat breakfast at the study site. You'll write down everything you eat and drink. 4. You will fill out a travel expense form before you leave.
11-18 days interval between each study period	<ul style="list-style-type: none"> • Consume any food you want; no restrictions are applicable during this time period.
End of the study (last study day)	<ul style="list-style-type: none"> • Participation compensation is arranged in person; • Exit questionnaire (optional) is answered in person or via email;

For a more detailed description of procedures summarized in the table refer to Appendix B.

The following procedures are experimental: we will ask you to consume one of the commercial blueberry varieties, or blueberry-rich protein bar or nutrient matched

control beverage, and we will collect some sample of your blood and urine to analyze for blueberry components absorbed after consumption.

Risks and benefits

There are minimal risks related to the research procedures and the data generated from the research.

Some participants may experience pain and/or bruising from the blood draws regardless of method of collection. We will draw your blood with the needlestick blood collection method for most of your study visits. When you come for your 9-hour study visit, you get to choose how your blood is drawn from your body: needlestick or cannulation.

- With a needlestick venipuncture, a single needle will be inserted into a vein in your arm to collect blood. The needle is inserted and removed fairly quickly, but the disadvantage of this approach is that we will need you to have multiple needlesticks during your 9.5-hour study visits.
- With cannulation, a small tube (“cannula”) is inserted into a vein in your arm so that a needle can be inserted into the tube and draw blood from there. The initial procedure to place the tube is a bit more complicated than the needlestick. There is also a risk that the tube can become blocked. We will wash the tube between blood draws so it is clean each time we use it. The advantage, however, of cannulation is that you will only experience a needle poking into your skin once instead of multiple times if you choose the needlestick collection. Keep in mind that with this method, you will have a tube in your arm for maximum 9.5h. If the tube becomes blocked at any point, we will remove the tube and revert to needlestick venipuncture for the blood draws with your consent. The cannula should be accessed by lab or study personnel only. If the cannula becomes dislodged at any time throughout the day, you should return to the lab immediately. You cannot leave the NRI building, where the phlebotomy laboratory is located, while the cannula is in your arm.

Infection, excessive bleeding, clotting, and/or fainting from blood draws are also possible, although unlikely, in both blood draw methods. We are mitigating these risks by having licensed, experienced medical professionals draw your blood within current standards of care for blood draws and ensuring that you have something to eat after each fasting blood draw. We also will limit blood draw attempts to 2 per vein on your arm. If we are unsuccessful drawing blood from a vein in your arm, 1 additional, experienced medical professional will try the venipuncture one time, for a maximum of 3 attempts at the venipuncture per arm.

There is a risk that your body may react to some of the food that we will give you to consume. We do not expect this to happen because the food will be prepared in commercial grade kitchens using food safe practices. We are also excluding people from participating in the study that have food allergies or intolerance to salicylates, berries, or berry derived products as a precautionary measure.

There is a risk to privacy if our data is breeched due to the private health information and biospecimens that you are sharing with us. We are implementing data security measures appropriate to the sensitivity of your personal data. We are also committed to securely and permanently destroying your health data if you drop out of the study

at any time for any reason. If you choose to complete the study, your identifiable information will be destroyed immediately following publication of the first manuscript from the study and or completion of the project grant.

There are no direct benefits to you participating in this study, You are, however, helping others to understand the nutritional value of berries.

Right to withdraw your participation

You may stop participating in this study at any time for any reason. In order to stop your participation, please contact the study coordinator, Monique Carvalho de Santana, mcarval@ncsu.edu and 704-250-5451 or the faculty point of contact for this protocol, Cheri Granillo, cdgranil@ncsu.edu and 704-250-5492. If you choose to withdraw your consent and to stop participating in this research, you can expect that the research team will take out your information and biospecimens from their data set, securely destroy your data, and prevent future uses of your data for research purposes wherever possible. This is possible in some, but not all, cases. You will also still receive compensation for all of the research procedures you completed.

Confidentiality, personal privacy, and data management

Trust is the foundation of the participant/researcher relationship. Much of that principle of trust is tied to keeping your information private and in the manner that we have described to you in this form. The information that you share with us will be held in confidence to the fullest extent allowed by law. Protecting your privacy as related to this research is of utmost importance to us.

How we manage, protect, and share your data are the principal ways that we protect your personal privacy. Protecting your privacy as related to this research is of utmost importance to us. There are very rare circumstances related to confidentiality where we may have to share information about you. Your information collected in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety. In other cases, we must report instances in which imminent harm could come to you or others.

All study records will be kept confidential. Hard-copy data will be stored in a locked cabinet in the PI's office which is also locked and accessible only by card access. Digital data will be stored in separate secure server locations on an NC State managed network and the files will be accessible only via account password protection and transferred via encrypted email on the NCSU web server.. All biological samples will be coded with a unique randomly generated identification number. Only the principal investigator, Dr. Colin D. Kay, will have access to the coded anonymized information. The study coordinator, Monique Carvalho de Santana, will be provided access to the data in the case of an emergency.

Data shared about you in this study will be de-identified. While we might be able to link your identity to your data at earlier stages in the research, when the research concludes, there will be no way your real identity will be linked to blood results or the data we publish. Study results will only be provided in a summarized fashion to our study sponsor and scholarly publications. No reference will be made in oral or written reports which could link you to the study.

Your de-identified blood and urine samples will be stored at the Plants for Human Health Institute of the North Carolina State University for a minimum of 3 and no more than 10 years from collection.

Compensation

For participation in this study, a \$312 maximum compensation will be provided to you (in addition to travel reimbursement) calculated in proportion to the number of blood collections, urine collections, and food records you complete for the study. The breakdown is as follows:

- \$5 for each 24-hours of urine collection samples
- \$7 for each blood sample
- \$4 for each 4-day food intake report

Compensation for participants who are H-1B visa holders and sponsored by NC State University is dependent upon the presentation of the below documents, or the participant can opt of receiving compensation to participate in this study:

- Copy of passport photo page
- Copy of entry stamp in passport
- Copy of I-797 approval notice with I-94 (Can be printed from: <https://i94.cbp.dhs.gov/I94/#/home>)
- Confirmation from sponsoring institution's Office of International Services indicating they are aware of the visitor's activity with NCSU and the payment s/he is receiving (University best practice).

However, H-1B visa holders who are sponsored by another Institution need to get the permission of their host Institution before participation and the compensation would be processed directly to the sponsoring Institution.

North Carolina State University does not authorize compensation to individuals under J-1 or F-1 visas. In this way, participation of J-1 and F-1 visa holders is voluntary.

You will be paid at the end of the study or when you withdraw from the study, whichever occurs first. If you choose to withdraw from the study prior to its completion, you will receive partial compensation based on the procedures undertaken during your participation.

Travel expense reimbursement will be paid up to a maximum of 40 miles at \$0.575/mile per study day (day 1 through day 3 of each study period). If you will be taking public transit, you can be reimbursed for those fares by giving your fare receipts for each attendance to campus for research procedures (i.e., bus or train) to the research coordinator. Your trips will be logged at every visit day, and you can process payment at every day 3 of each study period, or at the end of your participation in the study (last day). Travel reimbursement can be provided for everyone, and visa holders need to present some documents depending on his/her visa status (See appendix D in the Consent Form).

For any compensation or travel expense reimbursement, you will need to share your social security number and bank account information to Dona Miller, the NCSU Administrative Support Specialist in the North Carolina Research Campus'

administrative office. Her email is dona_miller@ncsu.edu and her phone number is 704-250-5449. The research team will provide you a form to complete and bring to Ms. Miller so that she can process the payment directly into your bank account or have a check issued and mailed to your home. Your banking information and social security number will not be shared or handled by any member of the research team.

Emergency medical treatment

If you are hurt or injured during the study session(s), the researcher will call 911 for necessary care. There is no provision for compensation or free medical care for you if you are injured as a result of this study.

What if you are an NCSU student?

Your participation in this study is not a course requirement and your participation or lack thereof, will not affect your class standing or grades at NC State.

What if you are an NCSU employee?

Your participation in this study is not a requirement of your employment at NCSU, and your participation or lack thereof, will not affect your job.

Sponsorship and Funding

This research is funded by the Foundation for Food and Agriculture Research (FFAR). This means that the sponsor is paying the research team for completing the research. The Foundation for Food and Agriculture Research (FFAR) is a unique federally funded, public-private partnerships to support innovative science addressing today's food and agriculture challenges. The researchers do not have a direct financial interest with the sponsor or in the final results of the study. If you would like more information, please ask the researcher listed in the first page of this form about the funding and sponsorship.

What if you have questions about this study?

If you have questions at any time about the study itself or the procedures implemented in this study, you may contact the research coordinator, Monique Carvalho de Santana, blueberrystudy@ncsu.edu, and (704) 250-5451, or the Faculty advisor Cheri Granillo, cdgranil@ncsu.edu, 704-250-5492.

What if you have questions about your rights as a research participant?

If you feel you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact the NC State IRB (Institutional Review Board) Office. An IRB office helps participants if they have any issues regarding research activities. You can contact the NC State IRB Office via email at irb-director@ncsu.edu or via phone at (919) 515-8754.

Participant**Initials**

1. Have you read the Participant Information Material?.....YES/NO	
2. Do you agree that you do not fall within the basic exclusion criteria listed for this research study (Appendix A)?.....YES/NO*	
3. Have you had an opportunity to discuss this study and ask questions; including the exclusion criteria and your responsibilities as a volunteer?.....YES/NO	
4. With whom have you discussed the information for this research study? _____	
5. Have you received sufficient information about the study?.....YES/NO	
6. Have you read the detailed description of the study procedures (Appendix B)?.....YES/NO	
7. Have you received satisfactory answers to all your questions?YES/NO	
8. Do you understand that you are free to withdraw from the study: At any time, without having to give a reason for withdrawing, without withdrawal affecting future participation in other research studies?.....YES/NO	
9. Do you agree to have blood samples collected from you either by single cannula or by a series of needle sticks (see Appendix C)?*YES/NO	
9.1 If you opt to have blood samples collected by single cannula, do you agree not to bend the arm in which the cannula is inserted for the entire period while it is in your arm?.....YES/NO	
9.2 If you opt to have blood samples collected by single cannula, do you agree to come back to the clinic (Human Research Core) every hour to have the cannula flushed with saline?.....YES/NO	
9.3 If you opt to have blood samples collected by single cannula, do you agree to allow the cannula to be accessed only by study personnel?.....YES/NO	
10. Are you aware that your personal information will be kept confidential?.....YES/NO	
11. Are you aware that your blood and urine samples will be stored anonymously at the Plants for Human Health Institute of the North Carolina State University for a minimum of 3 and no more than 10 years from collection?.....YES/NO	
12. Are you aware of the potential hazards of participating in this study such as momentary discomfort and/or bruising from the introduction of a needle into your forearm vein, and highly unlikely infection, excess bleeding, clotting, and/or fainting?.....YES/NO	
13. Will you inform the study coordinator or the clinical team of the start of any medication/medical changes while participating in the study*YES/NO	
14. Do you understand that all research is subject to inspection and audit? Although your records may be accessed for this purpose your personal information remains confidential.....YES/NO	
15. Do you agree to request travel reimbursement (for a maximum of 20 miles from the North Carolina Research Campus) with the North Carolina State University administrative office by yourself in case you decide to request it?..... YES/NO	
16. Do you agree to consume a control beverage of whey protein dissolved in water, corn syrup and vegetable oil?.....YES/NO*	
17. Do you agree to abstain from coffee or tea one day before and during the first and second study visit days?YES/NO	

<i>In the case where these are consumed accidentally, you are asked to simply write this in the food intake diary and try to avoid it in the future, or if its within one week of the study visit, to repeat it on subsequent visits for consistency.</i>	
18. Are you a visa holder?.....YES/NO If yes, which type of visa? _____	
18.1 Do you understand that compensation for your participation may depend on the type of visa that you hold?.....YES/NO	
19. Do you agree to request compensation for the study procedures with the North Carolina State University administrative office by yourself in case you decide to request it?.....YES/NO	
20. Do you agree to take part in the study entitled “ BAM (Blueberry Absorption Metabolism) Study: a study investigating the nutritional equivalence of fresh and processed berries ”?.....YES/NO*	
21. Would you like to have your contact information retained to be invited to participate in future studies?.....YES/NO	

* If you have said NO to the questions marked with “*” we are unable to accept you in this study.

Consent to Participate

By signing this consent form, I am affirming that I have read and understand the above information. All of the questions that I had about this research have been answered. I have chosen to participate in this study with the understanding that I may stop participating at any time without penalty or loss of benefits to which I am otherwise entitled. I am aware that I may revoke my consent at any time.

Participant’s printed name _____

Participant's signature _____ **Date** _____

Investigator's signature _____ **Date** _____

Appendix A: Inclusion and Exclusion criteria

You can participate in this study if you want to be in the study AND if you:

- are a male or female adult between 25-65 years;
- are non-smoker nor vaping or dipping (i.e., tobacco chewing) users (or ex-smoker who ceased ≥ 6 months ago);
- present no allergies to berries, red apple, red orange, purple onion and broccoli;
- if you present no allergies to dairy products, specifically whey protein, fructose or salicylates;
- are generally healthy and without chronic diseases including cancer, type 1 and 2 diabetes;
- not be prescribed thyroid or hypoglycemic medication or hormone replacement therapy (HRT) (due to the likely concomitant effects that these medications cause on the primary endpoint in the study);
- have not been consuming any supplements* rich in berries (e.g. berry, cocoa, tea or coffee extracts) for at least a month before the study and willing to not consume it during the study;
- live within 40 miles from the North Carolina Research Campus (NCRC) campus;
- agree to restrict dietary intake of foods similar to berries or dietary supplements* containing extracts from berry, cocoa, coffee or tea, during the week before the study and study days;
- agree to comply with the study procedures involving the collection of urine and blood samples, and to record your dietary intake over 2 days before each berry feeding study day, and two days after the berry or berry products intake;
- have body mass index (BMI) from ≥ 18.5 to ≤ 30 (lbs/in²x703);
- have a successful (i.e., within normal range for healthy individuals) biochemical, hematological and urine analyses assessed by our clinical advisor.

You cannot participate in this study if you do not want to be in the study or:

- are a current smoker [vape and dip (i.e., tobacco chewing) users included], or ex-smoker ceasing < 6 months before recruitment;
- are pregnant or breastfeeding;
- have current or significant past medical history of vascular disease or medical conditions likely to affect the study measures i.e. vascular disease, circulatory (i.e. Reynaud's), diabetes, hepatic, renal, digestive, hematological, cancer, or thyroid disease;
- are fructose intolerant or have allergy to salicylates, dairy products, specifically whey protein, or to berries;
- are unprepared to adhere to dietary restrictions for 1 week preceding and during each berry or berry product feeding visit or unwilling to comply with the study procedures;
- are in parallel participation in another research project involving dietary diet and/or sampling of biological fluids/material;
- are on therapeutic diets or having experienced substantial weight loss (to be judged by clinical advisor) within 3 months of screening;
- are taking supplements* rich in berries (e.g. berry, cocoa, tea or coffee extracts), unwilling to cease intake during, and 1 month preceding the study, or unwilling to stop existing intake of other supplements* (minerals, vitamins,

plant extracts, plant or animal oils, amino acids, energy drinks) or regular use of large-dose nutrient, herbal, and dietary supplements* during the past one to two weeks, or planning to use them during the study;

- are prescribed thyroid, hypoglycemic medication or HRT medication -other medications will be assessed for suitability by the clinical advisor;
- have donated blood in the last month;
- consume more than 1 and 2 drinks of alcohol per day for women and men, respectively, or more than 7 and 14 drinks per week for women and men, respectively (U.S. Department of Health and Human Services and U.S. Department of Agriculture Dietary guidelines 2015-2020);
- are currently on a weight-reducing plan or using weight-loss medications (e.g., selective serotonin reuptake inhibitors, steroids, Ritalin, appetite suppressants such as Diethylpropion or Amfepramone, and weight loss medications such as Alli, Xenical, Qsymia, Belviq, Contrave, and Saxenda), or planning to continue this treatment during the approximate 10-week period of the study;
- present abnormal biochemical, hematological or urinary results, and measurements considered to be counter-indicative for the study, including: kidney and liver function, fasting glucose (especially if indicative of diabetes), lipid abnormalities, full blood count;
- have BMI<18.5 or >30 (lbs/in²x703).

*A supplement or health supplement refers to any dietary ingredient which is consumed in a format which is extracted from a food or plant. For example, vitamins, minerals, amino acids, herbs or botanicals, or even components or extracts from plants or animals such as extracts from fruits or omega-3-fatty acids from fish are generally considered supplements. These are most often consumed as a powder, pill or beverage, like an energy shot or drink. You may consume vitamins or minerals or amino acids or protein drinks or bars consumed as a meal replacement as long as it is typical to your usual diet and you consume this regularly. In the case where these are consumed accidentally, you are asked to simply write this in the food intake diary and try to avoid it in the future, or if its within one week of the study visit, to repeat it on subsequent visits for consistency.

Appendix B: Detailed description of text summarized in Table 1

1. In the consent visit (½ hour) at the Plants for Human Health Institute (PHHI; North Carolina Research Campus, 600 Laureate Way; Kannapolis, NC 28081), you will be able to clarify any questions, it will be explained that volunteers will be given a list of foodstuffs to ‘avoid’ and/or ‘limit’, and alternatives to them during the wash-out week and visit days; the intake of tea and coffee will be limited to a combined intake of 2 medium cups (12-16 oz) per day during washout, but none on the day before the berry feeding visit, and the intake of alcohol will also be regulated based on the recommendations of the Food and Drug Administration (Annex 2- “Food Exclusion and Alternatives List”). You will also be required to refrain from taking supplements containing extracts of cocoa, coffee, tea or berries for at least 1 month prior to the assessment visits, and throughout the study. Volunteers will be advised to maintain their normal lifestyle during the study (e.g. dietary intake, exercise levels and non-smoking habits). It is especially important that participants do not experience substantial shifts in body weight/body composition during the study, as fluctuations can affect the observation relative to berry component utilization by the human body. If still interested in participating in the study, you will be asked to sign the study consent form. After signing the consent form, your inclusion/exclusion will be verified via confirmation of your BMI, measurement of resting blood pressure, and via a medical history and medication use questionnaire.

2. In the clinical screening visit (1 hour) at the Human Research Core (North Carolina Research Campus, 500 Laureate Way; Kannapolis, NC 28081), you will arrive fasted for 10 hours, and your BMI, measurement of resting blood pressure, medical history and medication use questionnaire will be assessed. You will also be asked to provide a midstream urine sample to establish kidney, liver and urinary tract health via a dipstick test (Urispec 11 way) for content of blood erythrocytes, urobilinogen, bilirubin, protein, nitrate, ketones, ascorbic acid, glucose, pH, specific gravity, and leukocytes. Female participants will also be assessed for pregnancy. After confirmation of eligible BMI, you will provide a fasting blood sample (approximately 0.29 oz. or ½ tablespoon) through venipuncture and used to establish health status via a comprehensive metabolic panel test to assess the blood content for glucose, calcium, albumin, total protein, sodium, potassium, carbon dioxide, chloride, blood urea nitrogen (BUN), creatinine, BUN/Creatinine ratio, total globulin, albumin/globulin ratio, alkaline phosphatase, alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT) and total bilirubin. The results of the urine and blood analyses will be available in approximately 24-48h, when the participants will be notified about their eligibility and scheduled for the berry feeding visits.

3. You will have one week washout before your berry or berry product feeding visits, when you will be asked to consume a diet low in berries and red or purple fruits and vegetables as directed by the dietary exclusion list (Annex 2- “Food Exclusion and Alternatives List”) provided. We will ask you record your diet (Annex 3- “Food Intake Record”), and collect 24h-urine during the two days preceding each berry feeding visit (Annex 4- “Urine Collection Instructions”). The study days will occur during weekdays. At the study day 1 (approximately 9.5-hour long) in the clinic, you will arrive fasted for 10 hours (having consumed only water), the clinical team will take receipt of previously collected urine (two 24-hour collections, one from each of the two previous days) and the food intake record from the two preceding days. Also,

you will complete a basic medications assessment/questionnaire to establish fitness to proceed (e.g. to assess if you have fasted correctly and are free from symptoms indicative of compromised health), and record any medications being used onto the medical questionnaire. Your resting blood pressure, height, and weight will be measured. Additionally, urine (0-9h, 9-24h and 24-48h) and blood samples (at 0, 1, 3, 6, 9, 24 and 48 h) will be collected across the three study days for each berry or berry product feeding visit. Failure to comply with fasting or dietary restrictions at first visit will require a further one week washout period; repeated failure to follow the fasting or dietary guidance during the study may result in withdrawal from the trial. This procedure will be repeated across 3 other study periods (for a total of 4 study periods). In the first feeding visit, you will receive either, in randomized order, one serving (approximately 5.3 oz) of two different commercially available blueberry varieties, a protein bar enriched with the equivalent of one serving of blueberries or a nutrient-matched control beverage. You will consume a dairy product, specifically whey protein beverage together with the berries in order to provide equivalent macronutrient contents across the feeding products. After the berry or berry product is given on day 1 of each feeding visit, breakfast will be provided, as well as lunch, dinner and snacks. Blood pressure, height, and weight measurements, and potential medication change record will be done on days 2 and 3 of after each berry feeding visit. Days 2 and 3 last about 1 hour. You will be provided with standardized berry-free meals and water during the visit days 1 and 2, and breakfast after blood collection on day 3. You will be asked to write your food and drink intakes during visit days 1 and 2, and to repeat these intakes on the following three feeding visits.

You will be asked to choose your standardized berry-free meals from a list of foods provided (breakfast, lunch, dinner and snacks) based on the following foods: white bread*/ bagel*/white roll* with cheese, cream cheese, honey, butter, salad dressing, ham, turkey, chicken, and or roast beef, chickpeas, plain breakfast biscuit, hard boiled eggs, chips, white rice, macaroni and cheese, noodles, cucumber, baby carrots, peas, canned or dried pineapple, canned or dried peaches, crackers (no wholemeal, dark chocolate, red fruits), butter cookies; and beverages: water (mineral, flavored or sparkling), milk (1% or 2% reduced fat, skim, lactose-free, or rice milk), diet soda, artificial fruit drink. During your full day visit to the clinic and the following day you will be asked to abstain from consuming coffee or tea which may result in the development of a headache which can be caused from caffeine withdrawal. Caffeinated diet beverages will be provided should you wish to consume them as an alternative to coffee or tea.

*Available as plain or gluten-free

The protein bars contain 27.5 g protein, dried blueberry (equivalent to 1 serving of blueberries), 20 g fat, 41.25 g carbohydrates, and 440 total calories /113 g. The protein bars do not contain any peanuts, soybeans or soy, eggs, fish, crustaceans, tree nuts, wheat, gluten, or sulfites.

4. Between the feeding visits, you will receive a “thank you” email about your participation and a reminder that you can observe approximately 2 weeks (11 to 20 days depending on scheduling constraints) of free-living compliance break, when you can return to your normal dietary habits and take a break from the study regime. This will be followed again by seven days of wash-out, urine collection and record of

your food intake for two days before the next feeding visit day. This will be repeated for the remaining three berry or berry product feeding visits.

You will need to come to the Human Research Core on the North Carolina Research Campus, in Kannapolis on 14 separate occasions over a period of 3 months (total of 54.5 hours commitment). The blueberries or blueberry product will be consumed on 4 different feeding days, each followed by 2 follow-up visits. This equates to 4 feeding days (across the 4 study periods) and 8 one-hour visits. Before you are enrolled in the study you will also be required to come to the research campus for a ½-hour initial presentation and one 1-hour screening visit. For the 4 blueberry product feeding days, you will be with us for approximately 9 and 1/2 hours, while the following two visits will be less than 1 hour. The 4 separate feeding days will be spaced out over 11 weeks with about 2 weeks break between each of the feeding days.

Appendix C: Description of IV Cannulas vs. Venous Blood draws at each time point

On the first day of each arm of the BAM study you will have 5 blood draws over a 9.5 hour period. The Human Research Core trained team will make every effort to ensure that this process is done with your safety and comfort as our top priority.

You will have the following options:

1. **Individual venous blood draws.** Blood from all five time points drawn from the most easily accessible, superficial veins in your forearms. We will make every effort to draw these samples from different sites in your arms if possible. Not everyone has accessible veins in both arms. If this is the case, all 5 samples may need to be drawn from one arm. We will, however, try to use the 2 different veins in your one accessible arm.

Note: There is the chance that we will have to try to find an accessible vein two times by one phlebotomist with 1 further attempt by a second phlebotomist if the first is unsuccessful.

2. **IV Cannula Placement.** You also have the option of having an IV cannula inserted into the vein in your arm at the beginning of the first day of each study period, or at any time during this day, except right before the last blood draw of the day. It will allow us to get the blood samples from the cannula, and minimize the inconvenience of sticking you multiple times. This is the same procedure that you have when you have an IV placed in your arm for hospital procedures. We will place a short, plastic cannula into the vein in your arm. This is done with a small needle covering the cannula that we place into the selected vein in your arm. We then remove the needle, leaving the plastic cannula in place. We will draw the blood that we need for your first time point out of the cannula, then we will flush the cannula with a small amount of sterile saline. Next, we will cap the cannula so that it is kept sterile and will not leak before your next time point. You will need to keep your arm as straight as possible in between time points to ensure the cannula stays in place. You must return to the lab every hour to have the cannula flushed. The cannula should be accessed by lab or study personnel only. If the cannula becomes dislodged at any time throughout the day, you should return to the lab immediately. You cannot leave the NRI building, where the phlebotomy laboratory is located, while the cannula is in your arm.

Note: There is a chance that the cannula cannot be used for all blood draws over the 9.5-hour period, as the cannula may get clogged or move out of place. If this happens, we must remove the cannula and perform individual blood draws for each remaining time point. We will make every effort to avoid this, but it does happen sometimes.

Appendix D: Required Documentation to Process Travel Reimbursements for foreign nationals

Collecting the documentation listed below ensures that NC State is processing payments to international visitors in valid immigration status, and in the assessment of possible tax liability based on the visitor's tax status.

Documentation is a requirement even if the payment falls under the Accountable Plan.

F-1 students, H-1B, and TN Visa holders can not be paid honoraria.

J-1 Visa

- Copy of passport photo page
- Copy of J-1 visa in passport
- Copy of entry stamp in passport
- Copy of I-94 (Can be printed from: <https://i94.cbp.dhs.gov/I94/#/home>)
- Copy of DS-2019
- Letter of payment authorization from the sponsoring institution's Office of International Services, if the visitor's J-1 visa is not sponsored by NCSU
- Original signed W-8BEN

B-1 visa

- Copy of passport photo page
- Copy of B-1 visa in passport
- Copy of entry stamp in passport
- Copy of I-94 (Can be printed from: <https://i94.cbp.dhs.gov/I94/#/home>)
- Original signed W-8BEN

Visa Waiver Business (VWB) (ESTA)

- Copy of passport photo page
- Copy of entry stamp in passport
- Copy of I-94 (Can be printed from: <https://i94.cbp.dhs.gov/I94/#/home>)
- Original signed W-8BEN

**** ICT requires the original signed W-8BEN/W-9 and the original copies of collected immigration documents. Please send them to us via campus mail to Campus Box 7233 ****
(Failure to comply mail result in the delay of payment)

Thank you!

Annex 2: Food Exclusion and Alternatives List (for 7 days preceding each clinic visit)

Version 1 09/24/2019

Food to Exclude from Diet	Foods to Limit*	Suggested Alternatives
FRUITS (including products containing them)		
Berries: Bilberry, Blackberry, Blueberries, Cranberry, Elderberry, Lingonberry, Raspberry and Strawberry Blackcurrant Currants Pomegranate Plum Prunes Raisins Redcurrant Red apples Red or blood orange Red/purple/blue grape	Apricots Orange/citrus Pear Mango Grapefruit Green apples Green grapes	Avocado Banana Clementine Kiwi Lemon Melon Nectarine Peach Pineapple
VEGETABLES (including products containing them)		
Barley Black olives Eggplant Nut skin Purple carrots Radish Red or black beans Red cabbage Red onion Red/purple potato Soybean and soy-based foods	Beetroot Brussel sprouts Green olives Oregano Parsley Purple basil Purple corn Rhubarb Spinach Sweet corn White onion	Broccoli Carrots Cauliflower Celery Cucumber Green/white cabbage Green bean Leek Lentil Lettuce Parsnip Pepper Peas Pumpkin Rice Tomato Shallot Zucchini
BEVERAGES (including products containing them)		
Juices derived from any above listed berries, fruits or vegetables Red wine Energy drinks, e.g. Red Bull, Powerade, Gatorade	Coke/cola -a maximum of 2 medium cups (12-16 oz.) a day of tea and coffee. -**Food and Drugs Administration recommends no more than 7 alcoholic drinks per week for women and 14 drinks per week for men	Carbonated drinks, e.g. Sprite Fruit juices from non-berry sources, e.g. green apple and white grape juice etc. Lager, cider, spirits/liquor Malted drinks Milk Water White wine
OTHERS (including products containing them)		
Cinnamon Dark Cocoa/chocolate Jams, sauces or dessert toppings derived from any above listed berries or fruits	Cured and canned meat (e.g. bacon, ham, sausage, corned beef), Smoked fish Purple corn tortillas or crisps	Milk or white chocolate Fresh meat (e.g. chicken, turkey, beef, pork, lamb) Fresh fish

Please, consume a maximum combined intake of 10 portions per week from the 'FOODS TO LIMIT' (yellow column). Portion Sizes:** a portion is about 1 medium sized piece of fruit such as an apple, 1 medium cup (12-16 oz.) of tea/coffee, or 3.5 oz. of vegetables. If you are in doubt about a specific food, a good rule of thumb is "if it's red or blue, then it's not for you". *A drink of alcohol is defined as about 12 fl oz. of 5% alcohol beer, 5 fl oz. of 12% alcohol wine, 1.5 fl oz. of 80 Proof distilled spirits (40% alcohol)** according to the U.S. Department of Health and Human Services and U.S. Department of Agriculture Dietary guidelines 2015-2020.



BAM (Blueberry Absorption Metabolism) Study

Blueberry
Absorption
Metabolism

NC STATE UNIVERSITY
**Plants for
Human Health**
INSTITUTE

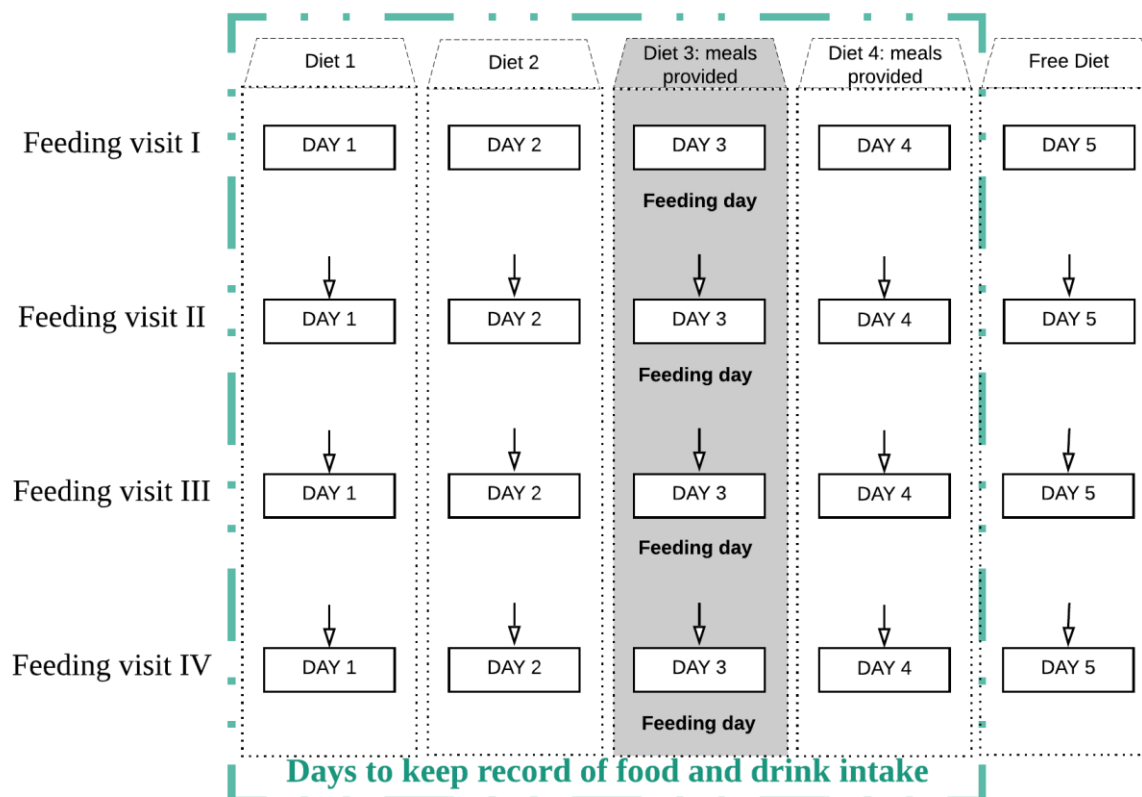


4-Day Food Intake Record

Volunteer ID: _____

Start date: _____ End date: _____

1. You are going to **record the time, kind and amount of your food and drinks** consumed during four days: **TWO DAYS BEFORE** (days 1 and 2 in the figure below) your feeding visit day at the Human Research Core (which is Day 3 in the figure below), also during your feeding visit day at the Human Research Core (**DAY 3**) and again during **VISIT DAY 4**.
2. Remember that you will need to **REPEAT** the diet, eating the same foods and drinks as previously recorded for Feeding visit I days 1-4, on the following interventions (Days 1-4 of Feeding visits II-IV, in the figure below). For example, the meal you have on day I of the intervention I should be the same as you consume on day I of intervention II, but you don't need to eat the same food in all the consecutive four days. Keep it consistent ☺



In this way, please, do **NOT** consume **too much exotic foods or drinks** during these 4 days because you might not find it easily to find these items again.

Instructions for Recording Food and Drink

- ✓ Start a new page for each day (midnight to midnight).
- ✓ Use as many pages as you need for each day and attach all together.
- ✓ At the top of each page, write down **the feeding visit period (I, II, III or IV), the day (1, 2, 3 or 4), and the date.**
- ✓ Write down the time when you eat/drink, including any alcohol consumed.
- ✓ Record everything you eat and drink, remembering to record **each item separately**.
- ✓ For example, a cheese sandwich is 3 food items, the bread, the spread (butter, margarine etc.) and the cheese.
- ✓ Record the amount of each item of food/drink that you eat. You can record the amount using household measures e.g. a teaspoonful of sugar, a mug of tea, a slice of bread.
- ✓ Record the brand of a packaged item.
- ✓ In case of food consumed at a restaurant, record the name of the dish and of the restaurant.
- ✓ Remember to record details of any vitamins, minerals or other dietary supplements that you take.
- ✓ For recipes e.g. beef casserole, spaghetti bolognaise, use the recipe sheets provided, noting down the individual ingredients and amounts. We can also use photocopies or computer files or photos of recipes if necessary.

An example of a completed sheet is given on the next page

Food intake record

EXAMPLE

Feeding visit period II

Day 2

Date: 19/02/2010

Volunteer ID (*Researcher use only*): XXX

Time	Food/Drink (description, brand, where bought, how cooked)	Amount served (weight, tsp, slices etc)
7:30AM	Granola (Nature Valley)	3 tablespoons
	2% reduced fat milk	$\frac{1}{2}$ 8 oz.-cup
	White toast	1 slice
	Low fat butter (Land O Lakes)	Spread thinly
	Cheddar cheese	1 slice
	Chamomile tea	8 oz.
10:30AM	Coffee (from machine), whole milk, no sugar	8 oz.-foam cup
1:00 PM	Whole wheat bread	2 thick slices
	American cheese	2 slices
	Spread (Utterly butterly)	Spread thinly
	Orange	1 medium size
	Diet coke	20 oz.
3:30PM	White grape juice (Welch)	8 oz.
	Twix bar	2 oz. bar
6:00PM	Beef stew (see recipe page)	$\frac{1}{2}$ amount of recipe
	Cauliflower, boiled	2 tablespoons
	Potatoes, roasted in vegetable oil	4 medium sized
	Low fat strawberry yoghurt (Yoplait)	6 oz.
8:00PM	Decaffeinated coffee	6 oz.
	Breakfast biscuits (Quaker)	2

Food intake record

Feeding visit period ____ Day ____ Date:

Volunteer ID (*Researcher use only*):

[illegible]

Recipe Sheet

Please use this sheet for any recipes you use whilst recording your intake for us. In the “Recipe Portion Served” box tell us how much of the recipe you actually ate.

Feeding visit period ____ Day ____ Date:

Volunteer ID (*Researcher use only*):[illegible]

Annex 4: Urine collection instructions

Version 1

09/24/2019



Blueberry
Absorption
Metabolism

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Dear Mr. (s)

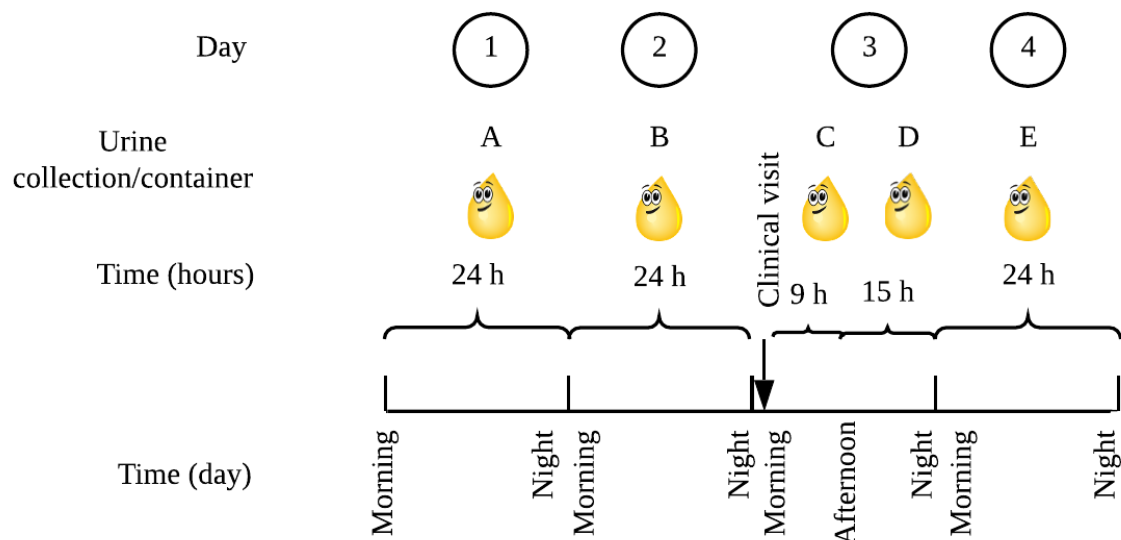
Please, follow these steps to collect your urine for **15 hours and 24 hours** while at home:

1. Use each of the provided containers to collect all your urine voids in the time (hours) indicated in the figure below as collections A, B, C, D and E;
2. Write date and time of the FIRST and the LAST urine collections on that container;

Example:

Collection A	
Start date: <u>02</u> / <u>15</u> /2020	First collection time: 7:15 AM
End date: <u>02</u> / <u>16</u> /2020	Last collection time: 7:00 AM

3. Please, store the container in the cooler provided, on ice packs. You received enough ice packs to freeze some while others are placed in the cooler to keep your container cool;
4. Please, return container(s) to the CLINIC on each scheduled visit.



5. Please, **DO NOT PLACE** any body parts into the container to collect your urine.

6. For **women**: place the urine hat provided under the toilet seat to collect your urine into this hat. Then, pour your urine into the container.

7. What exact time do you need to add your urine void into container A for example and not into container B?

a. Have the time you are coming to visit day 1, for example, you are coming at 7:00 AM.

b. **CONTAINER A** will be filled with the urine you generate from 48 hours before the time of visit day 1 until a total of 24 hours.

In other words, if your visit day 1 is at 7:00 AM on a Tuesday, you will need to fill container A with your urine from Sunday 7:00 AM until Monday 7:00 AM maximum. In case you urinate at 7:01AM on Monday, this urine already goes into container B;

c. **CONTAINER B** will be filled with the urine you generate from 24 hours before the time of visit day 1 until a total of 24 hours.

In other words, if your visit day 1 is at 7:00 AM on a Tuesday, you need to fill container B with your urine from Monday 7:00 AM until Tuesday 7:00 AM maximum. In case you urinate at 7:01 AM on Monday, this urine already goes into container C;

d. **CONTAINER C** will be filled with the urine you generate from the time of visit day 1 until the end of this visit, for a total of approximately 9 hours.

In other words, if your visit day 1 is at 7:00 AM on a Tuesday, you need to fill container C with your urine from 7:00 AM until 4:00 PM maximum. In case you urinate at 4:01 PM, this urine already goes into container D;

e. **CONTAINER D** will be filled with the urine you generate from the time you leave the clinic on visit day 1 until the time of your next visit (visit day 2), for a total of approximately 15 hours.

In other words, if you leave the clinic on visit day 1 is at 4:00 PM on a Tuesday, you need to fill container D with your urine from 4:01 PM until 7:00 AM of Wednesday maximum. In case you urinate at 7:01 AM, this urine already goes into container E;

f. **CONTAINER E** will be filled with the urine you generate from the time of visit day 2 until the time of your next visit (visit day 3), for a total of approximately 24 hours.

In other words, if you arrive in the clinic on visit day 2 is at 7:00 AM on a Wednesday, you need to fill container E with your urine from 7:01 AM until 7:00 AM of Thursday maximum.

Kind regards,

Monique Carvalho de Santana
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North Carolina State University
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Phone: (704) 250-5451