

IRB Project #: UNL-00024307

1. Study Title: Effects of Distinct Dry Beans Market Classes on the Gut Microbiota

2. Authorized Study Personnel

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3. Key Information:

If you agree to participate in this research study, the project will involve:

- Key inclusion/exclusion criteria (additional detail provided below):
 - Between the ages of 19 and 50 years.
 - A stable body mass index between either 18.5-24.9 kg/m² or 27.0-39.9 kg/m².
 - Not using any medications for diabetes, hypertension, heart, liver, kidney, gut, or immune conditions.
 - Not consuming beans from other sources during the study.
 - No known allergies or intolerance to beans.
 - Ability to wear mobile blood pressure monitor and limit physical activity over a 24-hour period.
 - Have a bowel movement at least every other day
 - Ability to deliver your stool sample to our study site within 4 hours of collection.
- Procedures will include (additional detail provided below):
 - This study consists of 3 phases: a Baseline Phase, 3 Intervention Phases, and 2 Washout Phases.
 - Sixteen study visits over approximately a 12-week period. Additional visits may be needed to deliver stool samples.
 - During this study:
 - We will ask you to complete several questionnaires during each phase of the study. This will include basic demographic and health information, diet, lifestyle, and digestive health information.
 - We will collect your blood pressure using 2 different machines and ask you to wear a 24-hour mobile blood pressure device.
 - Measurements of your height, weight, and body composition (amount of fat, muscle, and water in your body).
 - Four blood draws
 - Collection of fresh stool samples that need to be delivered to the research location (Innovation Campus) within 4 hours of collection.
 - Ask you to eat pre-cooked beans (pink, great northern, or a mixture of beans) daily for two weeks (1st week: ½ cup, 2nd week: 1½ cups). You will do this during the 3 Intervention Phases. There will be 2 weeks between eating each bean type that you can resume your normal lifestyle, with the exception of eating beans.
- The total time required to complete the study is estimated at 21 hours and 20 mins. This does not include the time wearing the mobile blood pressure monitor (~144 hours) or travel time.

- Informed Consent Document; 40 mins \times 1 = 40 mins
 - Diet History Questionnaire; 50 mins \times 1 = 50 mins
 - Diet 24-Hour Recalls; 20 mins \times 18 = 360 mins
 - Bowel Habit Journal; 15 mins \times 9 = 135 mins
 - Gut Symptoms Questionnaire; 10 mins \times 9 = 90 mins
 - Other Questionnaires
 - sleep habits; 10 mins \times 1 = 10 mins
 - physical activity; 10 mins \times 6 = 60 mins
 - quality of life; 10 mins \times 6 = 60 mins
 - demographic, medical, and medication history; 30 mins \times 1 = 30 mins
 - Height and Weight; 5 mins \times 1 = 5 mins
 - Body Composition; 5 mins \times 6 = 30 mins
 - Office Blood Pressure; 10 mins \times 12 = 120 mins
 - Mobile Blood Pressure; 20 mins \times 6 = 120 mins
 - Blood Collection; 20 mins \times 4 = 80 mins
 - Stool Collection; 10 mins \times 9 = 90 mins
- Stool samples will be used to assess your gut bacteria. The blood sample will be used to test different health markers.
 - In some cases, we may find interesting microbes in your stool sample that we will want to study. Microbes are isolated from your stool will be property of the University of Nebraska.
 - There is minimal risk associated with this study, as the study involves a blood draw and mobile blood pressure monitor. It is possible to experience mild pain, fainting, bleeding, bruising, and/or an infection at the insertion site. Bruising is common, but usually goes away after a few days after the blood draw. It is also possible to experience skin irritation, discomfort, bruising, or an interruption of your daily activities or sleep while wearing the mobile blood pressure monitor. Mild digestive symptoms may also arise when consuming the beans.
 - You will receive up to \$300 in electronic Amazon gift cards for your time and participation.
 - Your data collected from this study may be shared as described below.
 - You will be provided with a copy of this consent form.
 - Your participation is voluntary. You can decide not to participate at any time without any penalty or loss of benefits.

4. Invitation

You are invited to take part in this research study. The information in this consent form is meant to help you decide whether or not to participate. If you have any questions, please ask.

5. Why are you being asked to be in this research study?

You are being asked to be in this study because you are between 19 and 50 years with a stable body mass index that is between either 18.5-24.9 kg/m² or 27-39.9 kg/m². Also, you meet the initial inclusion/exclusion criteria through the pre-screening process. Finally, you are able to:

- i) eat the provided amount of beans and avoid eating other beans during the study,
- ii) wear a blood pressure monitor and limit physical activity for 24 hours
- iii) collect and deliver stool samples to the study site (Innovation Campus) within 4 hours of collection.

Inclusion criteria:

- Between the age of 19 and 50 years.
- Not currently pregnant or planning to become pregnant (*Females Only*).
- Stable body mass index between either 18.5-24.9 kg/m² or 27.0-39.9 kg/m² for the last month.
- Has not made any major dietary changes in the last month.
- Able to read and speak English.
- Requires no legally authorized representative (LAR).
- Not institutionalized (e.g., prison, psychological treatment center, etc.).
- Ability to wear mobile blood pressure monitor and limit physical activity over a 24-hour period.
- Have a bowel movement at least every other day
- Ability to collect and deliver your stool sample to our study site within 4 hours of collection.
- No known allergies or intolerance to beans.
- Ability to avoid consuming beans during the study, except for the provided beans
- Not using any medications for diabetes, hypertension, heart, liver, kidney, gut, immune, or chronic pain conditions.

Exclusion criteria:

- Has a cardiac device or history of organ transplant
- History of gastrointestinal surgery of disease diagnosed by a physician that involves the stomach, small, and large intestines.
- Recent history of cancer in the last year (excluding skin cancer).
- Current use of tobacco.
- Current or recent use of digestive enzymes, laxatives, dietary fiber, prebiotic, or probiotic supplements.
- Supplement regimen or dosage changed within the last 3 weeks.
- Medication regimen or dosage changed within the last 2 months.
- Taken antibiotics in the last 2 months.
- *BMI 18.5-24.9 kg/m²*: Current use of oral or injectable medications for the treatment of any chronic conditions.
- *BMI 27.0-39.9 kg/m²*: Current use of oral or injectable medications for the treatment of specific chronic conditions: diabetes, hypertension, heart, liver, kidney, gut, or immune.

6. What is the reason for doing this research study?

Dry beans are known to be good for our health in part by benefiting our gut bacteria. Yet, how they affect us depends on the diverse makeup of nutrients in different bean types. These bean types are called market classes. Some market class examples are pink beans and great northern beans. When we eat dry beans, about 35-45% of the nutrients remain undigested. These nutrients can include dietary fibers, proteins, and polyphenols. These different nutrients serve as food for our gut microbes and can impact our health. However, it remains unclear how different bean types change the health and gut bacteria of humans.

This study is designed to compare the effects of two dry bean market classes, pink beans and great northern beans, on health markers and gut microbes in humans. It is also designed to

compare these effects to a mixture of five beans: pink, great northern, black, pinto, and kidney beans. This study is being conducted by the University of Nebraska-Lincoln. Around 12 people will participate in this study.

This research may identify gut microbes that are linked to the benefits of eating beans. These microbes may be isolated from your stool sample for further testing and will be property of the University of Nebraska. Your participation will contribute to important discoveries about the health benefits of different beans.

7. What will be done during this research study?

You will be asked to complete 16 study visits over approximately a 12-week period. Additional visits may also be needed to deliver stool samples. The study visits will take place at the Nebraska Food for Health Center Research Clinic located at 1901 N 21st Street, Room 101, Lincoln, Nebraska.

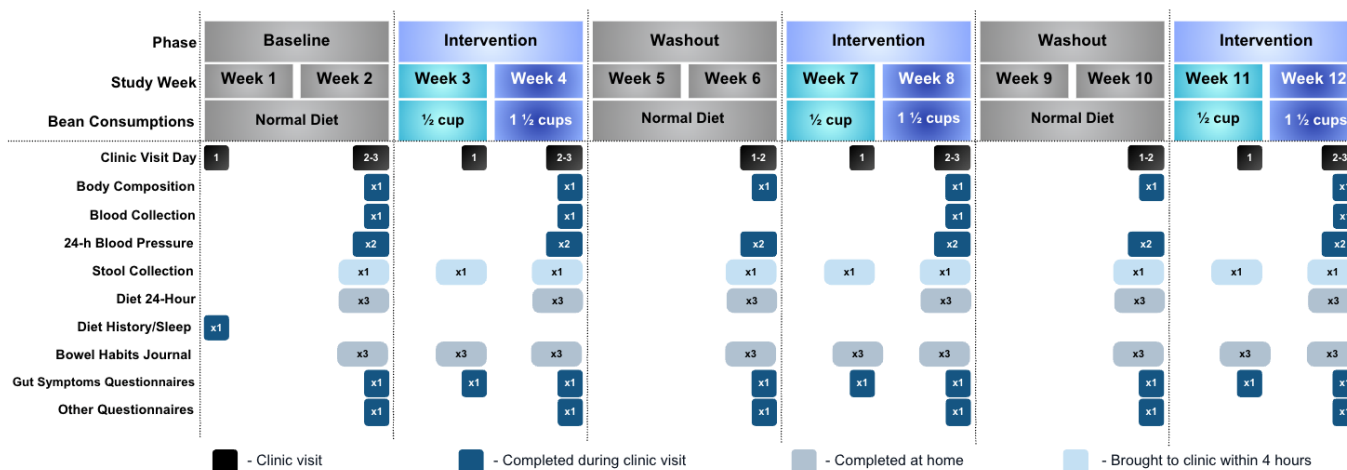


Figure 1. Layout of Study Visits and Tasks to be Completed.

Explanation of Study Visits (See Explanation of Each Study Task Below):

Baseline Phase:

- The study will start with a baseline phase. This consists of 3 visits over ~2-weeks.
- Visit 1:
 - Your first visit will be a screening visit. We will ask about demographic, medical, and medication information. We will also measure your height and weight to confirm study eligibility.
 - We will have you complete a diet history questionnaire and a questionnaire that asks about your sleep habits. We will teach you how to complete a bowel habits journal over a 3-day period and diet 24-hour recalls over a 3-day period. We will also provide stool collection supplies and teach you how to collect the stool samples.
- Visits 2 and 3:
 - We will ask you to complete 3 diet 24-hour recalls, a bowel habit journal over 3 days, and collect 1 stool sample during this study period.
 - At visit 2, we will check your blood pressure using two different monitors. Then, we will ask you to wear the mobile blood pressure monitor and abstain from

physical activity over the next 24 hours.

- At visit 3, we will recheck your blood pressure using the two different monitors and remove the mobile monitor. We will then measure your body composition and collect a blood sample. We will also have you complete questionnaires that ask about your digestive symptoms, quality of life, and physical activity. Finally, we will provide you with pre-packaged beans for the next week.
- Your stool sample will be collected over a 3-day window (Visit \pm 1 day).

Intervention Phases:

- There will be 3 intervention phases. Each intervention phase will last 2 weeks.
 - During the phases you will be asked to consume daily one of three types of beans.
 - Bean types include pink, great northern, or a mixture of pink, great northern, black, kidney, and pinto beans.
 - You will be asked to consume all 3 bean interventions but in random order. There are 6 different possible orders of consuming the beans. The order that you will consume the beans will be randomly determined at baseline.
 - Amount of beans will increase over the 2 weeks (1st week: $\frac{1}{2}$ cup, 2nd week: 1 $\frac{1}{2}$ cups). Bean will be handed out on a weekly basis and at the beginning of each study week.
 - The beans will be pre-cooked. They will be provided frozen in individual daily bags. You will be given an insulated lunch bag to bring the frozen beans home. Once home, the beans should be transferred to your freezer and then to your fridge to thaw. The beans can be kept in your fridge for up to four days before consumption.
 - You will be asked to reheat the beans before adding them to your normal diet. Remove beans from the bags before reheating.
 - We will ask you to avoid consuming all other beans during the study, except the beans provided.
 - We will also ask you to abstain from physical activity during the 24-hour mobile blood pressure monitor.
- Visit 1:
 - We will ask you to complete 2 questionnaires. The questionnaires will ask you about your digestive symptoms and bowel habits. We will also provide you with pre-packaged beans for the second week.
 - Your stool sample will be collected over a 3-day window (Visit \pm 1 day).
- Visit 2 and 3:
 - We will ask you to complete 3 diet 24-hour recalls, 1 bowel habit journal, and collect 1 stool sample during this study period.
 - At visit 2, we will check your blood pressure using two different monitors. Then, we will ask you to wear the mobile blood pressure monitor over the next 24 hours. Physical activity will be avoided during this time.
 - At visit 3, we will recheck your blood pressure using the two different monitors and remove the mobile blood pressure monitor. We will then measure your body composition and collect a blood sample. We will also have you complete

questionnaires that ask about your digestive symptoms, quality of life, and physical activity.

- You will be asked to bring in the empty bags and any unconsumed beans on this visit. This will be to monitor protocol adherence.
- Your stool sample will be collected within a 3-day window (Visit \pm 1 day).

Washout Phases:

- There will be 2 washout phases. During these phases you will be asked to return to your normal lifestyle, with the exception of avoiding eating beans, for 2 weeks. There will be two visits at the end of each washout phase.
- Visit 1 and 2:
 - We will ask you to complete 3 diet 24-hour recalls, 1 bowel habit journal, and collect 1 stool sample during this study period.
 - At visit 2, we will check your blood pressure using two different monitors. Then, we will ask you to wear the mobile blood pressure monitor over the next 24 hours. Physical activity will be avoided during this time.
 - At visit 3, we will recheck your blood pressure using the two different monitors and remove the mobile monitor. We will then measure your body composition and have you complete questionnaires that ask about your digestive symptoms, quality of life, and physical activity. Finally, we will provide you with pre-packaged beans for the next week.
 - Your stool sample will be collected over a 3-day window (Visit \pm 1 day).

Explanation of Study Tasks (Questionnaires and Measurements)

Questionnaires:

- Diet History Questionnaire: We will ask you to complete one diet history questionnaire. The questionnaire will ask you about what kind of food you ate over the past 12 months. This questionnaire will be completed online. It will take around 50 mins to complete.
- Diet 24-Hour Recall: We will ask you to complete 18 dietary 24-hour recalls throughout the study. The questionnaire will ask you about what food you ate the day before. This questionnaire will be completed online. It will take around 20 mins for each questionnaire.
- Bowel Habits Journal: We will ask you to complete a bowel habit journal 9 times throughout the study. The bowel habit journal will ask you to record each bowel movement you have over a 3-day period. It will also ask you to describe each bowel movement, such as its consistency. It will take around 15 mins for each questionnaire.
- Gut Symptoms Questionnaire: We will ask you to complete a questionnaire that asks you about your digestive symptoms over the last week. The questionnaire will be completed 9 times throughout the study. It will take around 10 mins for each questionnaire.
- Other Questionnaires: We will ask you to complete 3 other questionnaires.
 - The first will ask about your sleep habits over the last month. This will be done once and will take about 10 mins.
 - The second will ask about your physical activity over the last week. This will be done 6 times and will take about 10 mins to complete each questionnaire.

- The third will ask about your quality of life. This will be done 6 times and will take about 10 mins to complete each questionnaire.
- You will also be asked questions about your medical and medication history at the beginning of the study.

Measurements:

- Height and Weight: We will ask you to step on a scale to obtain your body weight and height. This will be done once and will be used to calculate your body mass index. This will take around 5 min.
- Body Composition: We will measure your body composition (how much fat and muscle your body is made of) 6 times throughout the study. This will be done using bioelectrical impedance analysis. The equipment measures how a low, painless electrical current travels through your body. You will be asked to remove your shoes and socks, jewelry, metal containing items, and items in your pockets. You will stand on the scale for the test. This will take around 5 mins each time.
- Blood Pressure: We will measure your blood pressure using two different monitors.
 - The first is an office-based blood pressure monitor. It will be used 12 times at the Nebraska Food for Health Center Research Clinic. It will take around 10 mins each time.
 - The second is a mobile blood pressure monitor. It will measure your blood pressure every 30 mins over a 24-hour study period. This monitor will be used 6 times throughout the study. It will take around 20 mins to set up and stop the blood pressure monitor each time. You will be asked to abstain from physical activity during this 24-hour test.
- Blood Collection: We will collect ~22.5-milliliters of blood (around 4.5 Teaspoons) from a vein in your arm or hand. This will be done 4 times throughout the study (~90-milliliters total). The blood draws will be done by trained personnel. The blood samples will be collected in the morning following an overnight fast, which means no food or drink, except water, 12 hours before the scheduled visit. Also, no alcohol 24 hours prior to the visit. Blood samples will be used to test health-related markers. This will take around 20 mins each time.
- Stool Collection: We will ask you to collect and deliver stool samples to the Nebraska Food for Health Center Research Clinic on Innovation Campus within 4 hours of collection. A total of 9 stool samples will be collected throughout the study. You will have a 3-day timeframe to collect each sample. The stool samples will be used to assess your gut microbes. It will take around 10 mins each time.

8. How will my data and biospecimens be stored or shared?

This study is collecting data and biospecimens (blood and stool samples) from you. We would like to make your data and biospecimens (stool samples) available for other research studies that may be done in the future. The research may be about similar study aims, topics, diseases, or conditions to this study. However, research could also be about unrelated diseases, conditions, or other types of research. These studies may be done by researchers at the University of Nebraska or other institutions, including commercial entities. Our goal is to make more research possible. Your name and identifying information will be removed from any data and biospecimens you

provide before they are shared with other researchers. Researchers that receive your information cannot easily link your identifying information to the data and biospecimens.

Participating in this study means you agree to share your data and biospecimens (stool samples). You can change your mind later, but researchers might still use your data and biospecimens (stool samples) if they have already been shared. If you do not want your data and biospecimens (stool samples) used for other projects, you should not participate in this study.

9. Will my biospecimens be used for genetic analysis and/or commercialization?

In some cases, we may find interesting bacteria in your biospecimens (stool samples) that are linked to the benefits of eating beans. These bacteria may be isolated from your stool sample(s) for further testing and may be used for commercial profit, even if identifiers such as your name, dates of service, or other identifiers are removed. Bacteria that are isolated will be property of the University of Nebraska. As a research participant, you will not share in this commercial profit.

Your biospecimens that are collected for this research study will include sequencing of your gut bacteria. It will not include whole genome sequencing. This means that the researchers have no plans to look at or try to “read” the protein information that makes up your genes (DNA) from your sample.

10. Will I be notified if my data and biospecimens result(s) in an unexpected finding?

When data and biospecimens are collected and analyzed, there is a chance of finding something unexpected. There may be benefits to learning such results, such as early detection and treatment of a medical condition. But there are also risks, such as feeling worried about a finding for which no treatment is required or appropriate.

The results from the data and biospecimens we collect in this research study are not the same quality as what you would receive as part of your healthcare. The data and biospecimen results will not be reviewed by a physician who normally reads such results. Due to this, the results of your data and biospecimens will not be placed in your medical record with your primary care physician or otherwise. If you believe you are having symptoms that may require care, you should contact your primary care physician.

For those individuals that are interested, we would like to make a portion of your data available to you after completion of the study. This information will be limited. But will include select health-related information collected at the beginning of the study. This may include body composition, blood pressure, and health marker information. Study personnel will not be able to review this information with you. Participants will be asked if they would like to receive this information.

Would you like to receive a copy of your health-related information at the end of the study?

If Yes, Initial: _____

If No, Initial: _____

11. What are the possible risks of being in this research study?

We will do our best to protect your data and biospecimens during storage or if they are shared. However, there remains a possibility that someone could identify you. There is also the possibility that unauthorized people might access your data and biospecimens. In either case, we cannot reduce the risk to zero.

Fasting: There is a possibility that you will feel hungry and uncomfortable during the fasting the night before each visit, and it may be inconvenient for you to not eat anything.

Blood Draw: The risks associated with blood draw include discomfort at the site of the blood draw, feeling dizzy and nauseated, and bruises and blot spots under the skin. Clotting of the blood, blocking of arteries, and infections are very rare but are also potential risks. These risks will be reduced or eliminated by having only trained staff draw blood.

24-Hour Blood Pressure Monitor: Wearing the blood pressure cuff for an extended period may cause discomfort or irritation, especially during inflation. The frequent inflation can be annoying and may interrupt daily activities or sleep. Prolonged or incorrect wear of the blood pressure cuff might lead to minor skin irritation, redness, and possibly minor bruising on the arm where the cuff is worn.

Dietary Changes: Intolerance or mild discomfort in your gut might occur from the daily addition of beans to your diet. This could include bloating, gas, or changes in bowel habits. Symptoms should resolve once the beans are not consumed.

Bean Allergy: Well allergies to beans are not commonly observed, they are possible. Allergies could range from mild (e.g., hives, nausea) to severe (e.g., anaphylaxis) symptoms. Symptoms should resolve once the beans are not consumed.

Stool Collection: There may be feelings of embarrassment when collecting stool samples and the risk of contamination. You can collect the sample in the privacy of your home. We will provide you with the supplies and instructions to minimize the risk of contamination.

It is possible that other rare side effects could occur which are not described in this consent form. It is also possible that you could have a side effect that has not occurred before.

12. What are the possible benefits to you?

You will not receive any direct benefit from sharing your data and biospecimens. However, we would like to share a portion of your data with you after you complete the study, if you are interested. These data will be limited but will include select health-related information collected at the beginning of the study. This information may be of interest to you.

13. What are the possible benefits to other people?

The benefits to science and/or society may include understanding how different dry beans can be used to precisely modify the gut microbiome. This will help inform precision nutrition efforts.

14. What are the alternatives to being in this research study?

Instead of being in this research study you can choose not to participate.

15. What will being in this research study cost you?

You will receive all study materials at no cost to you. You will be responsible for expenses associated with traveling to and from the Nebraska Food for Health Center Research Clinic on Innovation Campus.

16. Will you be compensated for being in this research study?

You will receive up to \$300 through electronic Amazon gift cards. At the end of each intervention phases, you will receive an Amazon gift card for \$100. This will be emailed to you.

In order to document your receipt of the payment, you must provide your name and address to the research team. Payment records will be stored for up to 7 years and may be stored with Financial Personnel at the University.

17. Who is paying for this research?

This research is being funded by the University of Nebraska-Lincoln and Nebraska Food for Health Center.

18. What should you do if you have a problem during this research study?

Your welfare is the major concern of every member of the research team. If you have a problem as a direct result of being in this study, you should immediately contact one of the people listed at the beginning of this consent form.

19. How will information about you be protected?

Reasonable steps will be taken to protect your privacy and/or the confidentiality of your study data; however, in some circumstances we cannot guarantee absolute privacy and/or confidentiality. To maintain confidentiality, you will receive a participant ID number that will be used throughout the study on sample containers, questionnaires, data reports, etc.

Physical research records will be stored in a locked cabinet in the office of the research coordinator or investigator and will only be seen by the research team and/or those authorized to view, access, or use the records during and after the study is complete. Electronic research records will be stored in secure, cloud-based servers authorized by the University of Nebraska and will only be seen by the research team and/or those authorized to view, access, or use the records during and after the study is complete. Study documents will contain only participant ID numbers. If files need to be shared among collaborating investigators, only the files containing coded data with ID numbers will be used. No names, phone numbers, or identifiable information will be reported or provided to any outside party.

Data collected using the online diet questionnaires will also be stored coded on the United States National Cancer Institute's server. It is encouraged to share gut bacteria data among researchers to promote our knowledge of the gut microbiome. Therefore, information about what gut bacteria are present in stool samples, how they change when eating different beans, and limited personal data (age, sex, body mass index, diet, etc.) will be uploaded completely deidentified to the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA).

Those who will have access to your research records are the study personnel, the Institutional Review Board (IRB), and any other person, agency, or sponsor as required by law or institutional responsibility. Information from this study may be published in scientific journals or presented at scientific meetings and may be reported individually, or as group or summarized data but your identity will be kept strictly confidential.

20. Public Information about this Study?

ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

21. What are your rights as a research subject?

You may ask any questions concerning this research and have those questions answered before agreeing to participate in or during the study. For study related questions, please contact the investigator(s) listed at the beginning of this form. For questions concerning your rights or complaints about the research contact the Institutional Review Board (IRB) at 1(402)472-6965 or irb@unl.edu.

22. What will happen if you decide not to be in this research study or decide to stop participating once you start?

You can decide not to be in this research study, or you can stop being in this research study (“withdraw”) at any time before, during, or after the research begins for any reason. Deciding not to be in this research study or deciding to withdraw will not affect your relationship with the investigator or with the University of Nebraska-Lincoln. You will not lose any benefits to which you are entitled.

If the research team gets any new information during this research study that may affect whether you would want to continue being in the study, you will be informed promptly. The researchers may also make the decision to take you out of the study, even if you want to continue, if:

- Your health changes and the study is no longer in your best interest.
- You do not follow the study rules or no longer meet the requirements to be in the study.
- The study is stopped by the sponsor, IRB, or researchers.

For your safety, please talk to the research team before you stop any research treatments. They will advise you how to stop the treatment most safely. If you withdraw you may be asked to undergo some additional tests. You do NOT have to agree to do these tests.

Institutional Review Board Approved

As a subject participating in this research study, you have certain responsibilities that may apply to this study, such as ensuring that you arrive at all of your scheduled appointments, adhere to the study protocol described in the consent form, and report any adverse reactions you may have during the study.

This study was approved by the UNL Institutional Review Board.

Documentation of informed consent

You are voluntarily making a decision whether or not to be in this research study. Signing this form means that (1) you have read and understood this consent form, (2) you have had the consent form explained to you, (3) you have had your questions answered and (4) you have decided to be in the research study. You will be given a copy of this consent form to keep.

Participant Feedback Survey

The University of Nebraska-Lincoln wants to know about your research experience. This 14 question, multiple-choice survey is anonymous. This survey should be completed after your participation in this research. Please complete this optional online survey at:
<http://bit.ly/UNLresearchfeedback>.

Participant Information:

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Name (please print)

Signature

Date

Person Obtaining Consent:

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Name (please print)

Signature

Date