Randomized, Controlled Trial of Resistant Starch in Stage I-III Colorectal Cancer Survivors Pilot Study: The Fiber for Health Cancer Study

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Study Protocol and Statistical Analysis Plan*

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*Please see Section 8 of the protocol for Data/Statistical Analysis Plan

Randomized, Controlled Trial of Resistant Starch in Stage I-III Colorectal Cancer Survivors Pilot Study: The Fiber for Health After Cancer Study

Standard Operating Protocol

Protocol Number: 10079 / RG1003387

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1. RESEARCH STRATEGY

Colorectal cancer (CRC) is the third most common type of cancer in the U.S.,[1] and diet is a recognized risk factor. Overall, CRC incidence and mortality rates have decreased in the past 20 years, attributed largely to use of CRC screening and polypectomy in adults over 50 years and improved therapy options. However, among adults younger than 50 years, for whom screening is not recommended if at average risk, CRC incidence rates have been increasing by ~2% per year since 1994 in both men and women.[1] While genetic factors account for some of the risk, environmental factors account for the majority of CRC risk.[2] Observational studies show inverse associations between dietary fiber and CRC risk,[3-6] with significant risk reduction found primarily in groups with fiber intakes >25 g/d.[7]

CRC risk, in addition to hyperplastic and precancerous adenomatous polyps,[8] could be greatly reduced through dietary modification, including increased dietary fiber intake and reduced intake of certain types of fat.[9] In the 2011 Colorectal Cancer Report, part of the Continuous Update Project, the World Cancer Research Fund/American Institute for Cancer Research Expert Panel classified evidence supporting consumption of fiber-containing foods and CRC protection as 'convincing'.[10] The Panel noted that for every 10 g/d increase in fiber, there was a 10% decrease in CRC risk. This recommendation was based on recent systematic reviews and meta-analyses of prospective cohort studies, which together showed significant CRC risk reduction with greater fiber intakes.[11, 12] Inconsistencies across epidemiologic studies are attributed in part to lower overall fiber intakes and narrow ranges of fiber intakes in Western populations.[13] For example, among ~135,000 women in the Women's Health Initiative (WHI) cohort, <10% of participants reported fiber intakes >25 g/d, with the majority of intakes falling between the narrow range of 10-20 g/d.[14] In fact, <5% of all Americans meet the recommended intake for dietary fiber, with mean intake ~15 g/d.[8, 15] As Bingham and others have pointed out, reductions in CRC are only apparent with fiber intakes >25 g/d.[7] Intakes at or above current recommendations (28 g/d for women and 35 g/d for men) show robust protection against CRC with relative risks ranging from 0.72 to 0.90.[8, 11, 12, 14] Animal models have also corroborated that dietary fiber reduces colon tumorigenesis.[16-18]

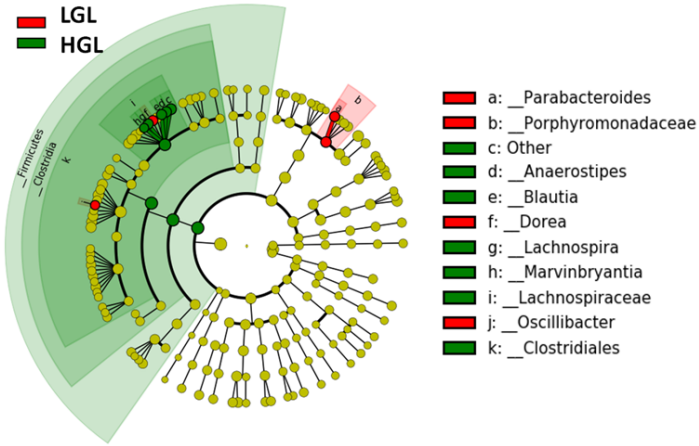
Several mechanisms have been hypothesized to explain how dietary fiber may reduce CRC. These include fiber fermentation to short-chain fatty acids (SCFA), particularly butyrate, by gut microbiota, reduced microbial production of secondary bile acids, faster intestinal transit time, and increased stool bulk.[19-21] Given the potential importance of fiber fermentation, consideration of fiber subtypes (i.e., soluble and insoluble or more and less fermentable) and their different microbial fermentation capacity to generate butyrate may be important. Few studies in humans have looked at fiber subtype and reports indicate both null [14, 22, 23] and inverse associations.[24-27]

Diet may affect CRC risk by contributing particular substrates (e.g., dietary fiber) that allow for production of chemoprotective microbial metabolites and modulating specific species in the gut microbiota.[28] For example, a key factor in determining the availability of butyrate and setting apoptotic and other regulatory events in motion is gut microbial fiber fermentation. Types of complex carbohydrates consumed (e.g., dietary fibers, resistant starch) influence prevalence of certain consortia of gut bacteria and subsequent metabolites to which the host is exposed.[29-42] The microbiome responds rapidly to dietary interventions,[43] although individual responses may differ.[44-47] Bacteria ferment fiber to SCFA, predominantly acetate, butyrate, and propionate, in a ratio of 3:1:1[48] via metabolic pathways unique to anaerobic gut bacteria.[49-52] Butyrate producers form a functional cohort, rather than a monophyletic group, distributed across four different phyla: *Firmicutes, Fusobacteria, Spirochaetaceae*, and *Bacteroidetes.*[53] Butyrate is produced via multiple pathways: the acetyl-CoA,[54, 55] glutarate,[56-58] 4-aminobutyrate,[59-61] and lysine pathways,[62-64] but distribution of these pathways in the human microbiome varies.[53] The dominant pathway associated with butyrate production is the acetyl-CoA pathway. The last step in conversion to butyrate across these multiple pathways is carried out by butyryl-CoA transferase (*but*) and butyrate kinase (*buk*).[53, 65-67] Vital et al[68] suggested that targeting the 21 human microbiome genes involved in butyrate production across these four pathways gives a comprehensive picture of microbial butyrate production or "butyrogenic potential." Propionate is also a major fiber fermentation product that can induce

differentiation and apoptosis.[69-71] Bacteria across several phyla produce propionate through the succinate and acrylate pathways.[72-75]

Preliminary Data and Experience in Support of Proposed Microbiome Analysis. Gut microbiome studies directed by Dr. Hullar have focused on measurement of GMC using sequence-based high-throughput methods, metagenomic approaches. We have optimized methods for fecal bacterial DNA preservation and extraction, and GMC analysis[76, 77] and applied these to studies of diet and the gut microbiome.[76, 78, 79] ***GMC is altered by dietary fiber.*** Directly relevant to our proposed study, we compared GMC in response to 28 d of controlled refined grain and whole grain diets (25 & 48 g fiber/d, respectively).[80] We analyzed stool samples collected at baseline and the end of 2 diet periods, characterizing GMC by sequencing the V1-V3 region of the16S rRNA gene. In total, we analyzed ~2 million sequences. After trimming, we identified bacteria using 700,000 high quality sequences for analysis.[81] Linear discriminant analysis (LDA) showed GMC differences between baseline and the 2 diets (**Figure 1**) and GMC was different between the 2 diets (perMANOVA, F=1.3, p<0.03, n=25) Further analysis showed an enrichment in Parabacteroides on the whole grain diet (**Figure 1**) whereas the refined grain diet enriched for Clostridiales.

Figure 1. Linear discriminant analysis of the microbiome at the end of the whole grain (WG, Green) and refined grain (RG, Red) dietary interventions.



Microbial butyrate-production genes are associated with carbohydrate intake. 16S rRNA genes. In 107 women, we evaluated associations between diet and GMC (pyrosequencing of V1-V3 region of 16S rRNA gene).[78] We analyzed ~1.4 million sequences. After trimming, we identified bacteria in 644,956 high-quality sequences.[81] Using 3-d food records, we detected differences in GMC structure between participants with low and high intakes (tertile 1 & 3) of refined grains (p<0.0001), starch (p<0.003), and insoluble fiber (p=0.034; Multi-Response Permutation analysis).[82, 83] Based on 16S rRNA gene content, we estimated the microbiome functional gene content.[84] There was significant enrichment in functional genes associated with the butyrate-production pathway in participants with higher fiber intakes (t-test, p=0.037).

The average U.S. consumption of dietary fiber is only 20-50% of recommended intake levels. Resistant starch (RS) is a naturally occurring type of fiber that is resistant to human digestion, but may be fermented by the human gut microbiota and affect systemic markers of health. RS is associated with decreased insulin resistance, systemic markers of inflammation, and serum endotoxin levels in both animal models and humans. Our aim is to see if a 2 months regime of eating common foods made with RS will change markers of inflammation and insulin resistance as well as the composition of gut bacteria in colorectal cancer survivors.

Study Overview

Patients with a history of stage I-III colorectal cancer who have completed colorectal cancer treatment will be recruited from the SCCA GI Oncology Clinic. Patients will participate in an 8-week randomized, controlled trial testing foods made with resistant starch vs foods made with corn starch. All food products will be made under strictly controlled conditions in the Fred Hutch Human Nutrition Lab and will include a variety of common foods such as scones, muffins, pudding and cookies. The only difference between the products is that some products will be made with resistant starch and some will be made with corn starch. Both investigators and participants will be blinded to their randomization assignment.

Eligibility criteria include: 1) history of diagnosed AJCC stage I-III colorectal adenocancinoma; 2) completion of all treatment of colorectal adenocarcinoma within the past 4-36 months; 3) ECOG Performance Status of 0, 1, or 2 (per physician); 4) age ≥18 years at diagnosis; 5) absence of any active inflammatory bowel disorder; 6) no use of antibiotics within 3 months prior to enrollment; and 7) ability to consent and follow study protocol.

Stool sample will be collected three times in the privacy of each person's home using a kit and instructions provided. Fasting blood samples will be collected at baseline and week 8 at the Fred Hutch Prevention Center.

After agreeing to participate and signing informed consent, participants will be randomized to receive the active intervention (daily foods containing resistant starch) or control intervention (daily foods containing corn starch).

The intervention will use commercially available RS-2 (Hi-Maize) that will be prepared into common bakery items, such as muffins, scones, as well as beverages such as smoothies. Control products will use the same recipe but will be prepared with regular corn starch. The RS will be Hi-Maize, a corn starch made solely from hi-amylose maize. This product is commercially available from, among others, King Arthur Flour, Honeyville and Amazon. It has been used in many published studies[85-93] as well as at least 20 studies currently listed in ClinicalTrials.gov. Dose range has been from 15g/day to 47g/day and study duration from 1 to 8 weeks. Some people may experience flatulence and perhaps some bloating due to the higher fiber content added to their diet, therefore we will have participants do a stepwise escalation that will allow for better adaptation to the target dose of 30g. RS per day. In the first two weeks participants will consume one study food with 15 g RS per day and starting in the third week participants will consume two study foods for a total of 30g RS per day for 6 weeks. Participants will be provided with a variety of options to allow for variety. No other dietary changes will be required. Participants may consume their usual diet and participate in their usual activity throughout the study. All products will be produced at the Human Nutrition Lab in the Fred Hutch Prevention Center. See Figure 2.

Systemic inflammation will be measured as serum C-reactive protein (CRP)/albumin ratio and adiponectin. Insulin resistance will be assessed using the homeostasis model of assessment-insulin resistance (HOMA-IR). Gut microbial community structure (16S rRNA sequencing), and gut metabolites will be compared between intervention and control groups for generation of hypotheses for protective prebiotic effects on gut microbial taxa.

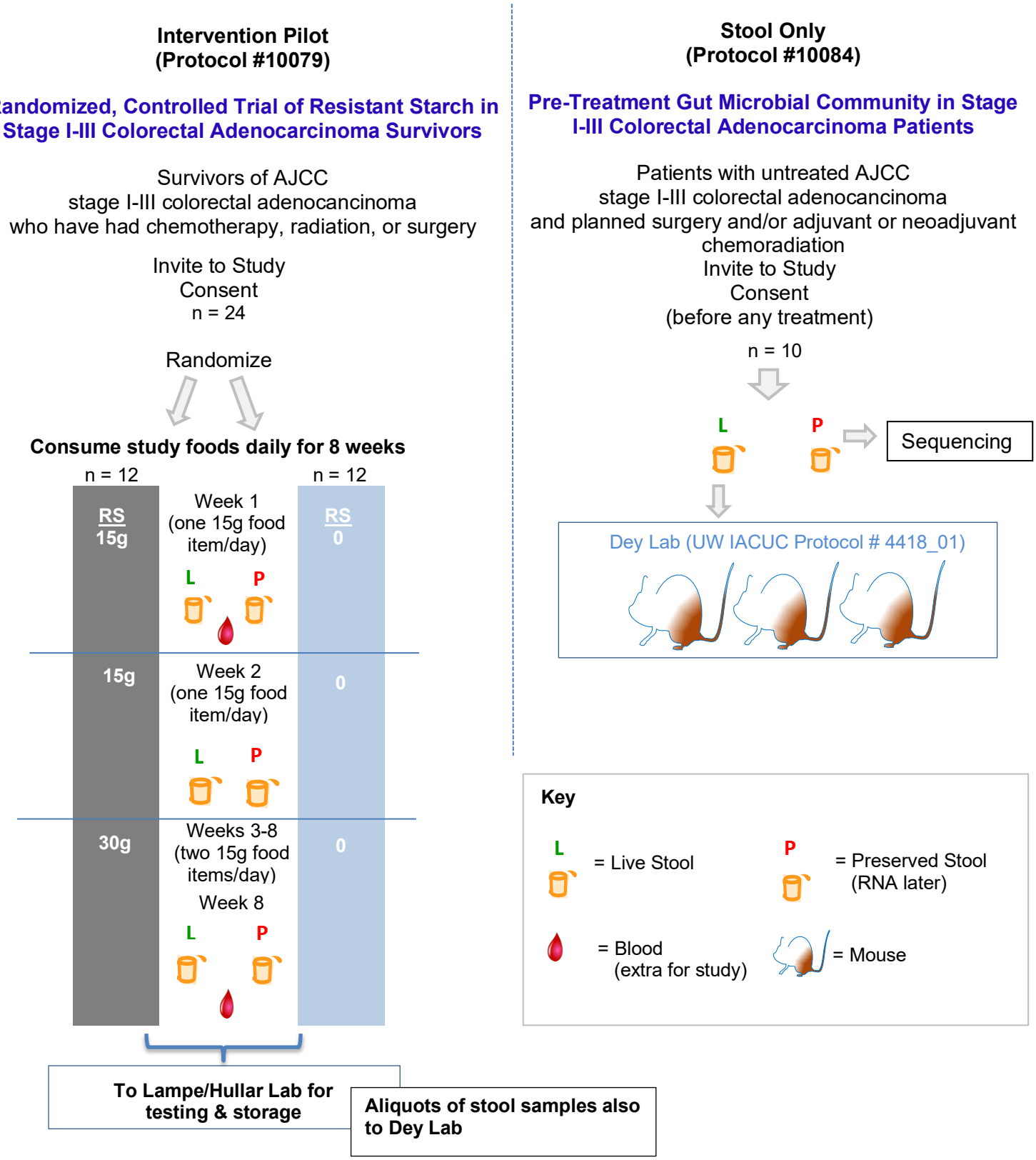
In order to determine whether an RS dietary intervention can potentially affect gastrointestinal tumorigenesis in high-risk individuals, participant fecal microbiota sampled will be transplanted into colorectal tumor-prone mice (Dr. Neel Dey, UW IACUC protocol # 4418_01).

Primary Aim: To determine the feasibility of an 8-week dietary intervention testing foods made with resistant starch compared to foods made with corn starch in patients who have completed treatment for stage I-III colorectal cancer. Feasibility will be assessed based on trial accrual, intervention adherence, and trial retention.

Secondary Aims:

1. To assess variability from baseline to 8 weeks in circulating markers of insulin resistance (glucose, insulin and HOMA-IR) and inflammation (CRP, albumin, adiponectin), by randomization arm.
2. To assess variability from baseline to 2 weeks and 8 weeks in gut microbial communities based on 16S rRNA gene sequencing, by randomization arm.
3. To explore whether resistant starch suppresses adenomas/carcinomas in human to mouse Fecal Microbiota Transplantation (FMT) studies.

Figure 2 – Study Design. Note: The Stool Only protocol and companion Intervention Pilot have separate protocols and IRB submissions. Dr. Neel Dey, Co-PI, will receive human fecal samples for the gnotobiotic mouse experiments under IACUC approval (UW IACUC protocol # 4418_01).



2. ELIGIBILITY CRITERIA

Inclusion:

1. History of diagnosed AJCC stage I-III colorectal adenocancinoma
2. Completed all treatment of colorectal adenocarcinoma within past 4-36 months.
3. Current ECOG Performance Status of 0, 1, or 2 (per physician)
4. Age ≥18 years at diagnosis
5. Ability to consent and follow study protocol

Exclusion:

1. Active cancer
2. Prior diagnosis of diabetes that is currently uncontrolled (defined as Hgb A1c >8.0)
3. Active inflammatory bowel disease (i.e., patients who are symptomatic despite medical therapy). This includes Irritable Bowel Syndrome, Crohn's Disease, or any other inflammatory bowel disorder
4. Known food allergy/intolerances to wheat, gluten, dairy or eggs
5. Use of antibiotic(s) within the last 3 months prior to enrollment
6. Women who are pregnant and/or breastfeeding
7. Current BMI < 18.5 kg/m^2
8. Uncontrolled constipation
9. Inability to speak and fully understand English

3. RECRUITMENT, APPROACH, AND CONSENT

Study investigators and staff will identify potentially eligible participants via the SCCA electronic health record. Eligibility and exclusion criteria will be checked and confirmed in the electronic health record. The SCCA physician caring for the identified patient will then be contacted to confirm appropriateness and grant permission for the patient to be approached for study participation.

In addition, potentially eligible participants will be ascertained by SCCA Survivorship Clinic staff who will screen survivor clinic records and notify study staff of potentially eligible participants. Survivorship clinic staff will provide eligible medical record numbers only for patients for whom the SCCA survivorship clinic care provider provides permission for the patient to be approached. Eligibility and exclusion criteria will be confirmed by study staff in the SCCA electronic medical record, upon which the approach process will be initiated as described below. In addition, the study brochure will be posted at the SCCA Survivorship Clinic. Persons interested in the study can contact the research study coordinator at the contact phone and/or email address provided in the brochure. The study coordinator will then conduct eligibility screening utilizing the telephone screening script procedure as per Appendix 3.

Patient contact will first consist of a mailed recruitment packet from study investigators introducing the study and inviting them to participate. This will be sent to the study candidate by both email and US Mail. The recruitment packet contains (1) an approach letter introducing the study and inviting the patient to participate and (2) a study brochure. In the letter, potential participants are invited to call study staff if they are interested in learning more about the study or if they wish no further contact. (See Appendices 1 and 2 for the approach letter and study brochure).

If a patient does not contact the study staff within 3 days of mailing the recruitment packet, study staff follows up with an email and screening telephone call. Up to 2 emails and 3 call attempts to reach the participant are made within a 1-week period. If the phone is not answered after attempt

1, a voice mail will be left, and a 2nd attempt will be made the next day. If the phone is not answered after attempt 2, a voice mail will **not** be left. If after 3 attempts to reach the participant contact does not succeed, no further attempts are made and the participant is released from study consideration.

The email and screening call is conducted as scripted (see Appendix 3). During the screening phone call, study staff assesses interest and reassesses and confirms eligibility. Eligible participants will be scheduled for a Consent Visit to be conducted either in person at the Fred Hutch Prevention Center, or virtually via telephone should telephonic consent be optimal to manage the visit in the context of the coronavirus/COVID-19 outbreak. The primary purpose of the Consent Visit is to sign informed consent. Height and weight baseline data, necessary for performing the block randomization, will be collected at the in-person Consent Visit or by participant self-report if the Consent Visit is conducted telephonically. In either case, the consent form is mailed to participants before the Consent Visit, along with driving/transportation directions and appointment confirmation information (see Appendices 4, 5, and 6).

Consent is obtained in person in the Prevention Center or by telephone by study staff at the participant's Consent Visit. If conducted telephonically, study staff conduct the Consent Visit using a *Telephonic Informed Consent Script* (Appendix 17). For telephonic Consent Visits, if after complete the informed consent discussion the participant verbally agrees to join the study, the participant is instructed to sign and date the consent form using the date of the consent call and to return the signed consent when s/he comes in for the next Study Visit. Study procedures proceed only after written informed consent is obtained.

4. DATA COLLECTION

4.a. CONSENT VISIT

As described in Section 3 above, potential participants schedule a Consent Visit with study staff to take place either in person at the Prevention Center (Arnold Building, Fred Hutch), or telephonically. After reviewing study procedures and being able to ask questions, interested participants will provide written informed consent and height and weight will be collected. After consenting, participants are randomized into the trial using a permuted block design, based on sex and 2 BMI categories (below and equal/above BMI = 25 kg/m$^{2)}$. Participants, investigators and study staff will be blinded to randomization condition.

At consent participants are provided with a stool collection kit and written and verbal instructions on their use as well as a blank 1) Food Frequency Questionnaire and 2) a baseline Study Questionnaire to fill out before and bring completed with them to their first intervention visit. Study staff schedule the Intervention Visit 1.

After consent, the following data will be abstracted by study staff from the SCCA/UW Medical Records.

A. Patient and tumor characteristics at time of **initial (*pre-treatment)*** presentation at SCCA:
   1. Demographics
   2. Height and Weight
   3. Comorbidities
   4. Resection Surgery Date
   5. Tumor site
   6. T stage
   7. Tumor size
   8. N stage

9. Number of positive nodes
10. Number of total nodes removed
11. Histologic type (i.e. adenocarcinoma)
12. Grade
13. Lymphatic invasion
14. Vascular invasion
15. Perineural invasion
16. Proximal/distal margins
17. Circumferential margin
18. Tumor-infiltrating lymphocytes
19. Peri-tumoral response

B. Colorectal cancer (CRC) treatment information:
   1. Neoadjuvant treatment received **prior** to CRC surgery:
      a. Chemotherapy Y/N and if Y, specific regimen and doses
      b. Radiotherapy Y/N and if Y, specific regimen and doses
      c. Dates administered
   2. Post-surgical treatment received **after** CRC surgery
      a. Specific treatment(s) received, dosages, dates
      b. Associated co-morbidities

4.b INTERVENTION VISIT 1 – START OF INTERVENTION

Participants collect a stool sample at home the day before or morning of Intervention Visit 1. Participants receive a reminder phone call the day prior to their intervention visit 1 to remind them about the appointment and to bring the baseline stool sample and completed baseline questionnaires with them to the visit. This call is conducted in accordance with the Visit Appointment Reminder Call Phone Script (Appendix 7). In addition, participants come to the Prevention Center to provide a fasting blood draw.

After the blood draw, participants are provided with their first set of foods and instructions for eating and logging the study foods and any associated symptoms, on a daily basis.

Summary of Intervention Visit 1 Activities:

Participants turn in their completed baseline questionnaires

1. Food Frequency Questionnaire
2. Baseline Study Questionnaire

Baseline biological samples are collected:

1. Stool
2. Blood (fasting) – 10 cc

Study staff provides the participant with study supplies for the next two weeks: study foods with instructions for storage and reheating; the Daily Study Food Consumption and Symptoms Log with instructions; and another stool collection kit with instructions for the 2nd intervention visit.

Study staff also reminds the participant that staff will call the participant weekly for the next two weeks, to check in and provide the participant opportunity to ask questions (see Adherence Call Script, Appendix 15).

Alternatively, if necessary for example during the COVID-19 pandemic, this visit may be conducted telephonically (using script per Appendix 19). Should this visit need to occur by telephone, the

baseline study fasting blood draw will not be collected. The participant will be instructed to collect the stool sample using the kit provided for the baseline collection.

The participant will be sent a pre-paid, same day courier return mailing label and box to return the frozen baseline stool sample and baseline questionnaires. Depending on accessibility of the participant to his/her neighborhood courier office, the participant can either take the shipment box to the courier office, or study staff can arrange for pick-up service by the courier from the participant's home. In the event of a 'shelter in place' mandate, the latter option will be used. In the event that the courier service is not operating, the participant will be instructed to keep the study materials at home, and the stool sample in his/her home freezer, until the courier service is again operational. The first supply of study foods will be couriered to the participant for receipt on the day of the Intervention Visit 1 telephone call.

4.c. DURING INTERVENTION

Participant activities during the intervention consist of the following which are completed by the participant at home.

- Daily consumption of study foods
- Daily completion of the Daily Study Food Consumption and Symptoms Log

Phone calls (once weekly through ramp up to 30g daily then every two weeks thereafter through end of study). Study staff contact the participants via telephone to monitor adherence, collect feedback about the study (feasibility, difficulties, etc.), and give a reminder about the participant's next Prevention Center Visit. See Adherence Call Script, Appendix 15).

4.d. INTERVENTION VISIT 2 – Week 2 - Day 14

Participants return to the Prevention Center on Day 14 for the Week-2 visit. Alternatively, this visit may be conducted telephonically (using script per Appendix 18). At this visit, the participant will bring in another stool sample, the completed Study Questionnaire (Week-2 Follow Up time point), and the Daily Study Food Consumption and Symptoms Log completed for days 1-13. If the participant is unable to come to the Prevention Center, the stool sample and questionnaires can be returned by mail.

<u>Summary of Intervention Visit 2 Activities</u>

Participants turn in their completed Week-2 follow-up questionnaires

1. Study Questionnaire (Week-2 Follow Up)
2. Daily Study Food Consumption and Symptoms Log (for Days 1-13)

Week-2 follow-up biological samples are collected

1. Stool

Study staff provides the participant with study supplies for the next six weeks. These supplies consist of: (1) instructions for weekly or every other week receipt of study foods from the Fred Hutch Human Nutrition Lab (foods will be mailed, couriered, or the participant may opt to pick up their foods in person); (2) the Daily Study Food Consumption and Symptoms Log with instructions to complete daily for days 14 through end of study; and (3) another stool collection kit with instructions for the 3rd intervention visit.

Study staff also reminds the participant that staff will call the participant every other week through end of study. The purpose of these calls is to check in, remind the participant to eat the study foods daily at the appropriate dosage levels, and provide the participant an opportunity to ask questions.

These calls also will ascertain what specific study food products the participant would like to order from the research kitchen for his/her next batch of study foods, and how he/she would like to receive them (delivery method). (See Adherence Call Script, Appendix 15).

4.e. INTERVENTION VISIT 3 - END OF INTERVENTION – Week 8 – Day 56

Participants return to the Prevention Center on Day 56 for their Week-8 and final visit. At this visit, the participant will bring in a final stool sample, the completed Study Questionnaire (Week-8 Follow Up time point), and the Daily Study Food Consumption and Symptoms Log completed for days 14 through end of study. If participant is unable to come to the Prevention Center, the stool sample and questionnaires can be returned by mail.

Summary of Intervention Visit 3 Activities

Participants turn in their completed Week-8 follow-up questionnaires

1. Study Questionnaire (Week-8 Follow Up)
2. Daily Study Food Consumption and Symptoms Log (for Weeks 3-8)

Week-8 follow-up biological samples are collected:

3. Stool
4. Blood (fasting) -10 cc

Study staff provides the participant with End of Study Thank You Letter and $50 Visa Gift Card.

Alternatively, if necessary for example during the COVID-19 pandemic, this visit may be conducted telephonically (using script per Appendix 20). Should this visit need to occur by telephone, the end-of-study fasting blood draw will not be collected. The participant will be instructed to collect the stool sample using the kit provided for the end-of-study collection.

The participant will be sent a pre-paid, same day courier return mailing label and box to return the frozen stool sample and end-of-study questionnaires. Depending on accessibility of the participant to his/her neighborhood courier office, the participant can either take the shipment box to the courier office, or study staff can arrange for pick-up service by the courier from the participant's home. In the event of a 'shelter in place' mandate, the latter option will be used. In the event that the courier service is not operating, the participant will be instructed to keep the study materials at home, and the stool sample in his/her home freezer, until the courier service is again operational.

The end of study thank you letter and $50 thank you payment will be mailed to the participant upon receipt of the end of study stool sample and questionnaires.

4.f. MANAGING POTENTIAL DELAYS OR DISRUPTIONS TO THE STUDY CALENDAR

This component of the study protocol is added as a result of the Fred Hutch laboratory ramp down in response to the coronavirus pandemic.

In response to the pandemic, or any other unanticipated natural disaster, it is possible that the start of intervention may need to be delayed. For other participants who may have already started the intervention, the intervention may need to be stopped early. Here we describe how each of these scenarios will be handled.

Should the start of the intervention need to be delayed:

In the event that a delay in the start of the intervention be necessary, the consented participant will be called and/or emailed (using script per Appendix 21). The reason for the need to delay the start of the intervention and the remainder of the study calendar will be explained to the participant. This call/email will involve either scheduling the new study intervention dates (if possible) or scheduling a date to check in with the participant again by phone to provide study status.

Should the intervention need to be discontinued early:
In the event that due to the pandemic or other natural disaster the investigators may need to terminate a participant's study intervention before the week 8 end of study timepoint, the participant will be called and/or emailed (using script per Appendix 22). The reason for the need to discontinue the study early will be explained to the participant. During the call/email, the participant will be instructed on how to complete his/her study activities, and the call/email will remind the participant that the end of study thank you letter and $50 payment will be sent upon receipt of the participants final study questionnaires.

5. INTERVENTION

Active group: Resistant Starch @ 15g/day during weeks 1 and 2, and @ 30g/d for weeks 3-8 (8 weeks total on study). If participant is not able to tolerate the higher doses they may stay at the maximum tolerated dose for the remainder of the study. (See Dosage Modification Instructions in Section 6, below). The Resistant Starch will be Hi-Maize, a RS2 corn starch from hi-amylose maize. This product is commercially available from, among others, King Arthur Flour, Honeyville, Amazon. It has been used in many published studies[85-93] as well as at least 20 studies currently listed in ClinicalTrials.gov. The dosage in our study is comparable to other studies and the product is well tolerated with the main issues being gastrointestinal discomforts such as flatulence and feeling bloated. Studies report few dropouts due to these symptoms.

Control group: will receive similar size and number of items as the active group, made with regular corn starch. If participant is not able to tolerate the higher doses they may stay at the maximum tolerated dose for the remainder of the study. (See Dosage Modification Instructions in Section 6, below).

Study Foods will be developed and produced by the Fred Hutch Human Nutrition Lab (HNL). The HNL facility, within the Prevention Center, is 2,900 ft^2 designed and implemented solely and specifically to conduct human feeding studies. It includes extensive food storage, food preparation, and service areas. Over a third of the square footage is devoted to storage, allowing for the purchase of food in lot-sized amounts, and consists of a walk-in freezer, refrigerator, and dry storage. The food preparation facility includes both cold and hot food preparation and production areas with a gas cook-top, convection ovens, steamers, microwave oven, blast chiller, Mettler-Toledo balances, and heat-sealer. A networked computer system, operated through the Division of Public Health Sciences, is used for data entry, diet development, and HNL food preparation documents. Available nutrition computer software includes ProNutra. Four offices are available for resource staff, including the HNL manager and research assistants. The commercial kitchen is inspected regularly by the Health Department and all workers have food handlers permits. Over the years, the HNL has delivered over 20,000 study participant meals for a variety of feeding studies. The HNL will design recipes for a series of products in order to give participants variety and satisfaction, ie. scones, muffins, puddings and smoothies. Development of the products will happen in conjunction with study investigators to balance nutritional macronutrient balance. Additionally, we will also consult with the SCCA Medical Nutrition Therapy group to obtain feedback on the nutritional needs of colorectal cancer survivors.

Instructions on study food dosing, safe food handling and storage procedures will be provided to participants (see Appendices 13 and 14).

We will work with each participant individually to plan how food items will be delivered to them during the course of the study. If a participant is willing and able to come to the Prevention Center once a week or once every 2 weeks, this gives us the opportunity to meet with them face to face and discuss any issues they may be having. If it is not practical for the participant to come to the Prevention Center to receive his/her study food items, we will arrange for delivery of the items via U.S. Mail, Federal Express, in person delivery by study staff, or courier. The HNL has done this procedure for other studies as well. The informed consent form will provide participants the opportunity to consent to their names and delivery addresses to be provided by study staff to the US Postal Service, Federal Express or courier service as needed.

Randomization into active or control group: Participants will be block randomized based on sex and 2 BMI categories. This will ensure that the two intervention arms have similar distributions of patients.

Active and control groups will be blinded, products will be labeled either A or B, and will be unblinded by the Human Nutrition Lab to investigators once study and data analysis has been completed.

Compliance measures: we will provide a calendar-driven, checklist-style daily log for participants to mark having eaten the study food items and, if not completely, how much. This log will also have a place for the participant to record any gastrointestinal issues they may be experiencing.

Interim compliance contacts: the research coordinator will contact the participant (via phone call once weekly through ramp up to 30g daily then every two weeks thereafter through end of study. (See Adherence Call Script, Appendix 15).

6. DOSAGE MODIFICATION

The dosage of the assigned study food will be modified if needed based on the participant's inability to tolerate the prescribed dosage.

Intolerance is defined as **any grade 3 or higher adverse event (AE) that is possibly, probably, or definitely attributable to the study food product.**

AEs for this study are assessed and graded in accordance with the NCI's Common Terminology Criteria for Adverse Events (CTCAE), version 5.0:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf (last accessed Mar 12, 2019).

Attribution of each AE will be determined and coded by the Principal Investigator(s), in collaboration with co-investigators as needed. Attribution to study dietary intervention is categorized as follows:

1=Unrelated 2=Unlikely related 3=Possibly related 4=Probably related 5=Definitely related

AE Grade and Attribution	Dose Modification
Occurring at any time on study	
< Grade 2, any attribution	No dose reduction, proceed as planned
≥ Grade 3, unrelated or unlikely related	No dose reduction, proceed as planned
Occurring during Week 1 or 2 of study	
≥ Grade 3, possibly, probably, or definitely related	Stop the dietary intervention and continue as follows **as soon as possible (within one week) after stopping the study food product:** ➢ Collect the Week 2 stool sample and study questionnaire. (Participant may send the Week 2 stool sample and Questionnaire by mail or deliver in person to the Prevention Center). ➢ Collect the end of study fasting blood draw (originally planned for Week 8), at the Prevention Center ➢ Omit all remaining activities. Provide EOS Thank You Letter and Gift Card.
Occurring during Week 3 - 8 of study	
≥ Grade 3, possibly, probably, or definitely related	Reduce the dietary intervention to 15g/day for the remainder of the study. Proceed with stool collection, fasting blood draw, and all other data collection as originally planned.
If **at any time** after dose reducing to 15g/day during Week 3-8 the participant experiences new or continuing ≥ grade 3 AE(s) that are possibly, probably, or definitely related to the study dietary intervention	Stop the dietary intervention and continue as follows **as soon as possible (within one week) after stopping the study food product:** ➢ Collect the end of study stool sample and study questionnaire (originally planned for Week 8). (Participant may send stool sample and Questionnaire by mail or deliver in person to the Prevention Center). ➢ Collect the end of study fasting blood draw (originally planned for Week 8), at the Prevention Center ➢ Provide EOS Thank You Letter and Gift Card.

7. PARTICIPANT RISKS AND BENEFITS

Overview of Risk
While we do not anticipate any serious adverse events during this study there are some known risks associated with participation. In addition to the known risks below there may also be some unknown risks associated with participation in this study.

Risk Associated with the Intervention
Risks associated with higher levels of either resistant starch or corn starch may include: gastrointestinal discomfort, increased gas, bloating, looser stools, and increased number of bowel movements.

Blood samples. Participants may experience lightheadedness or feeling faint when having blood drawn, and/or they may experience a temporary bruise at the site of venapuncture. All efforts will be made to minimize these risks.

Stool Collections. It may be embarrassing for people to collect their own stool.

Loss of confidentiality: This is a small change that there may be loss of confidentiality. We make strong efforts to protect confidentiality by using participant ID numbers instead of names and using password-protected electronic files.

Research Related Injuries
If a participant believes they have been injured as a result of their participation in these studies, the consent form directs them to contact the Fred Hutch IRO.

If the individual should require medical care (as determined by the intervention staff), due to research-related injury, it will be provided. However, the individual or their medical insurance will be responsible for the cost of such care.

Benefits
While there may be no direct benefit of this study to participants, the study could provide important information about future recommendations of fiber levels for colorectal cancer survivors. Because risks to subjects in this study are minor, the risks are most reasonable in relation to the expected benefits.

Compensation
Participants will each be given a $50 VISA gift card for completing the study.

Alternatives
Alternatives to participating in this research study include not participating.

8. DATA ANALYSIS PLAN

Feasibility Endpoints.
Feasibility of the study design and intervention will be assessed based on the following three criteria:

1. Accrual. The accrual rate will be estimated with a target accrual rate of full enrollment within 12 months after study activation.
2. Adherence. Adherence to the intervention will be defined as consuming 75% or more of study foods on ≥75% of days from baseline to 8 weeks.

3. Retention. Target retention is defined as ≥80% of enrolled participants providing blood and stool samples at 8 weeks.

Secondary Endpoints.
Secondary endpoint analyses will be considered exploratory and will be used to provide effect size estimates to be used in a future trial.

1. Assess variability in biomarkers of insulin resistance and inflammation will be assessed using descriptive statistics.
2. Assess variability in gut microbial communities from human stool samples collected during the study by changes in alpha diversity, global microbial community (beta diversity), and genera in response to the intervention. We will use multivariate and univariate approaches to assess significant changes in the microbiome in response to the intervention.
3. Fecal microbiota from "resistant starch responders" (i.e., individuals with significant resulting elevations in fecal short-chain fatty acids concentrations) will be selected as input samples in gnotobiotic mouse studies, in which mice will be fed diets with or without resistant starch in order to characterize effects on tumorigenesis in an otherwise controlled environment. We predict that the chemoprotective effect of this diet intervention will be dependent upon gut microbial metabolism.

9. DATA SAFETY MONITORING PLAN

Oversight for this study will be provided by the Principal Investigators at Fred Hutch, Drs. Marian Neuhouser and Heather Greenlee, with delegation of responsibilities to designated study personnel. Study PIs will ensure all entry criteria are met prior to the initiation of the protocol, and all study procedures and reporting of adverse events are performed according to the IRB-approved protocol.

In addition, in compliance with Fred Hutch/UW Cancer Consortium DSMP guidance, this study will be monitored by the Fred Hutch Clinical Research Support (CRS) Monitoring program. Study participants are previously treated colorectal cancer survivors in whom the study intervention is non-therapeutic. As such, the study is low risk in accordance with the Consortium DSMP guidelines. As the study is low risk, monitoring will involve a single visit by CRS Monitoring program staff or, at CRS discretion, a contracted external monitor. This monitoring visit will occur approximately 12 months after the first subject is enrolled, and will comprise, at a minimum, consent document review and eligibility review for 10% or 3 participants enrolled. Additional monitoring may occur if so recommended by the CRS Monitoring program.

This Data Safety Monitoring Plan (i.e., Section 8 of this protocol) is hereby submitted within this protocol document for IRB review and approval.

All adverse events related to the study procedures will be fully documented on the appropriate case report form(s) and entered in a study database. For each adverse event, the investigator will provide the onset, duration, intensity, treatment required, and outcome, including documentation of need for premature termination of any study procedures.

Anticipated adverse events related to this study are:

Intervention Foods Products made with resistant starch may have the same effects as a high fiber diet: mild bloating and abdominal distension might occur while on the study since the fiber content may be higher than participant's habitual diet. Most people adapt to a higher fiber intake within 3-5 days. Participants will be monitored by the study staff via telephone calls from study staff. Staff will be trained to monitor tolerance and abnormal events via short interviews and the completed daily study food and symptoms log. Study participants will be able to reach study staff by cell phone around the clock.

Preexisting Condition.
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period. Participants in this study have been diagnosed and treated for rectal cancer.

Adverse event grading scale:

0= No Adverse Event (AE) or within normal limits

1= Mild Severity: Transient laboratory test alterations; discomforts noted but no disruption of daily activities; no therapy, or only symptomatic therapy required

2= Moderate Severity: Laboratory test alterations indicating injury without long-term risk; discomfort sufficient to modify normal daily activity; specific therapy required (i.e., more than symptomatic)

3= Serious Severity: Laboratory test indicating a serious health threat or permanent injury; incapacity, inability to work, inability to perform normal daily activity; hospitalization required or prolonged; emergency treatment required; life-threatening events; death.

AEs for this study are assessed and graded in accordance with the NCI's Common Terminology Criteria for Adverse Events (CTCAE), version 5.0:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf (last accessed Mar 12, 2019).

Plan for unanticipated AE reporting: All unanticipated AEs related to the study procedures that are severe or serious will be reported by Drs. Neuhouser or Greenlee to the IRB within 7 days of notification of the investigator.

Plan for anticipated AE reporting: All serious anticipated AEs related to the study procedures will be reported by Drs. Neuhouser or Greenlee to the IRB within 7 days of notification of the investigator.

Plan for ongoing review of results: The PI will be notified within 24 hours by the research manager of any early terminations due to an adverse event.

Plan for safety review: the PI will perform a cumulative review of all adverse events and premature terminations review every 6 months after study initiation or after completion of 50% of participant visits, whichever occurs first.

Plan for annual reporting: A summary of the investigation including all adverse events and how they were handled, enrollment, drop-outs and reason for discontinuation and any protocol modifications will be provided to the IRB on an annual basis.

Annual Reports will contain:
a. The number of adverse events and an explanation of how each event was handled
b. The number of complaints and how each complaint was handled
c. The number of subject withdrawals and an explanation of why the subject withdrew or was withdrawn
d. The number of protocol deviations and how each was handled

Any questions of food safety will be immediately reviewed with the HNL manager. The occurrence of any serious and unexpected event may prompt changes in study protocol. Any such change will be approved by the IRB before implementation.

Monitoring for data integrity and safety will be the responsibility of the investigators and the CRS Monitoring program.

Investigators will include the following in routine monitoring review: A) Validity and integrity of data: Data are checked for missing, unusual, or impossible records when they are entered into the study's computer database. The investigators may consider modifications to the data collection protocol with permission from IRB if they deem the change(s) is necessary to ensure the validity and integrity of data collected. B) Enrollment rate relative to expectation: Early lags in recruitment will be rectified with increased recruitment efforts so that recruitment will be completed on time. The investigators will monitor this closely to ensure full enrollment of appropriate participants. C) Retention of participants and adherence to protocol: The investigators will monitor retention and adherence to study protocol. Adherence to the study diet will be monitored via the Daily Record that participants fill out and the uneaten foods they return.

In addition, a CRS monitoring visit will occur approximately 12 months after the first participant s enrolled, and will comprise, at a minimum, consent document review and eligibility review for 10% or 3 participants enrolled. Additional monitoring may occur if so recommended by the CRS Monitoring program.

10. BIOLOGICAL SAMPLES - PROCEDURES

Blood (fasting):
Phlebotomist at the Prevention Center will take one red top (serum) and one lavender top (whole blood)10 ml tubes. .

- Invert tube slowly, 8-10 times immediately after collection to ensure adequate mixing of the anticoagulant.

They will process plasma in accordance to normal procedure and aliquot into 6 (+) – 0.6 ml clear cryovials

STORING/COOLING

- The purple-top tubes are processed no later than 4 hours after collection.
- After processing, all cryovials will be stored at -80ºC.

BIOMARKER ASSAYS
Blood samples will be in a single shipment at the end of study to the Northwest Lipids Laboratory (fee for service) to assess changes in circulating biomarkers. Samples for each individual participant will be run in the same batch and all batches will include blinded duplicates as QC.

Stool
Participants will be provided stool collection kits that include:

1. Written instructions
2. A freezer pack to keep the sample cold
3. A folded paper "FecesCatcher" (Zeijen, The Netherlands) to place over the toilet (2 papers will be provided, one for use as a spare if needed)
4. A pair of gloves to keep hands clean
5. To collect stool sample:
    a. a plastic tube containing 5 ml. RNA-later and small glass beads. Tube has a scoop attached to the lid.
    b. a plastic tube containing materials necessary to maintain an anaerobic environment. Tube has a scoop attached to the lid.

6. A larger plastic tube with absorbent in it to protect the tubes with the sample
7. A black plastic zip-lock bag with an orange biohazard sign – to store it all
8. The Study Questionnaire to collect participant information as described in Section 4 above
9. A tote bag for the participant to use to carry their samples back to the clinic

Participants will be asked to return the samples to the clinic.

Preservation/Storage: Samples will be frozen in -80 freezer at Fred Hutch.

Samples in preservative (RNAlater) will be homogenized and aliquoted. DNA extraction will be conducted in batches under the supervision of Dr. Meredith Hullar in the JLampe lab according to established protocols.

Extracted microbial DNA will be sequenced by Dr. Neel Dey, Co-I on this project.

Samples in the anaerobic media will be sent to Dr. Dey to be transplanted into colorectal tumor-prone mice (under IUAC # UW IACUC protocol # 4418_01). A subsample will be transferred to Dr. Hullar for anaerobic in-vitro metabolism and culturing according to established protocols.

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APPENDICES

1. Recruitment/Approach Letter
2. Study Brochure
3. Phone Script -- Screening
4. Informed Consent Form
5. Driving/Transportation Instructions to Prevention Center
6. Study Visit Confirmation Letter
7. Phone Script -- Visit Appointment Reminder Call
8. Study Questionnaire – Baseline
9. Study Questionnaire – Follow Up
10. Stool Collection Instructions
11. Daily Study Food and Symptoms Record
12. Food Questionnaire
13. Instructions for Study Food Dosing, Handling and Storage – Version for Mailing
14. Instructions for Study Food Dosing, Handling and Storage – Version for Participant Pick Up
15. Phone Script – Adherence Call
16. End of Study Participant Thank You Letter
17. Phone Script – Consent
18. Phone Script – Intervention Visit #2
19. Phone Script – Intervention Visit #1
20. Phone Script – Intervention Visit #3
21. Phone Script – Delay in Start of Intervention
22. Phone Script – Early Termination of Intervention