Study Protocol

NCT04175106

Date: 19-Nov-2020

NORTH CAROLINA STATE UNIVERSITY

INSTITUTIONAL REVIEW BOARD FOR THE USE OF HUMAN SUBJECTS IN RESEARCH

SUBMISSION FOR NEW STUDIES

Protocol Number 19138

Project Title

CLOSED - 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.

IRB File Number:

Original Approval Date:

09/24/2019

Approval Period

07/29/2021 - 01/01/2100

Source of funding (provide name of funder not account number):

Foundation for Food and Agriculture Research

NCSU Faculty point of contact for this protocol:NB: only this person has authority to submit the protocol

Cheryl Granillo: Kannapolis Research

Does any investigator associated with this project have a significant financial interest in, or other conflict of interest involving, the sponsor of this project? (Answer No if this project is not sponsored)

No

Is this conflict managed with a written management plan, and is the management plan being properly followed?

No

Preliminary Review Determination

Category:

Full Board - Closed

In lay language, briefly describe the purpose of the proposed research and why it is important. Provide a brief synopsis of the study including who is targeted to participate and the data collection methods employed (limit text to 1500 characters)

The 4-phase crossover study focuses on within participant variation in the clearance kinetics of (poly)phenols in 2 different commercial blueberry varieties, a â€œminimally processedâ€• blueberry-rich protein bar, and a control beverage of matched-nutritive content in healthy human volunteers to identify the impact of berry content and processing on absorption and elimination kinetics. Sex will be used as a covariate in statistical analysis and as within participant variation is the primary outcome, population diversity is not required. There is no attempt to recruit or limit or stratify by socioeconomic statuses. We will establish clearance in blood and urine over a 48-hour period across 4 treatment phases. The entire study will last 3-months involving a consent visit, screening visit, four weeks of restricted food intake, four visits of 9.5 hours in the clinic, and additional 8 visits that will take 1 hour each. The total study requires a total commitment of 54.5 hours over the 3 month enrollment period.

Does any member of the project team who is responsible for the design, recruitment, consent, implementation of intervention, interaction with participants, or those handling identifiable private information under this IRB protocol - or any members of their immediate family (defined as spouse, dependent children - have any Significant Financial Interest or other types of conflict of interest (as described in SOP 14.3.a) related to the protocol?

If the answer is "yes," please provide the name of the investigator(s) with the potential or actual conflict and confirm that the relationship has been fully disclosed in the investigators most recent COI disclosure filed with NC State or disclosed through the collaborative research process. If there is a COI management plan in place with NC State University, please upload it with this application to ensure the IRB protocol meets the expectations of the COI plan and the COI is properly considered in the IRB review process. If you are uncertain how to respond or have questions, please contact COI-NOI-Compliance@ncsu.edu.

N/A

This research qualifies for Exemption. Review NC State's Exemption Research SOP for studies that may qualify. If you want to apply for an Exemption, download the Exemption Request Form and complete it. To the eIRB, upload the completed Exemption Request Form, all instruments, and if applicable a Data Access and Security Plan and the edited Consent/Opt-Out forms modified to fit the study design. Only complete the "Title" and

"Description" tabs in the eIRB, upload the aforementioned documentation, and submit the eIRB application. Do not complete any other tabs within the eIRB system.

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Is this research being conducted by a student?

Yes

Is this research for a thesis/disseration/capstone?

Yes

Is this research for a dissertatiion?

No

Is this independent research?

No

Is this research for a course?

No

Do you currently intend to use the data for any purpose beyond the fulfillment of the class assignment?

No

Please explain

If so, please explain

If you anticipate additional NCSU-affiliated investigators (other than those listed on the Title tab) may be involved in this research, list them here indicating their name and department.

Monique Carvalho de Santana, PhD student under the supervision of Dr. Colin D. Kay at the Department of Food, Bioprocessing and Nutrition Sciences in the NCSU, Cheri Granillo, Translational Nutrition Program Manager at the Plants for Human Health Institute of the NCSU at North Carolina Research Campus, Harry Schulz, Laboratory Managing Technician at Dr. Colin D. Kayâ€™s laboratory in the Plants for Human Health Institute of the NCSU at North Carolina Research Campus, Jessica Everhart, Research Technician at Dr. Colin D. Kayâ€™s laboratory in the Plants for Human Health Institute of the NCSU, and Atul Rathore, Postdoctoral Research Scholar at Dr. Colin D. Kayâ€™s laboratory in the Plants for Human Health Institute of the NCSU.

Will the investigators be collaborating with researchers at any institutions or organizations outside of NC State?

Yes

List collaborating institutions and describe the nature of the collaboration. If researchers from both institutions are doing any of the following activities: recruitment, consent process, data collection or handling of identifiable information/specimens a reliance agreement may be appropriate. For more information, please contact irb-coordinator-admin@ncsu.edu

University of North Carolina Chapel Hill, Clinical Services Facility at the Nutrition Research Institute-Human Research Core; located at the North Carolina Research Campus (Kannapolis, NC) will act as a sub-contracted facility providing clinical examination and leisure space, licensed kitchen and dining facilities, and phlebotomist and physician support. The NRI phlebotomists and physician will be responsible for phlebotomy services, consultation, blood pressure and anthropometric analysis, medical questionnaires, and review of abnormal clinical screening results and adverse events.

What is NCSU's role in this research?

Lead. NCSU investigators led the design of this intervention and will lead on recruitment, implementation, and data collection, handling and destruction. Investigators will follow Good Clinical Practice (GCP) and Good Clinical Laboratory Practice (GCLP).

Describe funding flow, if any (e.g. subcontractors)

The present clinical study is funded by the Foundation for Food and Agriculture Research, with the clinical intervention run (phlebotomy, clinical monitoring, MD oversight) under subcontract at the University of North Carolina Chapel Hill, Nutrition Research Institute-Human Research Core, located at the North Carolina Research Campus (Kannapolis, NC).

Is this international research?

No

Identify the countries involved in this research

An IRB equivalent review for local and cultural context may be necessary for this study. Can you recommend consultants with cultural expertise who may be willing to provide this review? Consultants may not be a part of the research team or have a stake in the research project. Provide email contact information for consultant(s). A local context review may lengthen the time it takes for your approval.

Adults 18 - 64 in the general population?

Yes

NCSU students, faculty or staff?

Yes

Adults age 65 and older?

No

Minors (under age 18--be sure to include provision for parental consent and/or child assent). If minors are included in your research, please read through the NC State University Regulation for your additional responsibilities. Following this regulation is a requirement of your affiliation with NC State.?

No

List ages or age range:

Could any of the children be "Wards of the State" (a child whose welfare is the responsibility of the state or other agency, institution, or entity)?

No

Please explain:

Does this study involve people who are also incarcerated, involuntarily detained or committed, or are in a program or hospital as an alternative form of sentencing?

No

Pregnant women?

No

Are pregnant women the primary population or focus for this research?

No

Provide rationale for why they are the focus population and describe the risks associated with their involvment as participants

Fetuses?

No

Students?

No

Does the research involve normal educational practices?

No

Is the research being conducted in an accepted educational setting?

Yes

Are participants in a class taught by the principal investigator?

No

Are the research activities part of the required course requirements?

No

Will course credit be offered to participants?

No

Amount of credit?

No

If class credit will be given, list the amount and alternative ways to earn the same amount of credit.Note: the time it takes to gain the same amount of credit by the alternate means should be commensurate with the study task(s)

How will permission to conduct research be obtained from the school or district? IRB approval is not permission to conduct the research. You need to access a gatekeeper. If you are implementing a survey with NC State populations, please make sure you follow the NC State survey regulation.

Will you utilize private academic records?

No

Explain the procedures and document permission for accessing these records.

Employees?

No

Describe where (in the workplace, out of the workplace) activities will be conducted.

From whom and how will permission to conduct research on the employees be obtained?

How will potential participants be approached and informed about the research so as to reduce any perceived coercion to participate?

Is the employer involved in the research activities in any way?

No

Please explain:

Will the employer receive any results from the research activities (i.e. reports, recommendations, etc.)?

No

Please explain. How will employee identities be protected in reports provided to employers?

Impaired decision making capacity/Legally incompetent?

No

How will competency be assessed and from whom will you obtain consent?

Mental/emotional/developmental/psychiatric challenges?

No

Identify the challenge and explain the unique risks for this population.

Describe any special provisions necessary for consent and other study activities (e.g., legal guardian for those unable to consent).

People with physical challenges?

No

Identify the challenge and explain the unique risks for this population.

Describe any special provisions necessary for working with this population (e.g., witnesses for the visually impaired).

Economically or educationally disadvantaged?

No

Racial, ethnic, religious and/or other minorities?

No

Non-English speakers?

No

Describe the procedures used to overcome any language barrier.

Will a translator be used?

No

Provide information about the translator (who they are, relation to the community, why you have selected them for use, confidentiality measures being utilized).

Explain the necessity for the use of the vulnerable populations listed.

There is no need for vulnerable populations in this study. No sex/gender or racial/ethnic group will be excluded.

Amendment request (November/2020): We are including participants who are 65 years old. The necessity for the use of vulnerable populations is because we have had volunteers who are 65 years old interested in enrolling in this study and fitting in every eligibility criteria of our study. Since we have been delayed extensively as a result of the COVID-19 pandemic, the inclusion of this population may facilitate our recruitment.

State how, where, when, and by whom consent will be obtained from each participant group. Identify the type of consent (e.g., written, verbal, electronic, etc.). Label and submit all consent forms. Adult Non-Exempt Consent Form Template Exemption Consent Form Templates

After showing interest in taking part in the study by response to a posted study advertisement (printed or online) via email or phone (Annexes 1A and 1B), potential participants will be contacted (by clinical coordinator or research technician) via email or phone for an eligibility pre-screen phone interview (Annex 2A), describing the protocol in further detail, including full inclusion and exclusion criteria.

If participants are deemed eligible (i.e., meeting inclusion criteria) at the initial interview, a package providing a description of the study and consent procedures (Annex 3), will be mailed or emailed to them (depending on their preference). Potential participants meeting the inclusion criteria and willing to participate will be invited (Annex 4) (via email or phone) to participate in a brief consent presentation (30 min) by the clinical study team at the Plants for Human Health Institute (PHHI; North Carolina Research Campus, 600 Laureate Way; Kannapolis, NC 28081). Here, participants will be provided a summary of the clinical and study procedures, be invited to ask questions to the clinical study team, review eligibility and describe consent practices. Briefly, the study coordinator (Monique Carvalho de Santana) or the clinical team will describe the procedures involved in this study (e.g. screening, dietary restriction, blueberries, blueberry product or matched-control feeding, blood and urine collection and fasting procedures, etc.) and encourage any questions from the participants.
Compensation will also be discussed. Those potential participants holding visas may fall under additional circumstances (see â€˜Compensationâ€™ tab).
Once the participant is satisfied and wishes to continue to the clinical screening stage, he/she will sign the consent form (Annex 5), pre-approved by the NCSU IRB. Participants may also sign the consent form at home if requested (returning via email, mail or in person). The consent form describes aims, methods, benefits, termination from the study, and potential hazards of participating.

The consent form must be signed prior to inclusion into the trial, and prior to any sample collection. Once consent form is signed, we will initiate a request (via email or phone) to schedule a clinical screening session. It will be made completely and unambiguously clear to each participant that they are free to refuse to take part in the study, or withdraw their consent at any time and for any reason, without incurring any penalty. The participant will be given another copy of the participant information materials (Annex 3) and signed consent form. The original signed informed consent will be kept on file by the Principal Investigator (Dr. Colin D. Kay) following established archiving and data handling protocols compliant with NCSU IRB procedures. No sex/gender or racial/ethnic group will be excluded, and there is no need to recruit vulnerable population in this study.

Participants giving their consent to participate will be assigned a numerical study code. The information linking codes to volunteers will have restricted access. All personal information at the study site will be kept confidential and stored securely. Only the Principal Investigator (Dr. Colin D. Kay), clinical coordinator, study phlebotomists and medical practitioner/consultant will be given access to participant identifiers if deemed necessary by the Principal investigator (Dr. Colin D. Kay) such as instances where adverse events are reported and blinding has to be broken to establish treatment group.

Participants who give consent to proceed in the study will be given a â€œcooling-offâ€• period of at least 24 hrs before arranging a clinical screening visit (when biological samples will be collected for screening) (Annex 7A). A reminder about the clinical screening visit will be sent to participants 48h prior to it (Annex 7B). Volunteers will be reminded that they are not obligated to participate further and that they can withdraw from the trial at any time. We estimate to start the feeding visits for the first cohort in March/2020.

Participants will also be informed that at the end of each feeding visit, they will have the option to have their travel expenses to and from the research facility (at the mileage rates applicable at the time of involvement) reimbursed via a NCSU reimbursement form (Annex 8). Participants are responsible for filling the travel reimbursement form out and submitting it to the PHHI Administrative Support Analyst, who will process their payment, via direct deposit or a single check.

We estimate to recruit volunteers (rolling-recruitment) in December/2019-January/2020, and March/2020 and May/2020 and through the end of 2020 as needed.Amendment COVID-19: the consent visit will occur in room 1308 of the UNC-CH Human Research Core clinical suite, on the North Carolina Research Campus (500 Laureate Way,

Kannapolis, NC 28081) or virtually via the study coordinatorâ€™s NC State University Zoom account. We will also offer the possibility of reading and signing the consent form online (using electronic signature or emailing us a scanned and signed document) or via mail.

If any participants are minors, describe the process for obtaining parental consent and minor's assent (minor's agreement to participate).Parent/Guardian Permission FormMinor Assent Forms

N/A

Are you applying for a waiver of the requirement for consent (no consent information of any kind provided to participants) for any participant group(s) in your study?

No

For each participant group that you are requesting a waiver of consent for, please state what method this waiver is needed for, why it is needed and address each of the above 5 criteria to justify why your study qualifies for a waiver of consent.

Are you applying for an alteration (exclusion of one or more of the specific required elements) of consent for any participant group(s) in your study?

No

Identify which required elements of consent you are altering, describe the participant group(s) for which this waiver will apply, and justify why this waiver is needed.

Are you applying for a waiver of signed consent (consent information is provided, but participant signatures are not collected)? A waiver of signed consent may be granted only if: The research involves no more than minimal riskThe research involves no procedures for which consent is normally required outside of the research context.

No

Would a signed consent document be the only document or record linking the participant to the research?

No

Is there any deception of the human subjects involved in this study?

No

Describe why deception is necessary and describe the debriefing procedures.Does the deception require a waiver or alteration of informed consent information?Describe debriefing and/or disclosure procedures and submit materials for review.Are participants given the option to destroy their data if they do not want to be a part the study after disclosure?

For each participant group please indicate how many individuals from that group will be involved in the research. Estimates or ranges of the numbers of participants are acceptable. Please be aware that participant numbers may affect study risk. If your participation totals differ by 10% from what was originally approved, notify the IRB.

Twenty-eight healthy participants will be recruited and randomized for 4 different treatments (in a crossover design), with the probability that 24 will complete the study (15% drop out rate). No between group comparisons will be made aside from using sex as a covariate in statistical analysis. The study focuses on within participant variation so population diversity is not required.

We will not target participants that are unemployed or from a low socioeconomic background. All who express an interest will undergo a telephone pre-screen and full screening visit, until participant enrollment numbers are met. We will exclude participants and/or alter the recruitment advertisement strategy if greater than 20% of the participant pool identifies their income is below 23k/y. No data on income will be recorded with the study documentation, we will only record a tally of numbers of people answering below 23k/y in a separate single column excel workbook conditionally formatted to detect when the proportion of individuals reaches 20%. At the screening interviews participants will be made clear the time commitment is approximately 55 hours; this includes 4 full 9h days over a 3 month period. This is described in detail in the participant information package.

We have extensive experience conducting this type of research over the past 15y and most recently successfully completed a 6mo intervention in over 100 individuals.

How will potential participants be be found and selected for inclusion in the study?

The recruitment strategy is extensive and includes initially posting on the NRCR campus, which targets individuals of high and low socioeconomic status and extensive ethnic diversity including professionals across the globe. The campus also employs locals of diverse ethnic and economic status. The planned recruitment areas have been pre-determined by NCSU extensions teams (including Extensions Health Alliance) to reach the broadest of community groups and have

been used by PHHI extensions for over 5 years now. Further planned recruitment through use of local newspapers and Magazines (Cabarrus County Magazine, Salisbury County Magazine) rounds out this recruitment strategy, if required. As the studies primary purpose is to establish the difference in bioaccessibility of blueberries in healthy, middle age individuals, we feel we have gone above and beyond attempting to be as inclusive as possible for a study requiring less than 30 individuals and not controlling for ethnic diversity or genetic differences in metabolism. We expect we will hit are recruitment target within weeks of posting the initial flyers and will therefore use a phased advertisement approach to avoid unnecessary expression of interest. Recruitment will be made on a first approached basis, provided eligibility criteria are met and having equal distribution of male and female participants.

Detailed Strategy:
Study recruitment will be conducted via posting flyers (Annexes 1A and 1B) on pre-approved bulletin boards on the NCRC buildings, NCRC public listservs; postings with the Parish Nurses at Carolina HealthCare System (Atrium Health) and "Faith Community Health Ministryâ€•; posting on local public bulletin boards: West Cabarrus YMCA, Harrisburg YMCA, Kannapolis YMCA, South Rowan - J. Fred Corriher Jr. YMCA, Salisbury - J. F. Hurley YMCA, Rowan Public Library-Headquarters, South Rowan Regional Library, Concord Library, Harrisburg Library, Kannapolis Library, Mt. Pleasant Library; Extensions and Health Alliances: Cabarrus Health Alliance, Rowan County Health Department, Cabarrus County Extension, Rowan County Extension, eNews at Salisbury Pediatrics; and/or newspapers and local magazines (Annex 1B): Independent Tribune, Salisbury Post, Cabarrus County Magazine, Salisbury County Magazine.

After responding to the recruitment flyer via phone or email, volunteers will be interviewed about their eligibility by phone (Annex 2A) or email (Annex 2B), or in-person interviewers for NCRC campus employees if requested (following interview script Annex 2A). For all study literature / advertising, the Clinical Coordinator will be identified as the first point of contact. Participants will be selected based on the inclusion criteria: male and female adults between 25-65 years. This age range was chosen because there is evidence of changes in absorption and metabolic process in adolescence and the elderly which would confound the findings of the present study; non-smokers or non-tobacco users [no vaping or dipping (tobacco chewing)], or who ceased it â‰¥ 6 months ago because it is known to affect metabolic processes such as phase II metabolism; who have a body mass index (BMI) â‰¥18.5 to â‰¤ 30 (lb/in2x703), as obesity is known to impact physiological processes associated with digestion, absorption and metabolic clearance/kinetics; successful biochemical, haematological and urinalysis assessed, as it can impact physiological processes associated with digestion and absorption; who present no allergies to fruits or vegetables containing polyphenolic (e.g. anthocyanins, flavonoids) and phenolic acids such as blueberries, red apple, strawberry, red orange, purple onion and broccoli because participants will be asked to consume blueberries; who present no allergies to whey protein or salicylates because participants will be served with blueberries, which contain traces of salicylates, and the blueberry-rich protein bar containing whey protein; who are generally healthy and without chronic diseases including cancer, type 1 and 2 diabetes, as individuals with diabetes have altered metabolism; who are not prescribed thyroid, hypoglycemic medication or hormone replacement therapy (HRT) (due to the likely concomitant effects that these medications cause on the objectives of the trial); who have not been consuming any phytonutrient or willing to cease intake during, and 1 month preceding the trial, otherwise, it would interfere with the analysis of the phytonutrients in the present study; those willing 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, and not planning to use them during the study, as it can impact physiological processes associated with digestion and absorption, however, one-month washout of supplements would make the participant eligible; those having donated blood at least more than 1 month before recruitment; individuals that do not 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), as excessive alcohol intake can alter phase II metabolism; who are not currently on a weight-reducing plan or using weight-loss medications (e.g., selective serotonin reuptake inhibitors, steroids, Ritalin, appetite suppressants, Xenical, Diethylpropion), or planning to continue this treatment during the 10-week period of the study, which are known to affect physiological processes associated with digestion and absorption; those agreeing to restrict dietary intake of rich sources of phytonutrients targeted in the study (Annex 9) during the wash-out and clinical visit periods otherwise, it can interfere with the analysis of the phytonutrients in this study; and who lives within 40 miles from the NCRC campus to avoid drop-outs and to comply with early morning scheduling/visits. The volunteers should also be willing to comply with the study procedures, which involves the collection of urine and blood samples, and the record of their additional

dietary intake over 2 days before each feeding visit.

For each participant group, how will potential participants be approached about the research and invited to participate? Please upload necessary scripts, templates, talking points, flyers, blurbs, and announcements.

Potential participants, from the diverse socioeconomic statuses represented by the surrounding communities, will be encouraged to take part in the study through the use of advertisements. Initially, flyers/posters (Annexes 1A and 1B) will be placed on pre-approved bulletin boards on the NCRC buildings, NCRC public listservs; postings with the Parish Nurses at Carolina HealthCare System (Atrium Health) and "Faith Community Health Ministryâ€•; posting on local public bulletin boards: West Cabarrus YMCA, Harrisburg YMCA, Kannapolis YMCA, South Rowan - J. Fred Corriher Jr. YMCA, Salisbury - J. F. Hurley YMCA, Rowan Public Library-Headquarters, South Rowan Regional Library, Concord Library, Harrisburg Library, Kannapolis Library, Mt. Pleasant Library; Extensions and Health Alliances: Cabarrus Health Alliance, Rowan County Health Department, Cabarrus County Extension, Rowan County Extension, eNews at Salisbury Pediatrics; and/or newspapers and local magazines (Annex 1B): Independent Tribune, Salisbury Post, Cabarrus County Magazine, Salisbury County Magazine. These locations (such as community centres, community magazines and newspapers) target populations from a variety of socioeconomic statuses. In all cases, the advertisement will encourage interested participants to contact the study coordinator for further information via phone or email.

Describe any inclusion and exclusion criteria for your participants and describe why those criteria are necessary (If your study concentrates on a particular population, you do not need to repeat your description of that population here.) Inclusion and exclusion criteria should be reflected in all of your recruitment materials and consent forms.

The inclusion of participants into the study will be established based on the inclusion and exclusion criteria through a preliminary inclusion questionnaire/interview, and the clinical screening session. The clinical significance of screening results, past and present medical conditions and medications, will be judged at the discretion of the studyâ€™s clinical advisor (Martin Kohlmeier, MD, PhD).

The inclusion questionnaire/interview will select the average healthy adult population for analysis of the clearance rate of blueberry components (phytonutrients) through the body over time. Thus, the study will include male and female adults between 25-65 years because there is evidence that adolescence and the elderly have different metabolic processes (particularly differing absorption and metabolism) compared to this age range; non-smokers, non-tobacco users [no vaping or dipping (tobacco chewing)], or who ceased it â‰¥ 6 months ago because it is known to affect metabolic processes; who present no allergies to fruits or vegetables containing polyphenolic (e.g. anthocyanins, flavonoids) and phenolic acids such as blueberries, red apple, strawberry, red orange, purple onion and broccoli because participants will be served blueberries; who present no allergies to whey protein or salicylates because participants will be served with blueberries, which contain traces of salicylates, and the blueberry-rich protein bar containing whey protein; who are generally healthy and without chronic diseases including cancer, type 1 and 2 diabetes; who are not 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 trial), otherwise, it can impact physiological processes associated with digestion and absorption; who has not been consuming any phytonutrient-containing supplements (e.g. with berry, cocoa, coffee or tea extracts) for at least a month before the study and willing to not consume it during the study, otherwise, it would interfere with the analysis of the phytonutrients after consumption of the treatments for this study; who lives within 40 miles from the NCRC campus to avoid drop-outs due to traffic and the required early morning visits; those agreeing to restrict dietary intake of rich sources of phytonutrients targeted on the study (Annex 9) during the wash-out and clinical sampling periods, agreeing to comply with study procedures involving the collection of urine and blood samples, and to record their additional dietary intake over 2 days before each feeding visit, and two days after the intake of the blueberries or blueberry product, otherwise, this study would not be suitable for them. The clinical screening will enroll/include those who have BMI â‰¥18.5 and â‰¤30 (lbs/in2x703) and a successful (i.e., within normal range for healthy individuals) biochemical, hematological and urine analyses assessed by the clinical advisor, as the design of the study is to establish bioequivalence in healthy middle aged humans and unhealthy, or adolescence or the elderly have altered absorption and metabolism. Studying these groups would require considerably higher participant numbers, which will be the focus of future investigations.

Exclusion criteria will include those who are current smokers [including vaping and dipping (i.e., tobacco chewing) users], or ex-smokers ceasing < 6 months before recruitment, pregnant or breastfeeding, subjects with existing or significant past medical history of vascular disease or medical conditions likely to affect the study measures i.e. vascular disease, circulatory (i.e. Reynaudâ€™s), diabetes, hepatic, renal, digestive, hematological, cancer, or thyroid disease as

any of these conditions can affect physiological processes associated with digestion and absorption; fructose intolerant subjects or those with known allergy to salicylates, whey protein or to berries because participants will be served with blueberries, which contain fructose and traces of salicylates, and the protein bar containing whey protein; those unprepared to adhere to dietary restrictions for 1 week preceding and during each feeding visit or unwilling to comply with the study procedures; who are in parallel participation in another research project involving dietary intervention and/or sampling of biological fluids/material for the safety of the individual; those on therapeutic diets or having experienced substantial weight loss (to be judged by clinical advisor) within 3 months of clinical screening because it can affect physiological processes associated with digestion and absorption; those taking phytonutrient-containing supplements (e.g. with berry, cocoa, coffee or tea extracts), unwilling to cease intake during, and 1 month preceding the trial, 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 because it can affect physiological processes associated with digestion and absorption, however, one-month washout of supplements would make participant eligible; those prescribed thyroid, hypoglycemic medication or HRT medication because it can affect physiological processes associated with digestion and absorption-other medications will be assessed for suitability by the clinical advisor; those having donated blood in the last month for the safety of the individual; individuals that 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) because excessive alcohol consumption alters metabolic processes such as phase I and phase II conjugation; 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 10-week period of the study because any of these situations can affect physiological processes associated with digestion and absorption. After the clinical screening, who presents 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, and who has BMI<18.5 and >30 (lbs/in2x703) will be excluded as the design of the study is to establish bioequivalence in healthy middle aged humans and unhealthy, or adolescence or the elderly have altered absorption and metabolism. Studying these groups would require considerably higher participant numbers, which will be the focus of future investigations.

Medical diets to control for metabolic disorders, including obesity are an exclusion criteria of the present study as it will negatively impact our primarily study focus; however healthy individuals on vegetarian or vegan diets will not be excluded and we will make concessions for their meal choices during the study days.

Here we define 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, participants are asked to simply write this in their 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.

Is there any relationship between researcher and participants - such as teacher/student; employer/employee?

No

What is the justification for using this participant group instead of an unrelated participant group? Please outline the steps taken to migitate risks to participants from the pre-existing relationship, including power dynamics of this relationship and/or perceived coercion.

Describe any risks associated with conducting your research with a related participant group.

Describe how this relationship will be managed to reduce risk during the research.

How will risks to confidentiality be managed?

Address any concerns regarding data quality (e.g. non-candid responses) that could result from this relationship.

In the following questions describe in lay terms all study procedures that will be experienced by each group of participants in this study.For each group of participants in your study, provide a step-by-step description of what they will experience from beginning to end of the study activities. Should you prefer, you can upload a detailed study procedure packet and refer us to that document in this text box. If you choose to upload a procedures packet, do not discuss procedures in the below text box.

1. After showing interest through response to study advertisements on previously mentioned pre-approved bulletin boards and listservs via email or phone call, volunteers will be recontacted to answer a questionnaire administered over the phone (Annex 2A) or email (Annex 2B) to establish if they meet the study eligibility criteria.

2. After eligibility is confirmed through phone or email inclusion questionnaire (Annex 2A or 2B), the potential participants will receive a package providing complete description of the study and consent procedures (Annex 3) via email or mail. If still willing to participate, they will be scheduled for a consent visit at the Plants for Human Health Institute (PHHI; North Carolina Research Campus, 600 Laureate Way; Kannapolis, NC 28081), when they will be provided a brief presentation of the study and volunteers will be encouraged to ask questions. A reminder will be sent 48h before this visit. It will be explained that volunteers will be given a list of foodstuffs to â€˜avoidâ€™ and â€˜limitâ€™, and alternatives to them, during the wash-out week and visit days (Annex â€Ž9); the intake of tea and coffee (as major providers of berry phytonutrients in the US diet) 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 feeding visit and on study visit days 1 and 2, and the intake of alcohol will also be regulated based on the recommendations of the Food and Drug Administration. Adherence to the low-berry-phytonutrient diet will be assessed using the food intake record (Annex 10), which the participants will be asked to provide during the two days preceding each feeding visit day, and the day of the feeding visit and following day. Participants will also be required to refrain from taking phytonutrient-containing supplements (e.g. with cocoa or coffee or berry extracts) for at least 1 month prior to the assessment visits, and throughout the trial. Volunteers will be advised to maintain their habitual 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 are likely to affect the endpoints in the study (i.e. phytonutrient metabolism). If still interested in participating in the study, they will be asked to sign the study consent form (Annex 5). After signing the consent form, inclusion/exclusion of participants will be verified via confirmation of their BMI, and via a medical history and medication use questionnaire (Annex 16). During this visit, participants will manifest availability for scheduling a clinical screen after a cooling-off period of a minimum of 24 hours. The clinical screening at the Clinical Services Facility at the Nutrition Research Institute-Human Research Core will be scheduled via email or phone (Annex 7A), and a reminder about it will be sent 48h before this visit (Annex 7B). Participants will be advised to follow 10-hour fasting (except for water) and to arrive hydrated at the clinical screening visit. Any sample will be collected only after the consent form is signed by the volunteer.

3. In the clinical screening visit, BMI, measurement of resting BP, medical history and medication use questionnaire will be assessed. Participants 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. Those of eligible BMI will provide a fasting blood sample (approximately 0.29 oz or Â½ tablespoon) through venipuncture and used to establish health status via 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. Abnormalities can indicate: bacterial, viral or parasitic infections, inflammation and inflammatory disorders, leukemia or bone marrow injury, trauma, stress or toxicity (drug or other), diseases of the immune system, lupus, HIV infection, congenital heart disease, dehydration, obstructive lung disease, anemia, bleeding, kidney disease, allergic disorders and hypersensitivity food reactions, kidney disease and cirrhosis of the liver, and malnutrition. Screening blood samples will be anonymized (e.g. identified with study ID, date of birth and gender) and sent to the Laboratory Corporation of America Holdings (Labcorp) for analysis. Volunteers will also be asked about their meal preferences for the study visits (Annex 6). At the end of the screening assessment,

breakfast will be provided to the participants. The clinical screening visit is expected to take no more than 1 hour with parking provided at no cost. 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 feeding visits via phone and/or email (Annex 11A), or a notification about their ineligibility (Annex 11B).

4. A total of 28 subjects that comply with the eligibility criteria will be recruited and will provide written, informed consent, with the anticipation that 24 will complete the study. A total of 3.79 oz (or 112 mL) of blood will be collected over the course of seven to thirteen weeks, depending on the participantâ€™s availability. We will examine the 48h (post-consumption) rate of phytonutrient clearance by the body following the consumption of the equivalent of one serving of blueberries (approximately 5.3 oz) per participant or protein bar enriched with blueberries or macronutrient-matched control, over four feeding visits. Participants will be asked to consume a whey protein beverage together with the berry treatments in order to provide equivalent macronutrient content across the treatments. UPLC-MS/MS techniques established in our group will be applied to track the recovery of phytonutrient metabolites in the participants' blood and urine.

The blueberry fruit (>10Kg) will be harvested by Dr. Chad Finn, a Research Scientist at the USDA-ARS breeding and research station within the Horticultural Crops Research Station. Corvallis. Dr. Finn has previously provided blueberry fruit for human consumption and human research involving sensory characterization analysis. The berries are grown following approved USDA farming practices; therefore, they are determined to be Generally Recognized As Safe (GRAS). These berry varieties are presently commercially available and chosen for their phytochemical content.

Protein bars (113 g each) will deliver 27.5 g protein and 789 mg total phenolics (equivalent to 1 serving of blueberries) and will be produced in a food-grade lab by hand mixing a 0.7:1.5:1 weight ratio of Crisco pure vegetable oil: Golden Barrel Corn Syrup: protein (from freeze-dried particles formed by homogenizing blueberry puree and 10% w/w whey protein isolate) for two minutes. Bars will be placed into silicon molds and refrigerated for 1 h at 4 Â°C prior to demolding. After demolding, bars will be placed in vacuum-sealed bags and stored at room temperature (25 Â°C). The protein bars will have the following composition: 27.5 g protein, 20 g fat, 41.25 g carbohydrates, and 440 total calories. The protein bars will not contain any peanuts, soybeans or soy, eggs, fish, crustaceans, tree nuts, wheat, gluten, or sulfites.

5. The participants will have at least one week of washout before starting each of the four feeding visits, when they will consume a diet low in berry phytonutrients as directed by the dietary exclusion list (Annex 9). Participants will be reminded (either via mail or email; Annex 12) about the starting day of the dietary washout procedure 48h prior to it. They will be scheduled (either via mail or email; Annex 12) to pick up two 24h-urine collection kits with further instructions for use (Annex 13). Participants will be provided with standardized blueberry-phytonutrient-free meals and beverages during the treatment visit days 1 and 2 of each feeding visit. The arrangement of meals, blueberries, protein bar and macronutrient-mathed beverages to be served to participants will occur in the Metabolic Research Kitchen at the UNC-CH Nutrition Research Institute, established on the NCRC campus. The standardization of the pre-feeding visit meals (two days prior to feeding visit) across the four pre-blueberry product feeding periods is necessary to control for any effect of recent background dietary intake on repeat biological assessments. Five days before the feeding visit day participants will be reminded (either via mail or email; Annex 14) to pick up two 24h-urine collection kits and be provided with further instructions for use (Annex 13), which will coincide with the last 48h of the washout. Three days prior to the feeding visit, at the time of urine collection kit pick up, participants will be reminded to fill out the two-day food intake record, and to come fasted for the visit day at the Clinical Services Facility at the Nutrition Research Institute-Human Research Core. A reminder about each feeding visit day will be sent via email or phone call 24h prior to that (Annex 15).

6. The visit days will occur during weekdays. In the first feeding visit day, volunteers will arrive at the research facility in a fasted state (having consumed only water for the preceding 10 hours), the clinical team will take receipt of previously collected biological samples (-48h and -24h urine collections) and the study coordinator will check the food intake record from the two preceding days. Also, a researcher or suitably qualified phlebotomist will complete a basic medications assessment/questionnaire (Annex 16A) to establish fitness to proceed (e.g. to assess if the participant has fasted correctly, is free from symptoms indicative of compromised health), and record any medications being used onto the medical questionnaire. Modification to these medications (e.g. dose changes) during the study must be reported, and

amended to this original record. The participantâ€™s resting blood pressure, height, and weight will be measured by the study coordinator, research technician or a suitably qualified phlebotomist. 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 to assess aspects of absorption, distribution, metabolism and excretion (ADME) of berry phytonutrients. On visit day 1 of every study period, participants will have the option to have their blood drawn via single needlestick or cannula. For either option, the phlebotomist will make two attempts per vein and, if unsuccessful, a second qualified person may attempt one time. If the participant opts for cannula placement, he/she will be instructed not to bend the arm in which the cannula is inserted. They are not to leave the building with the cannula in place and are to come back to the lab area every hour to have the cannula flushed with saline. 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 the consent of the participant. Otherwise, the participant who chooses to have blood drawn via a cannula and who is comfortable with it will have it up to the last blood draw on visit day 1, which is for up to 9.5 hours. The participant will be instructed not to access the cannula at any time. Access to the cannula should be by study staff only. Those failing 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 (Annex 17) and compensation (when applicable) for their inconvenience of potentially taking some time off from work for the study, disrupting their lifestyle via restricted consumption of certain foods during the study, and for any undertaken feeding visits (Annex 8) will be provided. This procedure will be repeated across 3 other visits. In the first feeding visit, the participant will receive either, in randomized order, one serving (approximately 5.3 oz) of a standard commercially available blueberry variety (i.e., cultivar), one serving of the blueberry variety screened for either having a high or low phytochemical content, a protein bar enriched with the equivalent of one serving of blueberries or a macronutrient-matched control beverage. After the blueberries, the protein bar or the control beverage is given on day 1 of each feeding visit, breakfast will be provided, as well as lunch, dinner and snacks. All food will be purchased pre-packaged at a local grocery store, including Foodlion LLC, stored within their expiration date at the UNC Metabolic Kitchen. Any food that requires preparation for single serving such as breakfast will be handled by personnel certified with ServSafeÂ® Food Handler certification. Food that needs to be taken home by participants will be pre-packaged, commercially available and transported in a cooler for safe handling. Blood pressure, height, and weight measurements, and potential medication and physical activity change record will be done on days 2 and 3 of each feeding visit. Participants will be asked to record their food and beverage intake during visit days 1 and 2, and to repeat these intakes on the following three feeding visits.

The participants will be asked to choose their standardized low-berry-phytonutrient 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 their full day visit to the clinic and the following morning, they 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 participants wish to consume them as an alternative to coffee or tea.
*Available as plain or gluten-free

7. Between the feeding visits, subjects will receive a â€œthank youâ€• email about their participation and a reminder that they can observe approximately 2 weeks (11 to 20 days depending on scheduling constraints) of free-living compliance break (Annex 18), allowing them to return to their normal dietary habits and take a break from the study regime. This will be followed again by seven days of wash-out, urine collection (one collection at -48h and another collection at -24h) and recording of their food intake for two days before the next feeding visit day. This will be repeated for the remaining three feeding visits, including the reminder emails or phone calls previously mentioned.

8. The circumstances by which researchers may initiate participant withdrawal from the trial will be stated at each visit, as well as the need for participants to inform the research team regarding adverse events and serious adverse events during the study. At the end of the approximate 3-month trial, an exit questionnaire will be completed by the participant (Annex 19), which will give an opportunity to capture information regarding the participant experience and including

questions related to adequate blinding during the two berry treatments. In addition, participants will receive an email about their contribution to the study, which will be conducted following Good Clinical Practice.

There is no intent to make a claim about the impact of a nutritional product on a diagnosis, cure, mitigation, treatment, or prevention of a disease or health related condition. Only clearance kinetics are being explored in the present trial.Amendment COVID-19: the consent visit will occur in room 1308 of the UNC-CH Human Research Core clinical suite, on the North Carolina Research Campus (500 Laureate Way, Kannapolis, NC 28081) or virtually via the study coordinatorâ€™s NC State University Zoom account. We will also offer the possibility of reading and signing the consent form online (using electronic signature or emailing us a scanned and signed document) or via mail.

In addition to the normal study procedures stated here, we will also follow all procedures and materials described in the COVID-19 Additional Procedures packet. Once a vaccine is developed to Address COVID-19, we will cease all COVID related procedures and communication and only implement normal study procedures as described in this IRB application.

Amendment request (November/2020): At the consent visit, date of birth is collected by the study coordinator into a password-protected Drive to label blood tubes that are used at the clinical screening and study visits to confirm the identity of each participant during blood draw by our UNC-CH partnerâ€™s phlebotomists. At the clinical screening, the blood tubes and paperwork that are filled out with the participantâ€™s date of birth by phlebotomists are sent to LabCorp.

Are you requesting the use of secondary information to be used as data for this research project? The secondary information can either currently exist or be generated in the future.

Discuss the following: permission to access the information (direct permission from the participant or records release), how researchers will access, transfer, store, and destroy the data.
Discuss the identifiable/re-identifiable nature of the data through either direct IDs, indirect IDs, or triangulation of datasets, data points, researcher access/expertise, or analysis. .
List all data categories to be requested (ex: age, race, student ID, GPA, ACT, Medical ID, diagnosis).
Discuss if the data requires a Data Use Agreement.
Discuss if the data are subject to FERPA, HIPAA, or the GDPR.

No

Social/Reputational?

No

Psychological/Emotional?

No

Financial/Employability?

No

Legal?

No

Physical?

Yes

Academic (affect grades, graduation)?

No

Employment (affect job)?

No

Financial (affect financial welfare)?

No

Medical (harm to treatment)?

Yes

Insurability (harm to eligibility)?

No

Legal (reveals unlawful behavior)?

No

Private behavior (harm to relationships/reputation)?

No

Religious Issues/Beliefs?

No

Describe the nature and degree of risk that this study poses. Describe the steps taken to minimize these risks. You CANNOT leave this blank, say 'N/A', none' or 'no risks'. You can say "There is minimal risk associated with this research." For each 'Yes' selected above, describe the probability of the risk occuring and the magnitude of harm should the risk occur. Discuss how you are mitigating those risks through participant selection, study design, and data security.

There are limited social or reputational risks associated with taking part in the present study as we are not actively recruiting individuals by societal or economic or ethnic status or diversity. Our open recruitment targets all healthy adult populations from the surrounding area. Nor will we recruit, screen, record, store or report data on psychological, emotional, financial or employability status. Our recruitment is targeted on healthy disease-free adults who are free from medications or supplements with alter metabolic processing involving digestion, absorption or metabolism. We are not collecting or accessing private or sensitive information aside for questions regarding healthy status or medication usage which might impact metabolic processing involving digestion, absorption or metabolism. This information will be destroyed immediately if individuals fail to meet our inclusion criteria or drop out of the study for any reason. Health questionnaire data will not be used as an endpoint in any analysis and is only captured to ensure health status remains consistent throughout the study or in any case, where it may not, this will be used as a coded confounding variable in statistical analysis or to identify outliers. This information will be destroyed immediately following publication of the first manuscript from the study and or completion of the project grant. No legal, behavior or insurability data will be used to establish eligibility nor stored for use as an endpoint in the present study. The only outcome parameters used in the present study include biological sample type (blood or urine), time of sample, dietary phytochemical (i.e., (poly)phenol metabolite content), age and sex.

There are minimal risks associated with this nutritional intervention which involves participants eating single portions of berries, a protein bar or control beverage matched for the protein, carbohydrate and fat, which are all food grade and comprised of foods or ingredients already present in the diet. There is a risk that the body may react to some of the food that is provided. This is not expected to happen due to the food being prepared in commercial grade kitchens using food safe practices. Minor risks relate to the collection of blood samples and storage of data (as discussed above). There are minimal risks involved in the urine collection and exit questionnaires will be stored in a secured location and only used internally to improve study practice. Risk for salicylate intolerance is minimal as the treatment will provide only 3.75 mg of salicylate (Aresta & Zambonin, 2016). Even though there are no expected adverse effects resulting from the consumption of blueberries, as a precaution, we have decided to exclude individuals in our study that have perceived food allergies or adverse reactions/intolerances to salicylates or any berries or berry derived products; which will be established during initial interview and again at the consent visit.

No adverse symptoms are anticipated following consumption of the blueberries or blueberry product because participants with allergies to the blueberries will not be enrolled and the foods and levels consumed fall within normal dietary patterns. Evidence indicates the foods explored in the present proposal have no adverse effects at the doses of individual phytonutrients they contain. We have reported studies that fed doses of single phytonutrients derived from berries far in excess of those in the present intervention (doses between 700 mg and 1.3 g), and presented no adverse reaction, high compliance and no indication of bioaccumulation or changes in clearance kinetics over 12 weeks (Kay et al. 2005; 2004). Presently licensed polyphenol/phytonutrient-rich supplements on the market derived from berry extracts often contain a single polyphenol/phytonutrient dose around 2000 mg/d.

Risks will be enumerated in the informed consent form and described orally during the consent process and relate to procedures involving blood and urine samples collection. The IRB Committee from North Carolina State University has an established adverse event reporting process which will be adhered too (Annex 20).

The consent process will inform the potential study participants about the study, indicate that participation is voluntary, and that he/she has the right to stop at any time. The procedures to protect against risks associated with blood collection

include a safe, hygienic environment for all biospecimen collection procedures, and an experienced clinical research group at the Clinical Services Facility at the Nutrition Research Institute-Human Research Core. Collected samples will be processed and analyzed in laboratories which are certified at biosafety level-2 (BSL-2), have chemical hygiene plans registered with environmental health and safety and follow GCLP. The Clinical Services Facility at the Nutrition Research Institute-Human Research Core has automated external defibrillator (AED), and key personnel (phlebotomists) are cardiopulmonary resuscitation (CPR) certified.

The study will involve the collection of a total of 4.07 oz (or 120.5 mL) of blood; approximately 8.5 mL (1/2 tablespoon) will be collected at the screening visit and 112 mL across 28 separate blood samples of 4-ml each over approximately 14-week period (i.e., the entirety of the study). Risks associated with drawing blood from the arm include momentary discomfort and/or bruising from the introduction of either a single needlestick or a single cannula into their forearm vein during venipuncture. With a needlestick venipuncture, a single needle will be inserted into a vein in the 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 the 9.5-hour study visits. With cannulation, a small tube (â€œcannula)â€• is inserted into a vein in the arm so that a needle can be inserted into the tube to draw blood. 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. The tube will be flushed between blood draws. The advantage of cannulation is that there will only be one needle stick once instead of multiple times. With the cannulation method, the cannula will remain in the arm for the entire 9.5h of the feeding day. If the tube becomes blocked at any point, it will be removed and the needlestick venipuncture will replace the cannula method for the remaining blood draws. The cannula can also be removed at any point before the final blood draw at the request of the participant.

To minimize risk, only trained phlebotomists will collect blood. Infection, excessive bleeding, clotting, and/or fainting are also possible, although unlikely. The choice of cannulation will also be offered to participants 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 the consent of the participant. Otherwise, the participant who chooses to have blood drawn via a cannula and who is comfortable with it will have it up to the last blood draw on visit day 1, which is for up to 9.5 hours. The amount of blood being collected will not influence the ability of the subject to participate in normal daily activities. Blood draws will be limited to attempts to 2 per vein. 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. The cannula should be accessed by lab or study personnel only. The participant will be instructed to return to the lab if the cannula becomes dislodged at any time throughout the day. The participant will also be told that he/she cannot leave the NRI building, where the phlebotomy laboratory is located, while the cannula is in his/her arm.

In case human blood or other potentially infectious material gets in contact with an unprotected break in the skin of the clinical research team, during any procedure, or splashes into the eyes, nose or mouth, the exposed area will be washed thoroughly for at least five minutes with soap and water and/or flush eyes or mucous tissues. Anyone who is exposed to it will be asked to proceed to the hospital emergency room or outpatient clinic within two hours, taking the Corvell WC Authorization/Physician's Report/Pharmacy Guide and filling it out, and to dial 919-515-3000 to report the exposure to the NCSU Emergency personnel. Employees must notify their supervisor and contact Rx Urgent Care (919-719-2250) the following day, and if possible bring the source individual with them.

References

Aresta, A., & Zambonin, C. (2016). Simultaneous determination of salicylic, 3-methyl salicylic, 4-methyl salicylic, acetylsalicylic and benzoic acids in fruit, vegetables and derived beverages by SPME-LC-UV/DAD. J Pharm Biomed Anal, 121, 63-68. doi:10.1016/j.jpba.2015.12.016

Kay, C. D., Mazza, G., & Holub, B. J. (2005). Anthocyanins Exist in the Circulation Primarily as Metabolites in Adult Men. J Nutr, 135(11), 2582-2588. doi:10.1093/jn/135.11.2582

Kay, C. D., Mazza, G., Holub, B. J., & Wang, J. (2004). Anthocyanin metabolites in human urine and serum. British Journal of Nutrition, 91(6), 933-942. doi:10.1079/BJN20041126

If you are accessing private records, describe how you are gaining access to these records, what information you need from the records, and how you will receive/record data. Private records may include: educational, medical, financial, employment. Some of these private records may be subject to laws such as FERPA and HIPAA. Your content here should match what you've discussed on the procedures tab.

N/A No private records are being accessed

Are you asking participants to disclose information about other individuals (e.g., friends, family, co-workers, etc.)?

No

You have indicated that you will ask participants to disclose information about other individuals (see Populations tab). Describe the data you will collect and discuss how you will protect confidentiality and the privacy of these third-party individuals.

If you are collecting information that participants might consider personal or sensitive or that if revealed might cause embarrassment, harm to reputation or could reasonably place the subjects at risk of criminal or civil liability, what measures will you take to protect participants from those risks?

We will be collecting data relevant to a person's health history, medication use, age, height, weight and BMI, which might be considered personal or sensitive. Personal data will be handled in line with the regulations of the Health Insurance Portability and Accountability Act of 1996. Each participant will be allocated a unique randomly generated three digit study code number. All personal data and biological samples will be coded with this number to ensure confidentiality. The printed information linking codes to participants will have restricted access to the Principal Investigator (Dr. Colin D. Kay), the study coordinator (Monique Carvalho de Santana) and assigned research team (research technician and associate working at the laboratory of the Principal Investigator), and will be stored in a locked cabinet at the Plâ€™s office. Only the code will appear on the acquired specimens/samples and outcome datasheets. Electronic identifiers will be stored in one NCSU network drive with access to the Principal Investigator and study coordinator, while analytical coded data will be stored in a secondary NCSU network drive with access to the Principal Investigator study coordinator, and assigned research team. It will not be possible for an individual to be identified solely from their code number.

If any of the study procedures could be considered risky in and of themselves (e.g. study procedures involving upsetting questions, stressful situations, physical risks, etc.) what measures will you take to protect participants from those risks?

To minimize risk, only trained phlebotomists will collect blood. Infection, excessive bleeding, clotting, and/or fainting are also possible, although unlikely. The choice of cannulation will also be offered to participants 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 the consent of the participant. Otherwise, the participant who chooses to have blood drawn via a cannula and who is comfortable with it will have it up to the last blood draw on visit day 1, which is for up to 9.5 hours. The amount of blood being collected will not influence the ability of the subject to participate in normal daily activities. Blood draws will be limited to attempts to 2 per vein. 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.

The study coordinator and Principal Investigator will report all adverse events occurring during the study in the case report form (Annex 20) to the North Carolina State University IRB. An adverse event is defined as any unfavorable and unintended sign, disease or symptoms related to the use of an intervention, or worsened during the study, regardless of a direct relationship with the intervention. An adverse event can become a serious adverse event if it results in death or persistent disability/incapacity, is life-threatening or medically significant, or requires hospitalization.

Describe the anticipated direct benefits to be gained by each group of participants in this study (compensation is not a direct benefit).

As the present study is intended to establish the concentration of the phytonutrients present in the participantâ€™s biological samples (urine and blood), the data and findings are not likely to be directly beneficial to the participant. In addition, after the study is completed and the data analyzed, participants will be sent a single A4 page letter summarizing, in lay language, that the study has been completed and the implication of their involvement in the study (Annex 21). Other possible benefits would include receiving details of their blood pressure and BMI. Also, each group of participants will benefit nutritionally from the blueberries and blueberry product phytonutrients, which have been reported to have positive effects on cardiometabolic health.

If no direct benefit is expected for participants describe any indirect benefits that may be expected, such as to the scientific community or to society.

This study will provide the researchers with previously unobtainable data about what happens to different blueberry varieties and processed blueberries in the human body after consumption. It is likely that the results of the data will

provide a significant advancement in the understanding of phytonutrient bioavailability and metabolism in humans and have potential implications for future studies evaluating the health effects of these compounds. Specifically, these findings will inform the design of clinical feeding intervention studies using phytonutrient-rich foods to establish the health benefits of phytonutrient consumption, including the relationship between consumption, metabolism and biological activity. The study findings will also directly benefit the researchers involved in this research as the results will be published in influential scientific journals and presented at local and international scientific conferences.
The knowledge gained through this study will be communicated openly and freely to the public through various channels, including media briefings (for the communication of major findings) and public presentations to ensure communication to the largest possible audience.

Will you be receiving already existing data without identifiers for this study?

No

Will you be receiving already existing data which includes identifiers for this study?

No

Describe how the benefits balance out the risks of this study.

Will data be collected in a way that would not allow you to link any identifying information to a participant?

No

Will any identifying information be recorded with the data (ex: name, phone number, IDs, e-mails, etc.)?

Yes

Will you use a master list, crosswalk, or other means of linking a participant's identity to the data?

No

Will it be possible to identify a participant indirectly from the data collected (i.e. indirect identification from demographic information)?

No

Audio recordings?

No

Video recordings?

No

Images?

Yes

Digital/electronic files?

Yes

Paper documents (including notes and journals)?

Yes

Physiological Responses?

Yes

Online survey?

Yes

Restricted Access (who, what, when, where)?

Yes

Password Protection (files, folders, drives, workstations)?

Yes

Suggestion of anonymous browsing?

No

Locks (office, desks, cabinets, briefcases)?

Yes

VPN (transfer, upload, download, access)?

Yes

Encryption (files, folders, drives)?

Yes

Describe all participant identifiers that will be collected from each data collection method (surveys, interviews, focus groups, existing data, background data collected via host site or software). Discuss why it is necessary to record identifiers at all and describe the deidentifying process

The investigator(s) will ensure that the subject's anonymity is maintained. No personal data will be included with any data submitted to the sponsor, or for publication. All biological samples will be coded with a unique anonymized

identification number randomly generated containing three digit study code to ensure confidentiality. It will not be possible for an individual to be identified solely from their study code number/identifier. The study coordinator (Monique Carvalho de Santana) will keep a separate confidential enrollment log that matches identifying codes with the subject's names and personal information (age, email, phone and home address) stored in a secure filing cabinet in the research office of the Principal Investigator (Dr. Colin D. Kay) at the Plants for Human Health Institute at NCRC, and electronic files will be stored in a password protected folder on a secured network drive where contents will only be accessible to him and may be requested by the study coordinator if unblinding is required due to a severe adverse effect. Thus, only the code will appear on the acquired specimens/samples and outcome datasheets. Any premature unblinding will only occur where serious adverse events require, when it would be immediately communicated to IRB.

Electronic data stored within the NCSU will be account password protected. Electronic data transferred via email will be encrypted by the NCSU web server. Only the Principal Investigator, the study coordinator and research team assigned by the Principal Investigator will have access to the coded anonymized information. The NCSU IRB will be informed of serious adverse events by the Principal Investigator and study coordinator as soon as they occur. All data files will be de-identified, with the file linking subject names and ID numbers which are stored in a separate secure locations. Hard-copy data will be stored in a locked cabinet in the Plâ€™s office which is also locked and accessible only by card access, while digital data (identifiable vs anonymized) will be stored in separate secure server locations.

For study compensation and for any travel expense reimbursement, the required forms containing confidential information about the participant will be submitted by the participant directly to NCSU Administrative office in the North Carolina Research Campus, addressed or handed to Dona Miller, the NCSU Administrative Support Specialist, and a direct monetary compensation for the study will be made to the volunteerâ€™s account, or a single check can be generated at the end of his/her participation (Annex 8). The research team will not handle or store any of this information, and it will be handled solely by the participant in person or via encrypted email.

Amendment request (November/2020): Date of birth is collected by the study coordinator into a password-protected Drive to label blood tubes that are used at the clinical screening and study visits to confirm the identity of each participant during blood draw by our UNC-CH partnerâ€™s phlebotomists. This data is not used to achieve any of this studyâ€™s purposes. At the clinical screening, the blood tubes and paperwork that are filled out with the participantâ€™s date of birth by phlebotomists are sent to LabCorp, which follows HIPAA guidelines for data destruction. At study visits, blood tube labels containing participantâ€™s date of birth are tossed after extraction of serum from the blood sample. This data is not retained by our UNC-CH partner. The date of birth of each participant is stored in a password-protected Drive with access to the Principal Investigator and study coordinators and deleted after each participant withdrawal or completion of participation in the study.

If recording identifiable information about participants, discuss any links between the data and the participants and why you need to retain them. Discuss destruction of links or removal of identifers.

The investigator(s) will maintain adequate clinical study records following GCP guidelines. All clinical study material will be archived for a period of at least 3 years after the completion of the study, and then destroyed. Biological samples (blood and urine) will be collected from participants, then analyzed using HPLC-MS/MS for phytonutrient composition, and stored for up to 5 years at Dr. Kayâ€™s laboratory before they are destroyed following NCSU biohazard waste procedures. Samples will be stored for this length of time in case any scientific dispute is raised by researchers in the field after this study is published, and samples need to be reanalyzed, or where new MS/MS technologies are developed which will lead to increased sensitivity and ability to detect a greater number of phytochemical metabolites. The data and samples from this study will not be used for future research, but only evaluated for phytochemical metabolites according to the primary endpoint of this study. Coded output data and data with participant identifiers will be stored in separate locations including electronic data (on secure NCSU network drives separated by firewalls) and including hard copy data. Participantâ€™s identifiable data will be destroyed after final data report to the sponsor and/or after the first publication of results, except in the case where consent is given to be recontacted for future studies, in which case we will retain volunteerâ€™s name, age, gender, telephone number and email address in a database stored on a secured network.

The investigators will ensure that all data obtained in the course of this study will be treated with discretion in order to guarantee the rights of the participantâ€™s privacy in accordance with the standards of the data protection law. The investigator will agree to allow the monitor/auditor/inspector/coordinator to have access to any or all the study materials

needed for source data verification and proper review of the study progress. If law does not allow direct source document verification, then the investigator agrees to assist the monitor/auditor/inspector/coordinator in the validation process of data quality.

Discuss if you'll be working with your departmental IT to create a data management plan and if you're using NC State managed devices, NC State Google Drive or other NC State non-networked device. If using a personal device, discuss data protection.

We will be working with our departmental IT to create a data management plan, which includes a NC State Google Drive and an email account dedicated for this study, and S departmental drive with restricted access to the Principal Investigator and research personnel that is assigned by him (identifiable and anonymized data will be separated by secure fire walls in separate password protected folders). In the email account, the identifiable information is the network IP and email account of the participant that is communicating via email, and this information will be in the domain of the North Carolina State University (NCSU). As soon as the email account is cancelled, which will be after final data report to the sponsor and/or after the first publication of results, the identifiable information will no longer be available, except in the case where consent is given to be recontacted for future studies, in which case we will retain volunteerâ€™s name, age, gender, telephone number and email address in a database stored on a secured network. One NCSU network drive and a locked filing cabinet will be dedicated for identifiable information with access to the Principal Investigator and the study coordinator.

Describe any ways that participants themselves or third parties discussed by participants could be identified indirectly from the data collected, and describe measures taken to protect identities. (Data can be reidentified by researcher access, technology employed, researcher expertise, and triangulation of data or other information. Discuss the probability of reidentification and the magnitude of harm to participants should the data be reidentified. Discuss the probability of reidentification occuring and the magnitude of harm should it occur).

Harm to participants though reidentification from triangulation of indirect identifiers is unlikely as data will only be provided to third parties as summarized group means and standard deviations, as pertaining to recorded age, gender, blood pressure, height, weight, medication history, and food intake. Furthermore, no participant identifiable data will be provided to third parties except in cases where a severe adverse event is reported and shared with the study clinical advisor/research phlebotomist, or a formal audit is requested. In the case of a formal audit the IRB will be contacted first to establish legal requirements for data sharing. The investigators will ensure that all data obtained in the course of this study will be treated with discretion in order to guarantee the rights of the participants' privacy in accordance with the standards of the data protection law. The investigator will agree to allow the monitor/auditor/inspector/coordinator to have access to any or all the study materials needed for source data verification and proper review of the study progress. If law does not allow direct source document verification, then the investigator agrees to assist the monitor/auditor/inspector/coordinator in the validation process of data quality.

For all recordings of any type:Describe the type of recording(s) to be made Describe the safe storage of recordings Who will have access to the recordings? Will recordings be used in publications or data reporting? Will images be altered to de-identify?Will recordings be transcribed and by whom?

No audio or video recording will be made during the present study. Images (PDF) of scanned hard copies of handwritten forms will be stored with coded output data and data with participant identifiers stored in separate locations on two distinct secure NCSU network drives separated by firewalls. Participantâ€™s identifiable data will be destroyed after final data report to the sponsor and/or after the first publication of results, except in the case where consent is given to be recontacted for future studies, in which case we will retain volunteerâ€™s name, age, gender, telephone number and email address in a database stored on a secured NCSU network. A hard copy of datasheets linking names, emails, phones and addresses with code numbers as well as coded â€˜non-identifiableâ€™ participant data will be kept in a locked filing cabinet at the Plants for Human Health Institute at NCRC with access restricted to the Principal Investigator (Dr. Colin D. Kay) and study coordinator (Monique Carvalho de Santana) during the feeding visit. Anonymized participant data will be stored on a secure NCSU network drive and will not be stored on any external drives (including desktop C drives, laptops or external storage devices). Files will only be accessible to the staff associated with this project. Biological samples will be stored as cell-free extracts for analysis, and retained at the study sites to be accessed by study staff for a minimum of 3 and a maximum of 10 years from collection and then destroyed using standard biohazard procedures in place at the Plants for Human Health Institute at the NCRC.

Amendment COVID-19: an initial screening survey for COVID-19 will be performed using Qualtrics, which results will be password protected. The survey information will be stored on a secure NCSU network drive and in a locked filing cabinet at the Plants for Human Health Institute at NCRC with access restricted to the Principal Investigator (Dr. Colin D. Kay) and study coordinator (Monique Carvalho de Santana), archived for a period of at least 3 years after the completion of

the study, and then destroyed.

Describe how data will be reported (aggregate, individual responses, use of direct quotes) and describe how identities will be protected in study reports. Reporting data may sometimes reidentify your participants. If needed, you can adjust how you report your data to protect the identities of your participants. Discuss.

Data will be reported as an aggregate (mean and standard deviation for groups of participants) of the phytonutrient concentrations in the blood serum and urine and reported across time points post-consumption. There will be no chance of linkage of reported mean and standard deviation concentration data to coded samples or to participantâ€™s identifiers.

Will anyone besides the PI or the research team have access to the data (including completed surveys) from the moment they are collected until they are destroyed? This includes sharing data with sponsors, journals, or using the data for future research endeavors. If you are sharing the data, this should be in your consent form.

No deidentified data will be shared with the study sponsor nor in publications, as participant and study outcome data will only be provided as summarized group means and standard deviations. We will retain volunteerâ€™s name, age, gender, telephone number and email address in a database stored on a secured network in the case where consent is given to be recontacted for future studies.

Describe any compensation that participants will be eligible to receive, including what the compensation is, any eligibility requirements for that compensation, and how that compensation will be delivered. Examples of compensation include: monetary compensation, research credits, raffle/drawing, novel items. Make sure to check with your department regarding issues of tracking payments as your department accounting office may have requirements that affect your human subjects privacy (such as the mandatory tracking of anyone who receives compensation). This tracking may influence the confidentiality/anonymity of your research and must be addressed in this application.

All the subjects of the study per protocol will receive a $312 maximum compensation (in addition to travel reimbursement) calculated in proportion to the number of collections or food records provided, 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 (Annex 8 details calculation breakdown by each intervention). This will be calculated at the completion of the study, or upon withdrawal, and includes urine and blood collections, and food intake records. The study coordinator will fill out a form (Annex 8) with all the procedures you have taken.

If the participant lives within 20 miles from campus (600 Laureate Way, Kannapolis, NC 28081) they may file for travel expense reimbursement, where he/she will be paid at the standard NCSU rates for a round trip by car, up to a maximum of 40 miles per attendance (currently at $0.58/mile) or via showing receipts of other transportation fares (i.e., bus or train) for each attendance to campus for research procedures.

Compensation for participants holding visas must also follow the following guidelines:

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 institutionâ€™s Office of International Services indicating they are aware of the visitorâ€™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). For any compensation or travel expense reimbursement, the required forms containing confidential information about the participant will be submitted by the participant to NCSU Administrative office in the North Carolina Research Campus, addressed or handed to Dona Miller, the NCSU Administrative Support Specialist, and a direct monetary compensation for the study will be made to the volunteerâ€™s account, or a single check can be generated at the end of his/her participation (Annex 8). The research team will not handle or store any of this information, and it will be handled solely by the participant in person or via encrypted email.

The sponsor (FFAR) provides the investigator(s) with sufficient material and support to permit the investigator(s) to

conduct the clinical study according to the agreed protocol.

Amendment request (November/2020): Please refer to the document â€œ19138 November 2020 Amendment Request Annex8-Travel reimbursement and study compensation forms_v3â€• for inclusion of participantâ€™s mailing address into travel reimbursement and study compensation forms and removal of the option for emailing W-9 and vendor forms.

Explain compensation provisions if the participant withdraws prior to completion of the study.

Participants may withdraw from the study at any time without explanation and they will be reimbursed based on the number of procedures or samples collected (when applicable). A record of withdrawals throughout the study will be kept for audit purposes (Annex 17).