STUDY TITLE: Evaluation of Ingestible Mini-pill for Gastrointestinal Regional Luminal Content Sampling

STUDY SPONSOR: NIH

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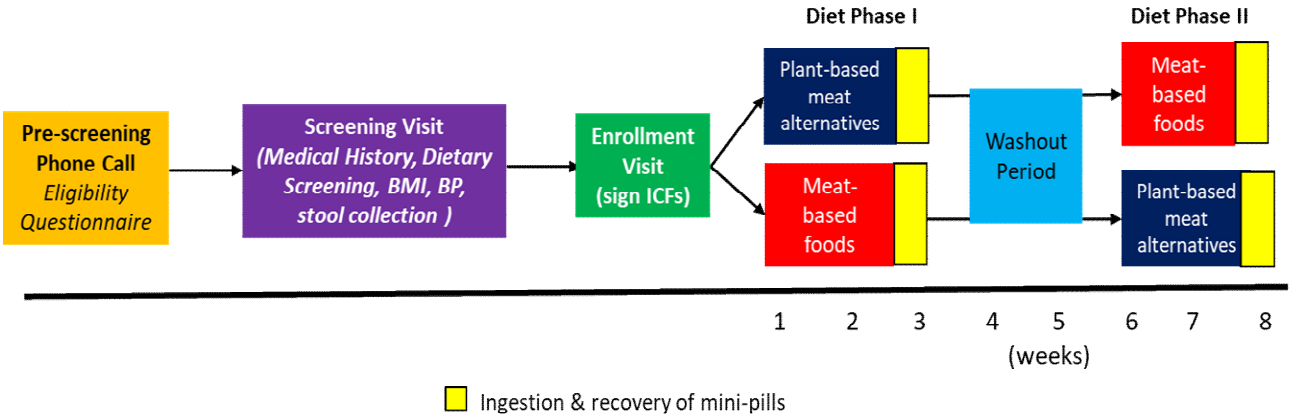
A. Study Schema



B. Introduction

B.1. Objectives

Describe the purpose, specific aims, or objectives of the study:
The purpose of this proof-of-concept study in humans is to determine if a noninvasive, ingestible device, called a "mini-pill", can collect gastrointestinal (GI) luminal content samples from 2 different locations along the GI tract after consumption of diets differing in protein source (meat and plant-based meat alternatives). The mini-pills will be recovered in the stool. We will analyze the microbial profile of the mini-pill contents and in stool, and also measure blood biomarkers related to cardiometabolic risk, to better understand the relationship between diet, microbiota and health.

Specific aims:

1. To assess the feasibility of sampling GI luminal content from targeted regions in humans using the mini-pill and recovering the sealed mini-pills post-elimination in the stool.
2. To analyze and compare the luminal content microbial population (microbiota) recovered from two GI regions and in stool, after consumption of diets differing in source of protein (meat and plant-based meat alternatives).

Exploratory aim:
To compare the effects of diets containing meat and plant-based meat alternatives on metabolites in stool and blood biomarkers associated with cardiometabolic risk.

The ingestible mini-pill is a non-invasive, single-use, passive collection device that is not intended for diagnosis or treatment. The mini-pills have already been developed, tested and validated by two Tufts researchers, Drs. Sameer Sonkusale (School of Engineering) and Giovanni Widmer (School of Veterinary Medicine), *in vitro, ex vitro,* and *in vivo* in animal models (*Supplement 1: Research Strategy and Supplement 2: Soft Autonomous Ingestible Device for Sampling the Small Intestinal Microbiome, Del-Rio-Ruiz R et al., manuscript in review*). A NSR device designation is being sought from the IRB to conduct a 2-phase randomized-controlled double blind crossover trial in humans by providing two entrées per day containing either meat or plant-based meat alternatives for up to 3 weeks each. After 2 weeks on each diet, participants will consume a provided breakfast meal and then be asked to swallow 6 mini-pills along with a blue food coloring dissolved in 50 mL of water, return home and collect stool samples until all the mini-pills are recovered (varies between 2 and 4 days, but can be up to 6 days). This protocol will allow us to describe the diet-induced progressive change in the GI tract microbiota prior to and including that in the stool.

B.2. Background and Rationale

1. *Describe the relevant prior experience and gaps in current knowledge*: The gut microbiota represents trillions of microogranisms and thousands of species.[1] Among the various microbial communities associated with the human body, the GI tract microbiota is noted for its high concentration and species diversity, and is thought to play multiple systemic roles in health and disease[2,3] In humans, the gut microbiota contribute enzymes not encoded by the host's genome and generate numerous bioactive compounds, such as short chain fatty acids (SCFA), secondary bile acids and trimethylamine (TMA) that elicit systemic metabolic effects. Dysbiosis (microbial imbalance) has been associated with conditions such as recurrent enteric infections, microbial overgrowth and a wide range of other disorders such as cardiovascular disease and type 2 diabetes.[4-8] External influencers of the GI tract microbiota include diet,[9,10] as well as other environmental factors, such as physical activity, ambident temperature or medication. Considerable interest has settled on diet.[11,12]

   Most studies infer the state of the GI tract microbiome from the analysis of microbial communities in stool samples. Healthy dietary patterns, characterized as being relatively rich in minimally processed plant foods and dietary fiber, and poor in animal products and ultra-processed foods, have been associated with a low prevalence of chronic diseases such as cardiovascular disease and type 2 diabetes,[9,13] but the response of proximal intestinal microbial communities to diet is difficult to study. Whereas rapidly evolving DNA sequencing technologies have facilitated the analysis of complex microbial communities, our capacity to non-invasively sample luminal content from different locations along the GI tract has not evolved.

2. *Rationale for the research and preliminary data:* The mini-pill development and subsequent validation work was funded through a NIH R21 phased innovation mechanism. NIH R33 funding for the second phase, the proposed human trial, was contingent on successful completion of the *in vitro, ex vivo* and *in vivo* work in pigs and hounds. As part of the R21 phase of the proposal, we have successfully validated a 3D-printed sampling device, referred to as a *mini-pill*, to retrieve luminal content at pre-determined regions of the GI tract in pigs and hounds (*See Supplement 2: Soft Autonomous Ingestible Device for Sampling the Small Intestinal Microbiome, Del-Rio-Ruiz R et al., in review*).

   The proposed study includes ingestion of six mini-pills per diet phase, three targeted to collect lumenal content from the upper GI, tract and three targeted to collect lumenal content from lower down in the GI tract. The rationale for three pills per location is to establish that sufficient qunatity of representative luninal content at each site is collected, and to determine reproducability. Sampling relies on uptake of intestinal content through the side inlets of the empty mini-pill as a result of natural GI peristalsis. Regional sampling of the mini-pill is initiated upon dissolution of the pH-sensitive enteric coating. Because the pH of the GI tract increases as the luminal content progresses from the duodenum to the colon, different pH-sensitive enteric material can be used to coat the mini-pills to sampling differ regions of the GI tract. Critical to minimizing contamination from downstream material, embedded absorbent beads located inside the mini-pill activate a valve which closes the intake inlet through which the sample has been acquired. (*See Supplement 3: Mini-pill for Collection of GI Tract Luminal Conten*t).

3. *Significance of the research objectives:* Because regions of the GI tract differ with respect to oxygen tension, pH and nutrient availability, the commonly sampled microbiota in stool is unlikely to reflect the microbial communities populating more proximal regions of the GI tract, particularly, the small intestine. To gain insights into the impact of the entire GI tract microbial environment on health and disease, it is essential to develop a methold to non-invasively sample luminal content from targeted locations, particularly upstream of the colon.[10]

4. *Specify if first time study device will be used in humans:* This is the first time this speicifc 3D printed mini-pill device will be tested in humans. However, a similar NSR device, albeit larger (NCT05749068), has been tested in humans after receiving IRB approval at another institution (See Supplements 5 and 6).[14,15]

5. *Is there an active control group?*
   ☐ **Yes** ☒ **No**

B.3. Risks to Subjects

List the reasonably foreseeable risks, discomforts, hazards, and/or inconveniences to the subjects related to their participation in the research, including risk of unintentional loss of confidentiality. There are no known risks associated with ingestion of the mini-pills. The results of in vivo safety testing of the mini-pills in hounds is provided in Supplement 2 (Soft Autonomous Ingestible Device for Sampling the Small Intestinal Microbiome, Del-Rio-Ruiz R et al., in review). A similar passive mini-pill has been tested in humans under non-significant risk designation after receiving IRB approval.[14,15] Our mini-pill (6 mm x 15 mm housed in a 6.4 mm x 17.0 mm gel capsule) is smaller than the one studied by others (6 mm x 30 mm). Hence, is expected to have a lower risk during administration, GI-transit and excretion. The safety data for the prior study conducted in humans using a similar devise indicated "All devices safely exited all participants and were successfully retrieved. No adverse events were reported." Our mini-pill contains (i) no mechanical parts, motors or gears, (ii) no electronics, cables, antennas batteries or passive components, and (iii) no active or passive magnet or metal. The mini-pill is 3D-printed using biocompatible resins avoiding the risk for possible allergic reactions or exposure to toxins.

There are the rare risks of microscopic bleeding, obstruction, perforation, infection and potential for unknown risks from the mini-pill. In the event of suspected aspiration, additional diagnostic (e.g. chest x-ray) or therapeutic procedures (e.g. bronchoscopy) may be required and the participant will be referred by the study MD to the TMC Emergency Department for evaluation or the emergency department preferred by their primary care provider.

There are no major risks associated with the short-term consumption of the meat containing entrées. Prior work has reported no adverse effects from consumption of the plant-based meat alternatives.[16] Some of the plant-based alternative entrées contain gluten, so participants with known sensitivity to gluten will be excluded. There are no known risks associated with obtaining the study measures (height, weight, waist circumference and resting blood pressure). There might be some discomfort from fasting for 8 to 10 hours prior to the study visits. There may be some nausea associated with swallowing the 6 mini-pills. The need to collect and return multiple stool samples at the end of each diet phase, may be inconvenient for the participants. There is a low risk of sensitivity to Betty Crocker Classic Gel Food Color, which contains Blue 1. Most allergic reactions to blue dyes are mild, described as grade I (69–87%) and present with urticaria (itchy skin), pruritus (hives), and/or generalized rash.[17,18] To address this issue participants with known sensitivity to food coloring will be excluded. Additionally, the nursing staff will monitor participants after ingestion of the pills and dye for any possible symptoms. There may be a small amount of bruising and discomfort, and rarely infection, associated with the drawing of blood samples. Subjects may become light-headed and faint. Every effort will be made to minimize discomfort to study subjects, with experienced nursing staff performing the blood draws. No known psychological or social risk are anticipated. There are no economic risks as study entrées will be provided to the study subjects, and there is no cost associated with the mini-pills. Protected health information will be collected as part of this study. Study participants may face risks related to inadvertent release of confidential information. This risk will be minimized through careful adherence to best practices for data collection and management. All research staff will be trained in principles and methods for assuring participant confidentiality and safety. Data will be used only in aggregate and no identifying

characteristics of individuals will be published or presented. Confidentiality of data will be maintained by using research identification numbers that uniquely identify each individual. Safeguards will be established to ensure the security and privacy of participants' study records. Databases will not use participants' names as identifiers. The files matching participants' names and demographic information with research ID numbers will be kept secure and only study personnel will have access to these files. After the study is completed, local data will be stored securely with other completed research studies.

1. *State which study interventions may have unknown risks:* None, reported by other investigators

2. *State which study interventions may have risks to an embryo or fetus (if a subject is or becomes pregnant) or to a nursing infant of a study subject***:** ☒ N/A

3. *Describe risks to people other than the participating subject, e.g., risks to family members, friends, others or risks to the community***:** ☒ N/A

4. *Are there any risks to study investigators or staff performing the study procedures due to research with high risk populations (e.g. prisoners, intravenous drug users, patients with major psychiatric issues, etc.)?* ☒ **No**

B.4. Potential Benefits to Subjects

1. *Describe the potential benefits that individual subjects may experience from taking part in the research. Include the probability, magnitude, and duration of the potential benefits. Discuss why the risks to subjects are reasonable in relation to the anticipated benefits to research subjects:* ☒ **N/A**, there is no direct benefit.

2. *Describe any benefit to the population from which the subjects are drawn:* The results of this study may allow for an advancement of our understanding about the relationship between diet, GI microbiome and health through development of a non-invasive methodology to analyze the intestinal microbiota from the duodenum to the colon.

3. *Describe any benefit to science, society, and humanity in general:* The results of this work may facilitate refinement of public health dietary guidance to optimize the microbiota throughout the GI tract for disease mitigation. This point is of particular importance as we shift towards personalized nutrition guidance. Ultimately, these types of studies, regardless of the diet comparison, can inform diet optimization with the intent of modifying the gut microbiota to improve long-term health outcomes.

B.5. Alternatives

1. *Describe alternatives to participating in this research study (e.g. alternative treatments, no treatment (palliative care), participating in an activity without data being used for the research, etc.):* Subjects may elect not to participate in the study.

2. *Describe the standard clinical care that may be an alternative:* ☒ **N/A**

3. *Describe how the subject can receive the research procedures/drug/device used in this study in a non-research setting:* ☒ **N/A**

C. Enrollment and Withdrawal

C.1. Inclusion Criteria

1) Men and postmenopausal women
2) Age ≥50 to ≤75 years
3) BMI ≥20 to ≤35 kg/m^2
4) Normotensive with or without medication
5) Normal gastrointestinal function with regular bowel movements at least once every other day
6) Normal kidney and liver function
7) Willingness to swallow the mini-pills
8) Willingness to collect and return multiple stool samples
9) Adequate refrigerator and freezer space to store study entrées
10) Intent to remain in the greater Boston area during the intervention periods

C.2. Exclusion Criteria

1) Individuals self-reporting adhering to any type of vegetarian diet
2) Lack of willingness to restrict fish intake to less than once per week during the dietary intervention phases
3) Allergy/intolerance/religious reasons to avoid study foods or food ingredients, including known hypersensitivity to Blue 1 food coloring and wheat gluten.
4) Regular use of prebiotics or probiotics within the past 3 months
5) Regular use of laxatives or fiber supplements
6) Chronic constipation
7) Chronic use of antibiotics (except topical)
8) Regular use of stomach acid lowering and weight loss medications such as GLP-1 agonists
9) Use of dental prophylaxis
10) Planned colonoscopy 2 months prior to or during the study period
11) Gastroparesis
12) Swallowing disorder, or inability or difficulty taking pills
13) Malabsorptive and inflammatory bowel disease, diverticulosis, and history of diverticulitis, gastric/ esophageal/intestinal surgery, including lap banding or bariatric surgery.
14) History of bowel obstruction, pancreas and liver disorders.
15) Any form of active substance abuse or dependence (including drug or alcohol abuse). This information will be stored in REDCAP in a subsection that has no identifiers.
16) Established major chronic diseases such as cardiovascular disease, diabetes, active cancer within the last 5 years, or any significant medical condition at the study MD's discretion
17) A clinical condition that, in the judgment of the study MD or principal investigator, could potentially pose a health risk to the subject while involved in the study.
18) Unwillingness to adhere to study protocol
19) Intent to increase or decrease body weight during the study period
20) No Social Security number (for payment and IRS forms).
21) Individuals who directly report to any member of the research team.

Can study subjects participate in another research study while participating in this research study:

☒ **No**

Please explain your response if needed: Introduction of additional diet and/or lifestyle interventions can affect the study outcomes. If participation in another research study involves blood draws in addition to those associated with this study, total may exceed allowable amounts.

C.3. Screening and Eligibility

1. *Describe in detail the methods that will be used to identify potential subjects and how the eligibility criteria will be assessed and satisfied. Specify what type of screening will take place prior to informed consent and screening that will take place after informed consent:*

Eligibility criteria will be determined via a prescreening questionnaire administered over the telephone, and an in-person screening visit that includes measurement of blood pressure, waist circumference, and height and weight to determine BMI. Additionally, at the screening visit, a nursing team member will review medical history and medication use to confirm eligibility. Dietary screening by a registered dietician will be conducted to determine food aversions, food allergies and/or sensitivities that would interfere with acceptability of the study entrées. The participant will be provided with a stool collection kit and must successfully provide a stool sample within 48 hours of screening to confirm regular bowel movements, as well as to familiarize themselves with the stool collection protocol after mini-pill ingestion.

2. *State who will confirm eligibility and ensure that eligibility and consent were documented prior to the subject receiving any study intervention. Note that those who are designated to determine eligibility must have appropriate training, expertise, and oversight, for example a physician PI or Co-I on a biomedical study:* Each subject's file will be reviewed by the PI, study doctor or designate prior to beginning the study to confirm eligibility and to ensure the Informed Consents were signed and the subject understands the study procedures.

3. *Will a screening data collection form/log be used in this research study?* ☒ **No**

C.4. Withdrawal of Subjects

1. *Describe anticipated circumstances under which subjects will be withdrawn from the research without their consent:* Non-compliance with study protocol based on not attending pre-scheduled study visits, not providing blood or stool samples, not picking up and consuming provided study entrées, change in medical status or use of steroids or oral antibiotics during the study. Additionally, if a subject does not follow all of the HNRCA volunteer rules and regulations, they will be withdrawn from the study without their consent.

2. *Describe procedures that will be followed when subjects **either** withdraw voluntarily **or** are withdrawn from the research, including the possibility of partial withdrawal from study intervention with continued data collection.* If a participant wishes to withdraw part-way through the study they are at liberty to do and no further data will be collected. Samples and data that have already been collected will be analyzed as per protocol.

3. *Describe any necessary safety precautions to be applied to subjects who withdraw or are withdrawn (tapering drug doses, evaluative x-ray, etc.):* ☒***N/A***

C.5. Recruitment and Retention

C.5.1 Local Recruitment Methods

Will subjects be recruited at the Tufts site for this study? ☒ **Yes**

If **Yes**, describe the following attributes of the recruitment plan for the Tufts site(s)[1]:

1. *When, where, and how potential subjects will be recruited:*
    a. *Source of subjects:* Boston and the surrounding areas will serve as the source of subjects. The HNRCA Metabolic Research unit (MRU) maintains a computerized database of participant information through Protocol Manager (volunteer database centralized under the Volunteer Services Department [IRB approved protocol # 6701] and contains the names of approximately 40,000 individuals) and nCoup Volunteer (secure SaaS participant database hosted on Amazon Web Services). In addition to this database, to

[1] If Tufts IRB is requested to be the Single IRB of Record for external sites (as per section K.3 Multi-Site Research), these external sites are included in "Tufts site(s)"

identify potential participants recruitment flyers will be posted in and around the HNRCA, Tufts Medical Center/Tufts University of Health Sciences campus, and surrounding area. Social media sites such as the HNRCA website and Facebook page, will also be used.

b. *Methods that will be used to identify potential subjects*: The HNRCA Protocol Manager and nCoup Volunteer database will be used to identify a pool of potentially eligible participants based on the protocol's inclusion/exclusion criteria and targeted mailings will be sent with information about the study. In addition to this database, to identify potential participants, recruitment flyers will be posted in and around Tufts University/HNRCA, and social media sites such as the HNRCA website and Facebook page will include a description of the study and links to forms that potential study participants can express interest in participating in this study.

2. *If potential subjects will be approached, specify where the recruitment discussion will take place to ensure subjects' privacy (e.g. a private clinic room):* Recruitment discussions will take place via a telephone call between the study staff and potential participant. There is a designated area in the HNRCA to make recruitment phone calls**.**

3. *If potential subjects will be* ***recruited by telephone****, describe how many times the research team will attempt to call / leave a voice message*: If the subject cannot be reached, a voice message will be left with information about returning the call. Three attempts will be made to call a potential subject. If no response is received, any further communication attempts regarding the study will cease.

    a. ☒ *Check to confirm that a script for* ***both*** *the telephone conversation and the voice message is included with the submission.*

4. *When subjects* ***respond to recruitment material****, describe the information that will be provided to them about the study and the information that will be collected from subjects (e.g. name, telephone number, etc.). Describe also, how many times you will attempt to respond to call the subject back / leave a voice message:* Upon the subject's response to recruitment material (targeted mailer, social media post or flyer), they will be informed of the general purpose of the study and procedures that will take place, in addition to the time commitment for the overall study as well as each in person study visit. A research team member will also administer a brief telephone prescreening questionnaire to determine eligibility. At this time, the following will be collected; name, telephone number, email address, and mailing address for future correspondence, height, weight, age, sex, gender, menopausal status, medication and supplement use, health history and if following a dietary pattern that excludes meat. The answers to the prescreening questions related to drug and alcohol abuse will be stored in REDCAP under a separate section that has no identifiers and cannot be linked back to the participants demographic information. They will also be queried as to whether they have adequate freezer/refrigerator space to store the study entrées.

    ☒ *Check to confirm that a script for* ***both*** *the telephone conversation and the voice message is included with the submission.*

4. *Will potential subjects be recruited at institutions that are not owned and operated by Tufts Medical Center, Tufts University, MelroseWakefield Healthcare, or Lowell General:* ☒ **No**

5. *If print and media advertisements will be used, specify when, where, how long and frequency of the advertisements that will be published/aired:* Recruitment material will be used for the duration of the study recruitment period. Advertisements will be published on the HNRCA website, as well as other social media sites. It is anticipated recruitment will occur over a 12

month period, or until 30 participants (15 females and 15 males) have completed each of the two diet phases.

a. ☒*Check to confirm that any necessary permission will be obtained for posting/airing these (for example, permission to post a flyer on a bulletin board).*

6. *If recruitment material is being mailed or otherwise distributed, submit the proposed material and describe where/how the distribution list will be obtained:* The list of potential subjects who will receive a targeted mailer will be identified via the HNRCA Protocol Manager or nCoup Volunteer database (IRB approved protocol # 6701).

7. *Describe how the recruitment methods described will be effective in attracting the targeted subject population. Address both planned recruitment activities as well as any proposed engagement strategies for retention:* Prior experience suggests that use of the HNRCA's database as well as the targeted nature of the mailings to a volunteer pool who meet the study inclusion criteria will be an effective manner to recruit subjects. Likewise, experience indicates that posting recruitment flyers in and around the Tufts Medical Center/Tufts University of Health Sciences campus and surrounding area, and social media sites such as the HNRCA website and Facebook page, are effective recruitment approaches.

C.5.2 Study-Wide Recruitment Methods

Is this is a multicenter study where subjects will be recruited by methods not under the control of the local Tufts site (e.g., call centers, national advertisements)? ☒ **No**

C.5.3 Projected Enrollment Numbers

We will enroll up to 60 participants, to reach our target of 30 completers (15 men and 15 women)

	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
Racial Categories	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	3	3	0	0	6
Native Hawaiian or Other Pacific Islander	1	1	0	0	2
Black or African American	2	2	1	1	6
White	15	14	4	3	36
More than One Race	4	4	1	1	10
Total	25	24	6	5	60

2. *Inclusion of individuals across the lifespan:*
   a. *Specify how you will ensure the inclusion of individuals across the lifespan. If applicable, provide a scientific or ethical rationale for the minimum and maximum age of study participants and for limiting inclusion of any age group (e.g., children or older adults)*: The objective of this project is to conduct a proof-of-concept study to test the feasibility of using 3D printed mini-pills to collect and characterize the microbiota in luminal content at 2 sites of the GI tract following consumption of 2 different diets. Our focus on

older adults relates to translational relevance, this age group presents with higher rates of chronic diseases/risk factors that may be responsive to dietary modification.

b. *Include a description of expertise of the investigative team for working with individuals of the ages included, the appropriateness of the available facilities to accommodate individuals in the included age range, and how the age distribution of participants will contribute to a meaningful analysis relative to the purpose of the study*: Older adults are the focus of research projects at the HNRCA. Drs. Lichtenstein and Matthan, as well as the HNRCA based research team has extensive experience working with this subgroup. The accessibility features in the HNRCA building were upgraded during the past 5 years to accommodate physical limitations. Given disorders of the GI tract occur throughout the lifecycle, knowledge gained from focusing on this age group will be generalizable to a broader age range of individuals.

3. *Inclusion of women and minorities:*
   a. *Describe the projected distribution of subjects by sex/gender, race, and ethnicity. Provide the rationale for the selection in terms of the scientific objectives and proposed study design. Describe the proposed outreach programs for recruiting sex/gender, racial, and ethnic group members. If applicable, provide a reason for limiting inclusion of any group*: Sex: 50% of recruited subjects will be postmenopausal women. Race/ethnicity distribution as depicted in the table above, reflects the general population distribution of older individuals (> 50 years) living in the greater Boston area, which serves as the potential pool of volunteers for this study.

   b. *Provide the rationale for the selection in terms of the scientific objectives and proposed study design. Describe the proposed outreach programs for recruiting sex/gender, racial, and ethnic group members:* Consistent with our study aims, the study population will focus on individuals age ($\geq$50 years) and BMI ($\geq$20 and $\leq$35 kg/m^2) cutoffs (females menopausal). Past experience has shown that our recruitment methods (targeted mailers, flyers and listing on social media sites such as the HNRCA website) are effective tools in reaching our recruiting goals by sex (50/50 split). We are not targeting any specific racial or ethnic group to meet study aims.

4. *Inclusion of Populations affected by the Disease or Condition being studied* ☒ **N/A**

C.5.4 Payment

Will subjects receive money, gifts, or any other incentive for participating in this study?
This does not include reimbursement for expenses, which is considered in the next section.
☒ **Yes**

If **Yes**, respond to all of the following*:*

1. *Describe any proposed payment or incentive for subjects.* Participants will be paid $25 for the screening visit; $140 for the enrollment visit and subsequent study visit procedures; $90 for food pickup/food intake checklist return; $220 for stool collection/return; and $100 for dietary assessments, for a total of $575.

2. *Payment amount:* $575 if they have their eligibility verified and complete the study.

3. *How payment will be made.* Payment will be made by check mailed to the participant at the end of each diet phase. It will take approximately 2 to 3 weeks for the participants to receive the check. The PI or designate will approve payment for each participant. Payment schedules, processing dates and payment log are stored in Protocol Manager (PM). Multiple levels of

University approval are obtained outside of PM and then checks are mailed from the University to each participant. Due to federal tax law, participants will be asked to provide a social security number in order to process payments. Participants will be requested to complete a Tufts University W-9 Form (Attachment 28) provided to the HNRCA by Tufts Support Services. If participants receive over $600 from Tufts Medical Center or Tufts University Health Sciences in a single calendar year (either in a single study or multiple studies), they will be issued an IRS 1099 form.

4. *To whom payment will be made (subject, parent [which one], legally authorized representative):* Participant

5. *Payment schedule:*
   a. *When payment will occur:* After the completion of each of the 2 diet intervention phases.
   b. *The payment schedule (amount at each time point), including details about the payment schedule and amount for subjects who withdraw or are withdrawn from the study:* Participants will receive a total of $575 if they have their eligibility verified and complete the study. All participants who participate in the screening visit (regardless of whether they qualify for the study) will still be paid $25 for their time and effort. Participants who withdraw from the study will be compensated for the diet phases that they completed prior to their withdrawal. Participants who withdraw partway through a phase will not be compensated.

C.5.5 Reimbursement

Will subjects be reimbursed for their expenses, such as travel, parking, meals, or any other study related costs? ☒ **No**

D. Costs to Subjects

Does the research involve any costs to subjects? ☒**No**

E. Study Design

E.1. Study Timelines

1. *Describe the duration of an individual subject's participation in the study:* The entire study period will be approximately 8 weeks, starting with the enrollment visit and including a 2-week wash out period. During that period, there will be 2 diet phases: meat entrées and plant-based meat alternative entrées. Each diet phase will be ~3 weeks, variability dependent on the post-injection mini-pill recovery in stool. All study procedures will be conducted in the MRU at the HNRCA.

2. *Describe the duration anticipated to enroll all study subjects at the Tufts study site(s):* 1.5 years

3. *Describe the estimated date for investigators to complete this study (complete primary analyses):* 3 years after receiving IRB approval.

E.2. Procedures

1. *Summarize the research design and sequentially identify all procedures to be performed to accomplish the specific aims of the project.:* Participants meeting the eligibility criteria will be randomly assigned to one of two randomization sequences (Phase I and Phase II), the meat or plant-based meat alternative entrées, or vis versa, to achieve a crossover design. There will be a 2-week washout period between the phases.

During each diet phase, participants will be provided with 2 study entrées per day and counseled by the research dietitian to consume them at two different times during the day. The length of each diet phase will be ~3 weeks (i) 2 weeks of meat or plant-based meat alternative entrées, (ii) ingestion of the mini-pills on week 2 (day 14), and week 5 (day 49) of the diet phases, and (iii) additional 2 to up to 6 days (consuming 2 entrées/day) until the mini-pills are recovered from the stool. In humans, prior work has established the adequacy of the intervention period to affect microbial communities.[16] A prior study in humans using a similar device indicated that all devices were recovered in stool using a collection window between 6 and 48 hours .[14,15] The mini-pill recovery window in our study using pigs and hounds was up to 72 hours. We plan to use the appearance of blue dye in the stool to better tailor the stool collection window for our participants.

Table 1: Summary of Study Timeline and Procedures

Study Procedures	**Diet Phase I**			**Wash out**		**Diet Phase II**			**Total #**
Study weeks (days)	1 (1-7)	2 (8-14)	3 (15-21)	4 (22-28)	5 (29-35)	6 (36-42)	7 (43-49)	8 (50-56)	8 (56)
Food pick up	X	X	X			X	X	X	6
Entrée check list return		X	X				X	X	4
Weight	X	X				X	X		4
Waist circumference	X	X				X	X		4
Blood pressure	X	X				X	X		4
Fasting blood samples*	X	X				X	X		4
Mini-pill ingestion		X					X		2
Stool collection/return**			XXX					XXX	up to 6
Diet 24-hr recalls	X	XX					XX		5
Payment			X					X	2

*Blood will be drawn in the morning after an overnight fast (8 to 10 hours, no food or drink except water and approved medications). The amount drawn on days 1, 14, 36 and 49 will be 12 mL (about 1 tablespoon) for a total of 48 mL over the entire study (about 4 tablespoons).

**The number of stool samples to be collected will vary depending on mini-pill recovery

Diet Phase 1

Week 1_Day 1*:* Following the screening visit, participants will come to the HNRCA after an 8 to 10 hour fast to return their stool sample. They will then be given the opportunity to review and sign the Study Informed Consent form and also be given the option to review and sign the Optional Tissue Banking Informed Consent form. Once enrolled, participants will have their weight, waist circumference, and blood pressure measured and provide a fasting blood sample. They will meet with a registered dietician to review instructions for entrée storage/consumption and pick up diet phase I specific entrées and checklist.

Week 2_ Day 8**:** Participants will come to the HNRCA to pick up another week of study entrées and also return and review their Entrée Checklist with a registered dietician.

Week 2-Day 14: Participants will come to the HNRCA after an 8 to 10 hour fast to provide a fasting blood draw. They will be given a breakfast containing a study entrée specific to the diet phase to be consumed within 30 minutes, and then asked to swallow 6 mini-pills along with blue dye dispersed in ½

cup of water within 15 minutes. Additional water may be consumed, if desired. Participants will be given study entrées for another week and stool collection kits with cooler and instructions.

Week 3-Days 15 to 21: Participants will continue to consume 2 entrées per day until the mini-pills are eliminated in the stool, which can vary between 2 and 4 days, but can be up to 6 days. Participants will be asked to collect and return all stool samples produced every 48 hours, until they are informed by study staff that all pills have been recovered.

Participants can stop consuming the entrées once all mini-pills are recovered. There will be a 2 week break at this point during which participants will have no study obligations and can consume their normal diet. If all of the mini-pills are not recovered after 6 days, participants can stop consuming the study entrees, and we will conduct a 2-week follow up telephone call to inquire about symptoms or concerns.

Diet Phase II

Week 6_Day 36: Participants will come to the HNRCA after an 8 to 10 hour fast to provide a fasting blood sample and have their weight, waist circumference, and blood pressure measured. They will pick up diet phase II specific entrées and checklist.

Week 7_ Day 43: Participants will come to the HNRCA to pick up another week of study entrées and also return and review their Entrée Checklist with a registered dietician.

Week 7-Day 49: Participants will come to the HNRCA after an 8 to 10 hour fast to provide a fasting blood draw. They will be given a breakfast containing a study entrée specific to the diet phase to be consumed within 30 minutes and then asked to swallow 6 mini-pills along with blue dye dispersed in ½ cup of water within 15 minutes. Additional water may be consumed, if desired. Participants will be given study entrées for another week and stool collection kits with cooler and instructions.

Week 8-Days 50 to 56: Participants will continue to consume 2 entrées per day until the mini-pills are eliminated in the stool, which can vary between 2 and 4 days, but can be up to 6 days. Participants will be asked to collect and return all stool samples produced every 48 hours, until they are informed by study staff that all pills have been recovered. Participants can stop consuming the entrées once all mini-pills are recovered. If all of the mini-pills are not recovered after 6 days, participants can stop consuming the study entrees, and we will conduct a 2-week follow up telephone call to inquire about symptoms or concerns.

Additional Study Procedures: Participants will complete one 24-hour dietary recall at the beginning of the first diet phase (baseline) and then two at the end of each diet phase over the phone. On day 1, a member of the HNRCA Dietary Assessment Unit will provide instructions and give participants a two-dimensional measuring aid (Food Amounts Booklet) to assist with estimating portion size. The first dietary recall will be completed by phone within 24 hours. Thereafter, they will be contacted by phone to complete additional 24-hour dietary recalls.

a. *Procedures, situations, or materials that may be hazardous, and the precautions to be exercised to maintain subject safety:* **N/A**.

Please also describe the following concerning procedures:

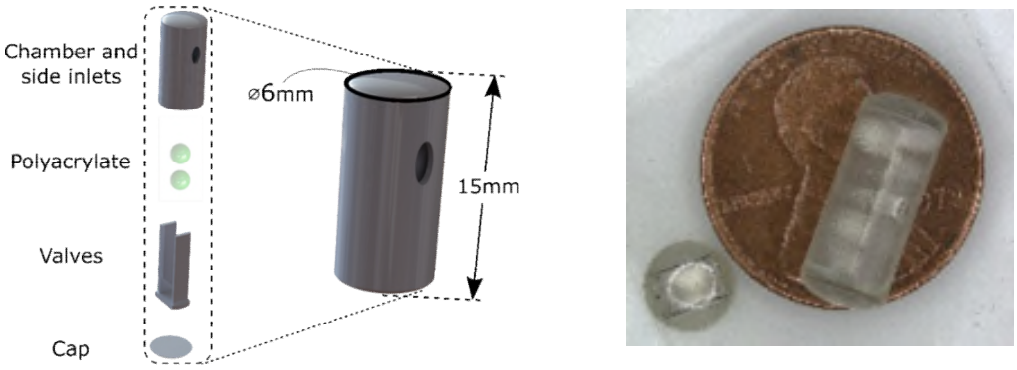
a. *How individuals will be screened for eligibility. Specify screening that will take place prior to informed consent and screening that will take place after informed consent*: IRB approved telephone prescreening questionnaire will be used to determine eligibility based on inclusion and exclusion criteria (see sections C.1 and C.2). Participants who meet prescreening criteria will be invited to the HNRCA for a screening visit to confirm eligibility. At the screening visit,

after obtaining consent, BMI will be confirmed by direct measurement of height and weight. In addition, blood pressure will be measured, and medical history/medication use and dietary habits will be reviewed.

b. *Procedures being performed to monitor subjects for safety or to minimize risks:* Every effort will be made to minimize discomfort to study subjects, with experienced nursing staff performing the blood draws and monitoring mini-pill ingestion. Post ingestion of the mini-pills, all participants will receive a 2 week follow up telephone call. During this call, we will review medical history in order to monitor any possible longer-term side effects including urgent/emergency visits, or hospitalizations specifically for abdominal pain/discomfort, GI obstruction, perforation, diverticulitis, GI bleeding.

c. *All drugs and devices used in the research, their regulatory approval status (whether they are approved by the FDA **and** being used within their FDA approved indication), and the purpose of their use. For drugs, specify the dosage of each drug, how they will be administered, and how often they will be administered. For devices, specify how they will be used/implanted:*
The mini-pill is a size 2 pill. It is not a wearable. It is not an implant. The mini-pill does not deliver any drugs. It is not intended for diagnosis or treatment. It is a passive, single-use, ingestible collection device that is recovered from the stool and its contents will be analyzed for microbial communities inhabiting the GI lumen. See Supplement 3 (Ingestible Mini-pill for Collection of GI Tract Luminal Content) which provides a detailed description, images, dimensions, designs and the safety profile of the device from both materials and our recent animal studies in hounds. An image of the pill is shown below.

Figure 2: Design of the mini-pill (size 2) including the component parts, dimensions and fabricated prototypes using fully biocompatible BioMed Clear resin (Formlabs). Right shows the size relative to a U.S. penny. Mini-pill will be standard size 2. Standard multi-vitamins are size 000.



A NSR device designation is being sought as part of this submission. The supporting evidence for NSR designation is based on the following: (i) It's demonstrated safety profile in hound studies (Supplement 2). (ii) Biocompatible materials are used in the making of the mini-pill (Supplement 3) (iii) Compared to the FDA approved electronic pills such as PillCam or SmartPill, our design contains no electronics, no battery and no chemicals. It is essentially an empty capsule that fills as it is traversing the GI tract. (iv) A NSR designation was given to a similar mini-pill used by Envivo Bio Inc. investigators (protocol downloaded from Clinicaltrials.com Supplement 4). Their results have appeared this past year in *Nature* and *Nature Metabolism*.[14,15] (Supplement 5 and Supplement 6)

d. *Specify which procedures, tests, visits, etc. described above are:*
   i. *Part of usual standard of care (procedures that would occur regardless of research participation) at the Tufts site(s):* **None**

ii. *Performed solely for research purposes (including "extra" routine tests):* All analysis to be performed as detailed above are solely for research purposes.

2. Will subjects be randomized? ☒ **Yes**

a. *Describe the randomization procedures, including the ratio of subjects randomized to each study arm:* The study design is a randomized cross-over trial. Each participant will receive both dietary interventions. Block allocation will be used to generate the randomization sequences, stratified by sex. Assignment to a sequence will be based on order of enrollment at time of consent. If a participant withdraws from the trial prior to completion, their randomization sequence will be locked and subsequent enrollees will be assigned to the next available slot in the allocation table. Only the MRU registered dietitian, kitchen staff and a member of the HNRCA Statistical Core Unit not involved in data analysis will be aware of participants' randomization sequence.

b. *Describe the blinding procedures:* This is a double-blind study. Only the MRU dietitian, kitchen staff and a member of the HNRCA Statistical Core Unit not involved in analysis will be aware participants' randomization sequence. No identifiers will be included on intervention food items provided to the participants.

c. *Is there a placebo control arm?* ☒ **No**

4. *Describe the following concerning pregnancy testing and birth control:* None as study participants will be postmenopausal females and males.

5. ☒*Check to confirm that any amendments to the protocol or informed consent documents will be reviewed and approved by the IRB prior to implementation, unless required to eliminate an apparent immediate hazard to subjects.*

E.3. Evaluations

Will you perform any laboratory tests for this study?

☒ Yes

If **Yes**, respond to all of the following:

1. *List all laboratory tests to be done as part of the study (e.g., hematology, clinical chemistry, urinalysis, pregnancy testing) as follows:* No screening laboratory tests will be conducted. Fasting venous blood will be drawn and stool collected at the beginning of the study and the end of each dietary phase for research purposes only.

2. *Describe laboratory methods to provide for appropriate longitudinal and cross-sectional comparison (e.g., use of consistent laboratory method throughout study, use of single, central laboratory for multi-site studies):* **N/A**

3. *If more than one laboratory will be used to perform study tests, specify which evaluations will be done by each laboratory:* Analysis of GI luminal content and stool microbiome will be conducted in the laboratory of Dr. Giovanni Widmer, Tufts School of Veterinary Medicine, a co-Investigator. Analysis of the blood samples for cardiometabolic risk factors (plasma lipids, lipoproteins, inflammatory factors, markers of glucose homeostasis) and stool and plasma metabolomic profiles will be overseen by Dr. Matthan in the Nutrition Evaluation and Cardiovascular Nutrition laboratories at the HNRCA.

4. ☐ *Check to confirm that laboratory tests that will be performed are in compliance with Clinical Laboratory Improvement Amendments (CLIA) of 1988.* All laboratory tests are being performed for research purposes only and test results will not be shared with the study participants or their physicians.

5. *If samples will be stored for the purpose of this study, describe the preparation, handling, and storage of specimens, including specific time requirements for processing, required temperatures, aliquots of specimens, where they will be stored, how they will be labeled, and what will happen to the samples after the study is over:* GI luminal content and stool samples will be aliquoted and stored at -80°C for microbiota analyses. A portion will be freeze-dried for metabolome analysis. Only samples from participants who have consented to optional banking will be retained for archival. All other samples will be destroyed after analysis. Fasting (8 to 10 hour) blood samples will be drawn (12 mL per visit) at the beginning and end of each diet phase for a total of 48 mL over the study duration. Serum and plasma will be isolated and aliquoted into vials by the staff in the HNRCA Nutrition Evaluation Laboratory, and stored in a -80°C freezer until analyses. Samples will be labeled with the date of collection, along with a unique subject identification number, specimen type and diet phase. After the proposed analysis, any remaining blood samples will be destroyed by the respective laboratories performing the analysis unless participants have consented to optional banking.

6. *Will biospecimens (including those collected for future unspecified research) be (check all that apply):*
    a. ☒ *Subjected to genetic testing (DNA, RNA sequence data, epigenetic data) (risks of genetic testing must be described in the ICF)*
    b. ☐ *Subjected to genome-wide analysis (must be described in the ICF)*
    c. ☐ *Genotype and phenotype data will be shared for research purposes (this must be described in the ICF, including with whom this data will be shared)*
    d. ☐ *Used to create a perpetual cell line - immortalized (must be described in the ICF)*
    Refer to the NIH's FAQ on Human Subjects Research - Human Specimens and Cell Lines for additional information.
    e. ☐ *None of the above will occur*

E.4. Collection, Storage, and Use of Human Biospecimens for Unspecified Future Research
Will biospecimens be collected and stored for unspecified future research?
☒Yes ☐ No
If **Yes**, respond to all of the following:
1. *Describe the role of biospecimen collection and storage in this study (check one):*
    ☐ *Biospecimen collection and storage for unspecified future research is the **primary purpose** of the study*
    ☒ *Biospecimen collection and storage for unspecified future research is an **optional part** of the main study*

2. *Describe the following:*
    a. *Where biospecimens will be stored:* Biospecimens will be stored in a -80°C locked freezer in the PI's laboratory (at the HNRCA) and Co-I's laboratory, Dr. Giovanni Widmer (at Tufts School of Veterinary Medicine).

    b. *How long they will be stored:* Subjects participating in the optional tissue banking may have their samples stored indefinitely and used for other research if approved by the IRB.

c. *How biospecimens will be accessed for future research:* Banked biospecimens may be distributed to investigator(s) located at other academic institutions for additional genetic and biomarker research studies. The distribution of biological materials to other investigator(s) will be subject to oversight by Tufts University/Medical Center IRB and require a material transfer agreement.

d. *Who will have access to the biospecimens for future research:* Samples transferred to external investigators will be de-identified and the code will not be available to the external investigators.

3. *Specify what types of biospecimens (blood, urine, stool, tumor, embryonic germ, or stem cells, etc.) will be stored and how they will be collected (blood draw, bone marrow aspiration, needle biopsy, etc.):* GI luminal content from the mini-pills and stool samples. Plasma, serum, red blood cells and buffy coat will be obtained from the blood draw

4. *The biospecimens and associated PHI are being obtained (check all that apply):*
   a. ☒ *Solely for research (would not be obtained as part of standard clinical care)*
   b. ☐ *As part of standard clinical care (leftover/discarded)*
   c. ☐ *In addition to that which would be obtained for standard clinical care. Specify how much more will be collected and stored for unspecified future research:*
   d. ☒ ***(When biospecimen collection, storage, and use for future research is an optional component of the main research study)*** *In addition to the amount of biospecimen to be obtained for the main research study, specify how much of this additional amount is for unspecified future research:(do this for each type of biospecimen to be collected and stored for future research):* For subjects that consent to tissue banking, remaining plasma, serum, red blood cells, buffy coat (white cells) and stool (fresh and freeze-dried) will be archived and stored in a -80°C freezer for unspecified future use. No additional blood will be drawn or stool collected specifically for tissue banking.

5. *Check/complete one of the following for how biospecimens are being released:*
   a. ☒ *The biospecimens and associated PHI will be de-identified (stripped of all identifiers) and coded; specify where the key to the code will be kept, how it will be secured, and under what circumstances the key to the code could be revealed:* Key to code will be stored in a locked cabinet and password protected computer. The key code will only be revealed if a subject asks to have their samples destroyed.

   b. ☐ *The biospecimens and associated PHI will be de-identified (stripped of all HIPAA identifiers) and* ***not coded****.*

   c. ☐ *The biospecimens and associated PHI will be labeled with, stored with, or associated with identifiers (for example, date of birth, date of biospecimen, zip code, etc.). Specify the identifiers and the rationale for collecting these. If identifiers will be gathered in the future and associated with the specimen, describe the frequency of gathering such identifiers.* ***Note:*** *If including dates or other HIPAA identifiers with the biospecimens is necessary, a limited data set may be required:*
   NOTE: For all applicable NIH funded research, all data and biospecimens are to be collected and stored according to the NIH Genomic Data Sharing Policy.

6. *Describe procedures to release biospecimens and associated PHI, including: the process to request the use of biospecimens for future research, approvals required for use, who can obtain*

the biospecimens: Specimens will only be released to investigators associated with this study. For collaborations with investigators outside Tufts, we will obtain a Materials Transfer Agreement.

7. *How will the biospecimens and associated PHI be labeled:*
   a. ☐ *Readily identified with the source individual's identifiers*
   b. ☐ *Limited data set (stripped of all HIPAA identifiers except for dates, age, city, state, and/or zip code)*
   c. ☒ *De-identified and coded: specify under what circumstances the key to the code will or could be revealed:* Per mandatory request as dictated by federal guidelines.
   d. ☐ *De-identified and not coded*
8. *Specify under what circumstances a recipient of the stored biospecimens and associated PHI would seek to contact the source individual (subject):* or ☒ N/A

9. *Describe the mechanism by which the research subject can withdraw permission to use the stored biospecimens and associated PHI for future research. Indicate what will happen to the biospecimens and related research data if permission is withdrawn (must be described in the ICF):* At any time after optional tissue banking permission has been granted, if the study participant wishes to withdraw consent to the research use of biological materials for future studies, he/she must inform the Principal Investigator in writing. The code will then be broken, and the specific samples will be retrieved and destroyed.

10. ☒ *Check to confirm that a copy of the biorepository's written policies are uploaded to eIRB, ensuring that items a through d below are addressed. If policies are not available, please complete the following:*
    a. *The types of investigators or entities to whom the biospecimens may be distributed:*
    b. *The types of research for which the biospecimens may be distributed:*
    c. *The measures taken to guard against disclosure of the source individual's private and protected health information:*
    d. *Who will oversee the distribution of biospecimens from the biorepository to other investigators or entities:*

11. *Describe potential risks to subjects and their families associated with the use of the subject's biospecimens or associated PHI for future research:* No risks are anticipated related to the subjects, or their families associated with tissue banking.

12. *Describe risks to groups or populations associated with the use of the subject's biospecimens or associated PHI for future research use (considering the subject population and the potential future research):* No risks are anticipated to the groups or populations associated with tissue banking.

F. Ethics and Protection of Human Subjects

F.1. Informed Consent Process

Will subjects be required to provide informed consent?

☒ Yes ☐ No

If **Yes**, respond to all of the following:

1. *Where the consent process will take place (e.g. a private clinic room):* The consent process will take place in a private space at the HNRCA MRU.

2. *Anticipated amount of time a potential subject will have to make a decision about participation in the study:* Potential subjects will have at least 90 minutes to make a decision about participation in the study.

3. *Processes to ensure ongoing consent throughout the study:* We will check in with study participants at the beginning of diet phase II to confirm that they still want to continue to participate in the study. We will also remind participants that participation is voluntary and they do not need to participate if they so choose.

4. *Role of each research team member involved in the informed consent process:* The study coordinator or MRU staff member will introduce the study to the potential subject and document the consent form process. The PI (Dr. Lichtenstein) and Co-I (Dr. Matthan) will be available to address any questions or concerns that the participant might have. The MRU staff and study coordinator performing the consenting process are fully trained and experienced with the consenting process.

5. *Check to confirm you will follow "SOP: Informed Consent Process for Research (HRP-090)".*

6. ☒ *Check to confirm the approved,* ***stamped*** *consent document will be used.*

7. *If the consent form includes a section for the participant to record their choice about an optional part of the research study, describe the plan for reviewing the consent immediately after it has been signed to make sure the participant's choice has been recorded. Please also describe the plan for honoring and following through with their choice:* ☒ **N/A**.

8. ☒ *Check to confirm that Non-English speakers will be enrolled using interpreters and IRB approved Short Forms per the IRB's Short Form policy. If IRB approved Short Forms will not be used, describe which languages the consent will be fully translated into, who will conduct the consent interview, use of interpreters, use of IRB approved translated documents, etc.:* Non-English Speakers will be included in this study if they are accompanied by a family member or personal friend (>18 years of age) who can translate the study ICFs and convey study instructions as well as help complete the dietary recalls and be present at all in person and phone study visits. For these subjects, the appropriate IRB approved short form will be used.

9. *If non-English speakers are not eligible (excluded from enrollment) for this study, provide the ethical and scientific justification, including whether this would be equitable. For example, if non-English speakers are eligible for the study and could potentially benefit from participation, it would not be equitable to exclude them:* ☒ **N/A**

10. ☒*Check to confirm you will follow "SOP: Written Documentation of Consent (HRP-091)". If* ***not****, describe how consent will be documented in writing:*

11. ☒*Check to confirm you will follow "SOP: Remote Consent Process (HRP-092)" if there is ever a situation where consent will* ***not*** *be obtained in person. If you will follow a different process if there is ever a situation where consent will* ***not*** *be obtained in person.* ☒ N/A.

F.2. Waiver or Alteration of Consent Process

1. Is a waiver or alteration of the consent process being requested for this study?
   ☐ Yes ☒ **No**

2. Is a waiver of written documentation of consent being requested?
☐ Yes ☒ **No**

3. Is a waiver of the consent process being requested for parents for research involving children?
☐ Yes ☒ **No**

4. Is a waiver of the consent process for planned emergency research being requested?
☐ Yes ☒ **No**

F.3. Confidentiality

1. *State where the study records, **both electronic and/or paper documents** including signed ICFs/assent forms, will be retained during the study (state the location for original document plus any copies that are made, e.g., if a copy of the ICF will be retained in the subject's medical record):* All data collected will be stored directly in REDCap. Informed consents will be captured electronically. Any paper informed consents and original source documents will be kept in a separate locked filing cabinet in the HNRCA MRU. All data collected will be stored in REDCap. The informed consents and original source documents will be kept in a separate locked filing cabinet in the HNRCA MRU.

2. *Will you be **coding** the study data (replacing identifiers such as name and MRN with a unique subject ID and keeping a **key** to link subject IDs with those identifiers)?*
☒ Yes ☐ No

   If **Yes**, respond to the following:
   Describe the coding mechanism in detail, including how the unique IDs (used to code the data) will be generated / assigned (sequential, random, other). If not sequential, explain how you will avoid duplicates: **Note:** The IDs used to code the data should not include initials, dates, or any identifiers.

   a. *State which identifiers (if any) will be retained in the coded dataset to be used for analyses:*
   ☒ **N/A**, all HIPAA identifiers will be removed from the coded dataset

   b. *Explain how you will keep the coded data and the key separate:* Any paper informed consents and original source documents will be kept in a separate locked filing cabinet in the HNRCA MRU. All data collected will be stored in REDCap. Subjects will be identified on documents where study-related data will be collected, by a participant number only. The PI will maintain a confidential list of the subjects involved in the study, separate from the consent forms, that will serve as a means of linking the subject ID number to the study records. All data used in analysis and reports will be used without identifiable reference to the subject. Only the PIs will have access to the key code for the consent forms and subject ID numbers. Only the research team will have access to the study files and data. The coded data and the key will be kept in separate location with different passwords.

   c. *Describe the plan to destroy subject identifiers (or links/keys) at the earliest possible time and specify when this will occur. If there is a justification for retaining the identifiers, provide the information here:*
   Note: Study data should be retained for 7 years in accordance with *"*SOP – Records Retention Timeframe – Investigators". Only the identifiers or links should be destroyed at the earliest possible time.

3. *State where study records will be retained when the study has been closed (long-term storage):* All study records will be kept for a minimum of 7 years after the study has ended. Data generated by the study will be maintained on the HNRCA's shared network storage. To facilitate loss prevention all network storage directories are continuously mirrored on an off-site disaster recovery server. The network storage directories are also backed up locally twice daily and backed up to off-site tape storage weekly. Upon study completion, all data and supporting documentation will be organized and compressed into a zip file, which will then be maintained on the laboratory's shared network storage at the HNRCA. This archival file will be periodically checked to ensure data integrity during archival period. In the event corruption is identified via the checksum it will be reverted to the most recent non-corrupt version. This combination of routine backups and data integrity checks will ensure the data is viable and accessible for the duration of the ten-year archival period mandated by the HNRCA data archival policy.

4. *State who, in addition to the research team, will have access to the study files, data, and/or specimens, or key to the study code:* No one.

5. ☒ *If you are utilizing REDCap to enter your identifiable data, check here to confirm you will consult with the Tufts CTSI at https://informatics.tuftsctsi.org/pims/request.htm#/ on proper REDCap confidentiality procedures*.

6. *In this study, will research data be entered or stored using a computer software application (on a computer or any other electronic device)?*
   ☒ **Yes** ☐ No

   If **Yes**, respond to the following:

   b. For **Tufts Medical Center** studies:
   Check to see if all software applications used in this study are already on the list of approved software. Please review the version type and/or described use-case of each application to confirm approved use. If the software you are using is not already on the list of approved software (or will not be used in accordance with the approval details on the list), you are required to complete this IT Security form so Tufts Medical Center can review the security of the software.
   ☐ *Check this box if the IT Security form has been completed and submitted.*
   ☐ *Check this box if you have confirmed that the software applications used in this study are already on the list of approved software (and will be used in accordance with the details on the list)*

   c. **For Tufts University studies:**
   Check to see if all software applications used in this study are already on the list of approved software. If the software you are using is not already on the list of approved software (or will not be used in accordance with the approval details on the list), Information Security review is needed.
   ☐ *Check this box if Information Security review is needed.*
   ☒ *Check this box if you have confirmed that the software applications used in this study are already on the list of approved software (and will be used in accordance with the details on the list)*

7. *Will data (or specimens) be sent outside of Tufts Medicine or Tufts University and/or sent between Tufts Medicine and Tufts University?* Samples will be sent to Tufts University School of Veterinary Medicine

   ☐Yes ☒ No

   If **Yes**, respond to all of the following:

   a. *Describe the nature of the data/specimens to be transferred:* GI luminal content and stool samples
   b. *State to whom or what entity the data/specimens will be transferred:* Dr. Giovani Widmer at Tufts Veterinary School.
   c. *Explain how data and/or specimens will be transported (e.g. fax, mail, delivery, email, etc.):* Dr. Lichtenstein or her designee will courier the stool samples and recovered mini-pills to Dr. Widmer's laboratory at the Tufts School of veterinary Medicine, Grafton Campus.
   d. *Confirm that you have consulted with Tufts MC Grants & Contracts (TuftsMCGrantsContracts@tuftsmedicine.org), Tufts University Technology Transfer, or LGH/MWH Legal Counsel to determine whether an agreement is needed to permit the transfer of data/specimens, and one of the following is true:*
      i. ☐ *An agreement (e.g. contract, Clinical Trial Agreement, Collaboration Agreement, Data Use Agreement (DUA), or Material Transfer Agreement (MTA)) has been or will be established that will cover any transfer of data/specimens, and the agreement will be executed **prior** to any transfer.*
      ii. ☐ *Grants & Contracts, Technology Transfer, or Legal Counsel has determined that an agreement is not needed for this research. (The appropriate office listed above should make this determination, not the Investigator.)*
      iii. ☒ *A determination from the appropriate office is still pending. (The study cannot be approved by the IRB until the appropriate office makes a determination, but it can be approved prior to the execution of an agreement.)*

8. *Will you use the Tufts Medicine electronic medical record system EPIC for this study?*
   ☒ No

9. *Explain whether audio/videotapes and/or photographs of subjects could potentially identify the study subject. If so, indicate who will have access to (be able to view) these items, in addition to the research team, how long the videotapes or photographs will be retained for the study, and what the plan is for their destruction:* ☒ **N/A**

10. ☒*Check to confirm that study records will be retained for the timeframe described in the record retention policy of the "SOP – Records Retention Timeframe – Investigators".*

11. ☒*Check to confirm that you will follow the "Confidentiality and Data Security Guidelines for Electronic Research Data" for collecting, storing, transferring, and securing electronic data. If not, describe how your plan differs from these guidelines and what steps that will be taken to secure the data (e.g., training, authorization of access, password protection, encryption, physical controls, and separation of identifiers and data) during storage, use, and transmission:*

12. *A Certificate of Confidentiality will be issued (for NIH studies) or obtained:* ☒ **N/A**

F.4. Provisions to Protect the Privacy Interests of Subjects

1. *Describe the steps that will be taken to protect subjects' privacy interests (e.g. ensuring that discussion of the study will take place in a private area where subjects cannot be overheard):*

The consent interview will take place in a private room in the HNRCA MRU. Family members will accompany Non-English-speaking subjects.

2. *Describe the steps that will be taken to make the subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might or might not experience in response to questions, examinations, and procedures (e.g. ensuring that subjects are comfortable with the research team members performing the study procedures):* Subjects will have at least 90 minutes to review the study documents and consider whether they wish to participate. It will be emphasized that study participation is entirely optional. Additionally, subjects' comfort during questions and research procedures will be ensured by clarifying each step of the process and easing any potential concerns they may have with regards to privacy and the research study process. The HNRCA MRU staff has extensive experience in this area and understanding the importance of subject comfort in terms of psychological well-being, study compliance and study retention.

F.5. Data Management

1. *Describe the data that will be collected during the study and how the data will be obtained including what source records will be used (submit all surveys, scripts, and data collection forms.):* The data generated as part of this proposal will consist of primary data spanning several types of digital formats dependent on the method of data generation. Anthropometric and blood pressure measures will be recorded in a clinical research management system and provided as de-identified and coded spreadsheets for analyses. Gut microbiome sequences will be stored in fastq format on the Tufts University research computer cluster. GI and serum metabolite measures, cardiometabolic risk factors and all other measurements will be stored as numeric spreadsheets. Due to the varied nature of the data associated with this proposal, the use of several open file types will be necessary to ensure the data is both machine readable, and readily accessible and usable for the greater scientific research community. To that end, all data files will be stored in open formats such as TIFF for the image files, and comma-separated values (csv) files for spreadsheet data.

   Additionally, plain text readme and metadata files will be developed and included with the study data to provide supporting documentation and descriptions of data collection methods. These will include abbreviations, coded values and other conventions used when recording the data. The supporting documents will be configured in a way that provide all the information necessary for independent investigators to use, validate and understand the data associated with this proposal.

   a. *If there are plans for long-term follow-up (once all research related procedures are complete), describe the data will be collected during this period:* ☒ **N/A**

2. *Describe the data management plan including the data types (e.g. biomarker, imaging, clinical events, etc.) and amount (n participants) of data, how data are collected (e.g. REDCap) and processed (e.g. analysis of imaging), any specialized data tools and software and code, and who will be responsible to monitor compliance with this plan:* All research data will be stored on either the secured, internal HNRCA network storage assigned to the research group or REDCap. Access to data is restricted based on study and team role and controlled through robust user- and role-based access rights. All access to identifying information is recorded and logged. Two-factor authentication is enabled and required in order to access research data and individual staff undergo training in data security best-practices.

3. *Describe any procedures that will be used for quality control of collected data:* Review of data by PI.

F.6. Provisions to Monitor the Study to Ensure the Safety of Subjects

1. *Describe the plan to periodically evaluate the data regarding both harms and benefits to assess subject safety as follows:*
   a. *The data that will be reviewed, including safety data, untoward events, and efficacy data:* The study subject will be monitored for discomfort during the study visit and will be accompanied at all times by a research team member. There are no medically anticipated immediate adverse effects associated with the assessments (height, weight, waist circumference, blood pressure) taken at the study visit, with consumption of the food items, or oral ingestion of the mini-pills.

   Who will review the data: The study doctor will evaluate any GI or other issues reported by participants. An independent physician, Joel B. Mason, M.D. Professor of Medicine and Nutrition, Tufts Medical Center/Tufts University will act as Safety Monitor and will review AEs and SAEs, if they occur and sign off on annual safety reports to the NIH.

   b. *How the safety information will be obtained and documented (e.g., case report forms, by telephone calls with participants, printouts of laboratory results, etc.):* Any instances of GI discomfort/side effects self-reported by participants to MRU staff via phone or at an in-person visit will be documented in REDCap.

   c. *The frequency of data collection, including when safety data collection starts:* If nursing staff determine a report is an AE or has potential of being an AE, the study doctor will review and the PI or designee will be sent a summary of the complaint/issue by email on the day the report has been made to the IRB and Safety Monitor.

   d. *The frequency or periodicity of review of cumulative data**:*** Yearly.

   e. *The statistical tests for analyzing the safety data to determine whether harm is occurring:* N/A

   f. *Any conditions that trigger an immediate suspension of the research or other action for the research:* N/A

   g. *Describe the entity responsible for monitoring the data, and their respective roles (e.g., the investigators, the research sponsor, a coordinating or statistical center, an independent medical monitor, a Data and Safety Monitoring Board (DSMB) /Data Monitoring Committee (DMC), and/or some other entity, and the timeframe for reporting events to this entity:* The study doctor will monitor any GI or other issues reported by participants. The Safety Monitor will review AEs and SAEs and sign off on annual safety reports to the NIH.

2. *A copy of the DSMB/DMC Charter if the study is enclosed with the submission:* **N/A**

G. Adverse Event Monitoring

G.1. Definitions

Define adverse events (AEs), serious adverse events (SAEs), and unanticipated problems (UPs) for your study: ☒ **N/A**, no physical risks are anticipated with this research study, and we will use the definitions in the Tufts Health Sciences IRB's **Reportable New Information policy**.

G.2. Reporting Procedures

1. *Describe the protocol-specific reporting procedures, including who will be responsible for each step (e.g., PI, Data Coordinating Center, Medical Monitor), which forms should be completed, timeframes for reporting, how reports will be distributed, and what follow-up is required:* We will comply with FDA requirements for records and reports (21 CFR §812.140, 21 CFR §812.150).

2. *Include specific details of reporting procedures for:*
   a. *Deaths, life-threatening events, pregnancies:* Deaths and life-threatening events that meet specified categories in RNI policy will be reported using eIRB (Report New Information button) within 5 business days.

   b. *Other SAEs:* SAE's that meet specified categories in RNI policy will be reported using eIRB (Report New Information button) within 5 business days.

   c. *Other AEs:* AE's that meet specified categories in RNI policy will be reported using eIRB (Report New Information button) within 5 business days.

   d. *Other UPs:* UP's that meet specified categories in RNI policy will be reported using eIRB (Report New Information button) within 5 business days.

G.3. Reportable New Information

☒ *Check to confirm that reportable new information will be reported to the IRB per the Tufts Health Sciences IRB's Reportable New Information policy.*

H. Statistical Considerations

H.1. Study Endpoints

1. *Describe the primary and secondary study endpoints:* GI luminal content and stool microbial composition.
2. *Describe any primary or secondary safety endpoints:* Recovery of the 12 mini-pills.

H.2. Sample Size

1. *Specify the number of subjects to be enrolled in total across all sites:* 60
2. *Specify the maximum number of subjects to be enrolled at the Tufts site(s)[2]. Subjects who sign an ICF are considered "enrolled". For studies that have a separate screening ICF, this number is the number of subjects who sign a screening ICF:* 60
   a. *Specify the number of subjects expected to be enrolled at the Tufts site (i.e. sign the screening or study ICF):* 60
   b. *Estimate the minimum number of subjects needed to **complete** the study at the Tufts site(s):* 30
   c. *Provide the rationale for enrolling this number of subjects at the Tufts site:* Based on our sample size calculation, we need 30 subjects (aiming for 15 postmenopausal women, 15 older men) to complete both diet phases, and have analyzable data. To account for potential drop-outs, and based on our prior experience with conducting dietary interventions, enrolling 60 participants should allow us to meet our target.
   d. *If a large number of withdrawals and/or dropouts is expected, explain why:* We have estimated the number of drop-outs expected based upon prior dietary intervention studies

[2] If Tufts IRB is requested to be the Single IRB of Record for external sites (as per section K.3 Multi-Site Research), include all sites in these enrollment numbers.

performed at the HNRCA. We have considered the time commitment for the various study components as well as the accounted for participants who inevitably have unavoidable unrelated issues that may cause them to stop participation (e.g., moving to another location, schedule change and altered availability for participating).

3. *Provide a sample size justification:* Based on microbiota β diversity values obtained from samples collected from hounds, the UniFrac distance between small intestine and stool averages 0.4 distance units with a SD of 0.05. Using these estimates, and assuming similar values apply to humans, a power ≈1 will be achieved with 10 clinical trial participants/group assuming $\alpha = 0.05$. Data dispersion in human participants may well exceed what we observe in hounds. The power calculation based on double the SD returns power of 0.93 for the same n of 10. Since the clinical trial uses a crossover design, UniFrac distances will be obtained from intra-individual comparisons, likely reducing 16S data variability compared to inter-individual distances. In relation to diet, power calculations are tentative because data on the effect of diet on human GI microbiota other than microbiota in stool are rare. Hence, a sample size of 30 (15 male, 15 female) is assumed to be adequate.

H.3. Statistical Analyses

1. *Describe the statistical analyses that will be performed for this study:* Microbiota taxonomic profiles across GI locations will be characterized using standard diversity measures as well as relative abundances of specific taxa.[9] Alpha diversity and beta diversity measures will be compared across regions using repeated measures ANOVA to account for multiple samples from the same individual. Effects of a plant-based and meat diet on GI tract microbiota will be compared, by region, using zero-inflated Gaussian mixed models, implemented in the R package "glmmTMB".[9] These models can include fixed and random effects to account for dependencies from the crossover design, and evaluate sequence and period effects on the microbiota taxonomic profiles. To compare the effect of the of the plant-based and meat diets on cardiometabolic risk factors, absolute values at the end of each diet phase and change from baseline to 2 weeks after randomization will be compared using a two sample t-test or Wilcoxon Rank Sum test.

I. Subject Populations & Vulnerable Populations

If the research involves individuals who are vulnerable to coercion or undue influence, describe the rationale for their inclusion and the additional safeguards included to protect their rights and welfare. Each group you plan to include should also be listed in section **C.1 Inclusion Criteria**

1. Can or will pregnant women be enrolled?
   ☐ Yes ☒ **No**

2. Can or will the research involve neonates of uncertain viability or non-viable neonates?
   ☐ Yes ☒ **No**

3. Can or will subjects who are not yet adults (neonates, children, teenagers) be enrolled?
   ☐ Yes ☒ **No**

4. Can or will minors who are:
   i) married, widowed, divorced; or
   ii) the parent of a child; or
   iii) a member of any of the armed forces; or
   iv) pregnant or believes herself to be pregnant; or
   v) living separate and apart from his/her parent or legal guardian, and is managing his/her own financial affairs

   be approached for study participation for either themselves or their child?

☐ Yes ☒ **No**

5. Can or will wards of the state and/or children at risk of becoming wards of the state be enrolled (this includes foster children or any child that is in state custody)?
   ☐ Yes ☒ **No**

6. Can or will cognitively impaired adults (adults with impaired-decision making capacity) or adults who may lose the capacity to consent be enrolled?
   ☐ Yes ☒ **No**

7. Can or will prisoners be enrolled?
   ☐ Yes ☒ **No**

8. Can or will students and/or employees be **targeted** for enrollment in this research?
   ☐ Yes ☒ **No**

9. Transgender Subjects: Are you recording sex or gender for your study?
   ☒ **Yes** ☐ No
   a. Is there a scientific and/or safety rationale for collecting information on whether a subject is transgender? ☐ Yes ☐ No
      If **Yes**, respond to all of the following:
      i. *Provide the scientific/safety rationale for collecting information on whether a subject is transgender*: Sex as a biological variable is a pre-specified exploratory objective of the funded study.

      ii. Are transgender individuals eligible for participation in this study? ☒ **Yes** ☐ No
      Transgender individuals may participate only if they are not taking hormonal therapy. Sex hormones are known to affect the study outcomes and use of hormonal therapy will confound the study results

      iii. ☒ *Check to confirm that relevant questions for transgender and gender nonconforming individuals have been incorporated into relevant study documents (i.e. protocol eligibility, screening forms, demographic questionnaires, surveys), per the website guidance.*

J. Drugs or Devices

1. Will the research involve drugs?
   ☐ Yes ☒ No

2. Will the research involve devices?
   ☒ Yes ☐ No
   If **Yes**, respond to all of the following:
   a. *If the device has an IDE or a claim of abbreviated IDE (non-significant risk device) identify the holder of the IDE/Abbreviated IDE:* We are requesting No Significant Risk (NSR) designation and abbreviated IDE determination from the IRB as part of this submission. The study will comply with FDA requirements for records and reports. (21 CFR §812.140, 21 CFR §812.150) and will not market or promote the device. (21 CFR §812.7).
   b. *Describe your plans to store, handle, and administer study devices so that they will be used only on subjects and be used only by authorized:* The devices will be ingested by the

participant under supervision by a MRU nurse. Each mini-pill will be scribed with a number using a laser to enable identification after recovery. Each participant's pills will be provided in a sterilized pouch labeled in accordance with FDA regulations. (21 CFR §812.5)

c. *Specify who will be responsible for the costs of implantation or placement of the device in subjects' bodies?* *or* ☒**N/A**, This device is ingested and is recovered from stool. The device will not be implanted or placed in the body**.**

d. *Specify who will be responsible for the costs of removing the device from subjects' bodies?* *or* ☒**N/A,** The device is recovered from stool samples.

e. ***Specify the cost*** *of the device and who will be responsible for the cost*: *or* ☒**N/A,** The device will be provided free of charge.

f. *Who on the research team, in addition to the Principal Investigator, will be accountable for device(s):* Dr. Sameer Sonkusale

g. *Who will interface with the sponsor:* Dr. Sameer Sonkusale

h. *The study device or procedure (including beneficial health care procedures) will be available to subjects after participation in the study:* ☐Yes ☒ **N/A**

i. *Handouts or instructions sheets that will be given to subjects on how to use study device(s) have been submitted to the IRB:* ☐Yes ☒ **N/A**

K. Study Administration

K.1. Setting

1. *Describe the sites / locations where your research team will conduct the research,* ***including recruitment activities****:*
   a. *Tufts MC / Tufts University locations (specify which facility/clinic if applicable****):*** Tufts University, Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA)

2. *The research will take place at an international site (including virtual participation such as an online survey or registry open to participants outside the U.S.), and the International Research Guidance and International Checklist were utilized:* ☐ Yes ☒ **N/A**
   a. *Include all relevant information described in those documents in this protocol:*

K.2. Resources Available

1. *Describe the roles/tasks of each research team member here (or alternatively, you may submit any current Delegation of Authority Log you may have which already has this information completed):*

   Oversees research activities: Dr. Alice Lichtenstein, Dr. Nirupa Matthan and Ms. Jean Galluccio (study-coordinator)

   Reviews subject eligibility, signs subjects into study, Study MD, Dr. Edward Saltzman

   Performs prescreening, eligibility determination: and arranges study visits: HNRCA MRU staff

   Performs assessments (height, weight, waist circumference, blood pressure diet history) and supervises ingestion of mini-pills: HNRCA MRU nursing and dietary staff

   Processes blood samples and makes aliquots: HNRCA Nutrition Evaluation laboratory (NEL) staff

   Performs cardiovascular disease biomarker analysis: HNRCA NEL staff

   Performs GI luminal content and stool microbiome analysis: Dr. Giovani Widmer

   Oversees/Performs metabolome analysis: Dr. Matthan

2. *Describe the qualifications (e.g., training, experience) of the PI and research team to perform their roles. Provide enough information for the IRB to determine the PI and research team are qualified to conduct the proposed research:* *or* ☒ current CVs for the research team members have been submitted.

3. *Describe the coverage plan to address any issues (including subject safety issues) that occur while the PI is away and/or unavailable. The research team member designated to serve as the acting PI in the PI's absence should have similar training and expertise as the PI:* Dr. Matthan, a Co-I is the designated team member to serve as acting PI. She is experienced and has similar training.

4. *Describe the process to ensure the research team members have adequate oversight and are adequately trained regarding the protocol, study procedures, and their roles and responsibilities:* PI will meet with the research team weekly to review enrollment, visits, data and any study related concerns**.**

5. *Medical or psychological resources that subjects might need, such as for emergencies or medical issues, are available for the study:* ☐Yes ☒ **N/A**

K.3. Multi-Site Research

Is this a multi-site or collaborative study where **one or more** of the following is true?

☐ Tufts IRB is requested to be the Single IRB of record for non-Tufts sites or collaborators,
☐ Tufts is the sponsor,
☒ Tufts is the primary grant recipient,
☐ Tufts is the coordinating center and/or data coordinating center

K.4. Community-Based Participatory Research

Can or will this study involve community-based participatory research?

☐ Yes ☒ **No**

K.5. Sharing Results with Subjects

Will results (overall study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings, ***including those collected and stored for future, unspecified research***) be shared with subjects or others (e.g., the subject's primary care physician or the subject's treating physician)?

☐ Yes ☒ **No**

L. Nursing Involvement

Will your study require the involvement of Nursing?

☒ **Yes** ☐ No

If **Yes**, respond to all of the following*:*

1. *Describe the research procedures Nursing will do for this study above and beyond their usual nursing practice:* Nursing will be performing blood draws and taking physical measures during the study (height, weight, waist circumference and blood pressure). They will also supervise the ingestion of the mini-pills by the study participants.

2. *Describe what measures have been taken to notify nursing, plan for nurse education, and mitigate the increase in nursing responsibilities:* Nursing is aware of their responsibilities for this study through their participation in protocol meetings which include all study staff and a representative of all departments involved with the study (including the nursing supervisor). At

the protocol meetings, services provided by nursing have been reviewed and the nursing supervisor has had all questions answered regarding nursing duties. Study visits will be scheduled according to nursing staff availability. No extra education is required of nursing staff in order to perform blood draws, administer pills, and take physical measures. The MRU at the HNRCA oversees nursing and balances study visits with nurse staffing to ensure that the appropriate staff are available to provide the nursing services needed for this study.

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