Synbiotic Supplementation to Reduce Anxiety Symptoms in Female Breast Cancer Survivors

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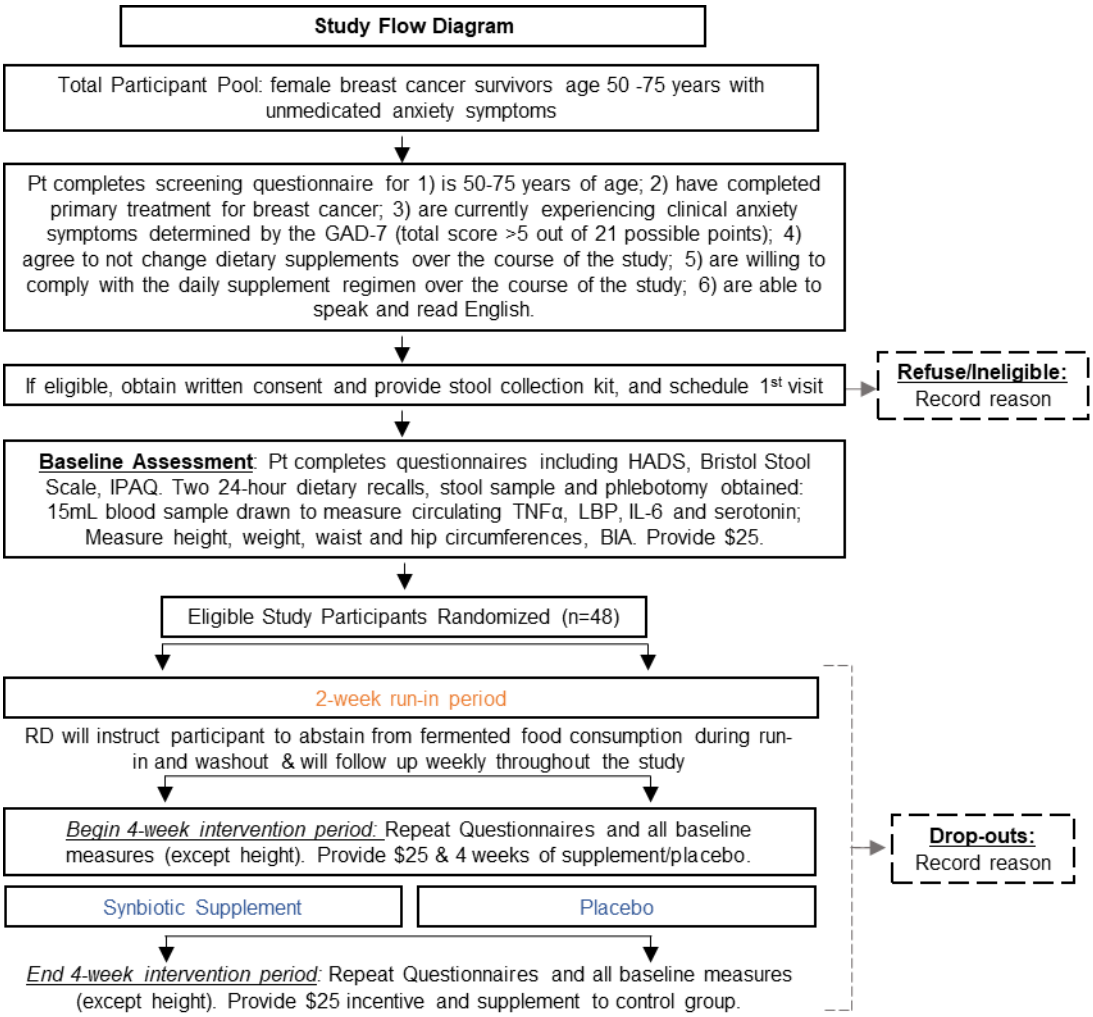
Schema



MATERIALS AND METHODS

1.1 Study Design: This is a double-blind, placebo-controlled clinical trial among 48 female breast cancer survivors with unmedicated anxiety symptoms. This study will determine whether daily synbiotic supplementation is feasible and explore the effects of the synbiotic on systemic inflammation, the fecal microbiota, as well as anxiety symptoms. Consenting patients will be randomized to the synbiotic intervention or placebo intervention. During the intervention period, participants will be educated and counseled to consume 5 tablets (synbiotic or placebo) each day and will be provided with these supplements to accomplish this goal. This study will evaluate feasibility (measured by accrual, retention and adherence) and reported adverse events will serve as the primary outcome measures, however, we also seek to explore changes in: 1) anxiety symptoms, measured via Generalized Anxiety Disorder-7 (GAD-7); 2) serum serotonin and inflammatory cytokines: lipopolysaccharide binding protein, interleukin-6, and tumor necrosis factor-alpha; and 3) fecal microbiome composition and diversity.

Prior to any in-person interactions, the following questions must be asked:

1. Have you had any of the following symptoms in the past two weeks, even if they were mild?
   - Fever or chills
   - Cough
   - Shortness of breath or difficulty breathing
   - Fatigue
   - Muscle or body aches
   - Headache
   - New loss of taste or smell
   - Sore throat
   - Congestion or runny nose
   - Nausea or vomiting
   - Diarrhea
2. In the past three weeks, have you visited another state, country, or facility with sustained (ongoing) occurrence of COVID-19?

3. Have you had close contact with a person that has tested positive for COVID-19 or who is under investigation for possible COVID-19?
4. Is there any additional information you would like to provide related to your possible exposure to COVID-19?

Any YES answers require that the appointment be rescheduled.

Study Flow

All in-person interactions require the following PPE:
Participant - Cloth face covering
Research Personnel – disposable lab coat, gloves, face shield or goggles, and a surgical mask.

PRE-STUDY

1. Distribute information letter, detailing study and directions to online Qualtrics screening survey.
    a. If completing Qualtrics survey online, call or email potential participant within 3 business days to follow up.
    b. If potential participant calls in to inquire about study, briefly describe the study using the information letter, then screen over the phone. Let them know their answers will be reviewed and you will follow up with them as soon as possible.
2. Determine initial eligibility, and follow-up with ALL participants within 3 days of last contact.
    1. If NOT interested collect reason for refusal, age, race, & miles from Auburn and document this information in the tracking database.
    2. If INTERESTED conduct further screening, but if screen-out, record reason & enter into tracking database.
    3. If INTERESTED and eligible, send the consent form via mail or email.
        a. After potential participant has reviewed the consent form and wishes to begin the study, schedule a time for them to come to PSB 101E to sign the consent form.
        b. Consent meetings can be conducted virtually if participant desires. Study supplies will be mailed to the participant if needed.
    4. OBTAIN CONSENT. Review consent form with participant and after they have signed, provide them with i) baseline questionnaires, ii) appointment reminder form, and iii) stool specimen kit.
    5. Schedule Baseline visit at least 3 days from current date to allow for one weekend and one weekday 24 hour dietary recall.
    6. The day before the clinic visit call the participant to remind them about their appointment and to fast for 8 hours.
    7. Provide them with your cell or office number and ask if they would call when they arrive at the parking lot.

BASELINE & 2-WEEK RUN-IN

1. Greet participant (either at their car or the entrance). Take their stool sample and questionnaires.
2. Complete intake form & anthropometric measures – height, weight, waist, hips, BIA
3. Blood draw.
4. Double check to make sure all questionnaires and intake forms are complete.
5. Take the appropriate randomization envelope from PSB 101E. Open randomization envelope & record assignment on intake form & in database.
6. Participants randomized to the synbiotic or placebo intervention arm will receive synbiotic capsules or placebo capsules, respectively, to take with them.
7. Provide $25.
8. Participants will avoid fermented food consumption for the next 2 weeks for the run-in period.

PRE- & POST-INTERVENTION

1. Repeat all procedures taken at baseline, but do NOT re-measure height.
2. Update intake form (ie, changes in medications and anthropometrics).
3. Check questionnaires for completeness.
4. Provide $25.

WEEKLY CALLS

1. Twice weekly phone calls with study staff to all participants to ensure adherence.
2. Schedule the participant's follow-up appointment within 3 days of each four-week interval.
3. The week prior to pre- and post-intervention appointments, conduct dietary recalls on the same corresponding days of the week collected at baseline (e.g. Thursday and Saturday or Sunday and Tuesday) & make sure that stool kit and questionnaires are mailed to arrive at least 3 days before the follow-up visits if not hand-delivered.

1.2 Study population

1.2.1 Source of Research Participants

The local newspaper will feature advertisements in print and online to recruit participants. This advertisement also includes direct-to-subscriber emails. The flyer used for newspaper ads will be accessible on Dr. Frugé's research website: https://cws.auburn.edu/eatyourgreens/pm/study . The study population will come from the general public in the Auburn/Opelika area but is open to any eligible person willing to travel to Auburn for the three visits.

1.2.2 Criteria for Eligibility

We will recruit female breast cancer survivors who:
1) are 50-75 years of age;
2) have completed primary treatment of breast cancer;
3) currently experience clinical anxiety symptoms determined by the GAD-7 (total score >5 out of 21 possible points);
4) Agree to not change dietary supplements over the course of the study;
5) Are willing to comply with the daily supplement regimen over the course of the study;
6) are able to speak and read English.

1.2.3 Criteria for Exclusion

1) changes in anxiety treatment (i.e., initiation of Cognitive Behavioral Therapy within the last 4 weeks)
2) current use (within 12 weeks) of anxiolytic medications
3) Use of the following drugs within the last four weeks (unless indefinitely prescribed): systemic antibiotics, corticosteroids, immunosuppressive agents, or commercial probiotics, which can drastically affect the gut microbe composition.

- All patients screened should be entered into the tracking database. Breast cancer stage, screening eligibility, demographic information, and reason for not participating should be determined and entered into the database located on the OneDrive.

1.2.4 Screening/Enrollment

The study coordinator will contact (either face-to-face or by telephone) all potentially eligible participants. The study will be explained (see study schema) and the patient will be given full opportunity to ask questions. Screening will be assisted via Qualtrics Eligibility Questionnaire.

If the patient is NOT interested thank them for their time and ask if they would provide the following information: breast cancer stage screening eligibility, demographic information, and reason for not participating

If the patient is interested, screen them using the Qualtrics Eligibility Questionnaire. Additionally, if they answer yes to any of the exclusion criteria, take note of when they completed or will complete the drug or probiotic therapy, and postpone their study enrollment if they are otherwise eligible (do not assign them a study ID at this time). If they screen-out record the reason and thank them for their time, but inform them that they are ineligible.

If the participant is eligible and remains interested, obtain consent as noted above. Assign the subject a study ID number (sequential number beginning at 100 and increasing as individuals are recruited, note that individuals who fail to keep baseline appointments will retain their ID numbers, but will not be counted as enrollees). Schedule the participant for their baseline appointment (see procedures under Phlebotomy and Blood Processing). Attempt to schedule a baseline appointment in which the participant would come in fasting within the next 3 days. If this is not possible or inconvenient schedule them at a later date.

Obtain the first 24-hour dietary recall reflecting a weekend day if possible. If baseline appointment is on

Monday, then the first can be a weekday from the previous week and the second can be the Sunday or Saturday prior to the visit.
The day before the clinic visit call the participant to remind them of their appointment and to come fasting. Also obtain a 24-hour dietary recall. The dietary recall and the previous intake will need to be input into ASA24 as soon as possible.

1.2.5 Randomization procedures

After the baseline visit and after ALL baseline data and samples are collected the study coordinator will access the stacks of sealed envelopes. Randomization is stratified by race, thus there will be a stack for non-Hispanic Black participants and a stack for non-Hispanic White participants, each randomly generated in blocks of four. The sealed envelope next in sequence will be opened and the study coordinator will record this assignment on the intake form. All participants will receive education pertaining to goals for daily consumption of their blinded supplement.

1.2.6 Supplement distribution

The probiotic and prebiotic are both commercially available and manufactured under the Swanson family of companies. The probiotic is Swanson Probiotic for Digestive Health. The prebiotic is Source Naturals FOS. We will purchase all of these for the intervention and control groups at study start (control group will receive placebo pills during the study and receive the 4 week supply at the conclusion of the study). We will record the lot numbers of each bottle and who receives pills from which lot #s. At the conclusion of the study, we will disclose which products the participants received so that they can obtain if desired. Study staff members that are not involved in randomization process will place the 5 intervention or control pills in each of the 28 day pill containers that will be associated with each randomization card.

1.2.7 Adherence

A two-fold approach to adherence will include encrypted, HIPAA compliant communications from the study staff using OhMD software (https://www.ohmd.com/) for participants who are willing to communicate via text. Study staff will check in twice weekly to collect subjective adherence and adverse effects data.

1.2.8 COVID-19

Prior to any face-to-face visit with research personnel, all participants and potential participants will be screened related to COVID-19 symptoms and exposure. We will continue to ask these questions throughout the study prior to any face-to-face interactions with research personnel. Research personnel will also be screened for COVID-19 symptoms and exposure. Hand sanitizer and hand washing stations will be available to participants and research personnel for general precautions. All involved (research personnel, potential participants, study staff, etc.) will be required to wear a face mask at all times, and will be asked to maintain 6-feet of social distancing during study procedures. Study staff in data collection room will be limited to ensure social distancing can be accomplished.

1.3 Scheduling

Scheduling will be dependent on 1) participant availability (morning is ideal since 8 hour fast is needed) and 2) Kristen's availability as the phlebotomist.

1.4 Sequence of Events at Clinic Assessments

The sequence of measures is as follows: 1) Patient is called the day before to remind them to fast for 8 hours prior to their appointment and to bring all medications in with them (so these can be accurately recorded); 2) confirm that the patient is fasting (if they have eaten within 8 hours record what they consumed and when they consumed it – use judgment as to whether the blood can be drawn or if another visit needs to be scheduled. For example if the patient ate a piece of toast and had a cup of coffee three hours ago make a note that this must be repeated exactly at follow-up and go ahead and draw the blood – if the patient ate the breakfast buffet at Golden Corral 30 minutes ago, then another appointment is warranted); 3) Collect stool sample and questionnaires from participant and perform anthropometric measures and blood draw.

1.5 Questionnaire Data

1.5.1 DIETARY RECALLS 2-day dietary recalls will be collected at baseline and follow-up. Recalls from one weekday

and one weekend day will be collected at each time point. At baseline, an effort will be made to collect the first recall soon after the participant is found eligible and indicates willingness to participate in the study. Verbal consent will be obtained prior to collecting this information and this data will be destroyed if indeed the patient is not ultimately enrolled. Day 2 of the recall will be collected as soon as possible after the first, making certain that the set includes one weekday and one weekend day. The days of the week will be recorded and follow-up recalls will be scheduled on identical days of the week the week prior to the final visit. Dietary intake data will be entered into ASA24 database for analysis.

1.5.2 GAD-7 Generalized Anxiety Disorder-7 item will be completed at screening, after 2-week run in and post-intervention period.

1.5.3 IPAQ International Physical Activity Questionnaire will be completed after 2-week run in and post-intervention period.

1.5.4 HADS Hospital Anxiety and Depression Scale will be completed at baseline, after 2-week run in and post-intervention.

1.5.5 BSS Bristol Stool Scale will be completed by participant upon collection of stool sample.

1.5.6 INTAKE FORM – This form will be completed at the baseline appointment and updated at the time of follow-up

1.6 Anthropometric measures

1.6.1. HEIGHT – Height will be measured without shoes (participant in stocking feet) and upon inhale using a fixed stadiometer and a Frankfort plane or level. It will only be measured at baseline and recorded in cm to the nearest 0.1 of a centimeter.

1.6.2. WEIGHT – Weight will be measured without shoes (participant in stocking feet) and in light clothing with pockets emptied and jackets, sweaters, etc. removed. Participants will be told to stand upright and on the center of the scale. It will be recorded in kg to the nearest 0.1 of a kilogram.

1.6.3. WAIST CIRCUMFERENCE – Waist circumference will be measured with a non-stretch tension controlled tape measure upon exhale making sure that the tape is placed at the top of the iliac crest and parallel to the floor. It will be measured twice at each time point (to the nearest 0.1 of a centimeter) and recorded on the intake form. The average will be taken for analyses.

1.6.4. HIP CIRCUMFERENCE – Hip circumference will be measured with a non-stretch tension controlled tape measure making sure that the tape is placed at the most distal point of the buttocks and parallel to the floor. It will be measured twice at each time point (to the nearest 0.1 of a centimeter) and recorded on the intake form. The average will be taken for analyses.

1.6.5. BODY IMPEDENCE ANALYSIS – study staff will program the handheld BIA analyzer using the patient's height in inches, weight in pounds, age, and gender and use the "normal" setting (as opposed to "athlete"). The participant will grip the analyzer with thumbs on top, holding the analyzer with arm straight forward. Study staff will record BMI and Body fat % from the output screen.

1.7 Processing

1.7.1. PHLEBOTOMY: Pre-labeled blood tubes and cryovials will be stored in PSB 101E. Take the appropriate tubes to the phlebotomy lab on the morning of the clinic visit. 2 vacutainers will be collected: 1-7ml serum separator (tiger-topped) and 1-8ml plasma tube (purple top). Patients should have blood drawn after an 8 hour fast. Serum tubes are transported to PSB and allowed to clot for 30 minutes and centrifuged at 3000 rpm at RT, then aliquoted into 4 cryovials each and placed in the appropriate freezer box in the -80° in PSB 130.

1.7.2. DOWNSTREAM PROCESSING: Stool sample should be transported to PSB in a cooler box and placed in -80C freezer in PSB 130. Samples will be batch processed at the midpoint and/or end of the study.

1.7.3 COVID-19 Precautions: During blood draws and stool sample collection, study staff will wear personal protective equipment, such as gloves, face covering, and goggles or face shield. Physical equipment (chair, table, etc.) will be decontaminated between participant.

2.0 STUDY PLAN : Study Calendar

Table 2. Measures/Measurement Points	Base	2-W	POST

Primary Outcomes:			
Feasibility – enrollment, adherence, retention	X	X	X
Adverse effects			X
Secondary Outcomes:			
Generalized Anxiety Disorder-7 item	X	X	X
International Physical Activity Questionnaire	X	X	X
Hospital Anxiety and Depression Scale	X	X	X
24-hour dietary recalls -- diet composition	X	X	X
Anthropometrics – height (only at baseline), weight, waist, hips, BMI, BIA	X	X	X
Fecal samples -- gut microbiome	X	X	X
Blood samples -- serum inflammatory cytokines	X	X	X
Demographics	X	X	X

3.0 DATA AND SAFETY MONITORING

3.1.1 Frequency of Adverse Events

Adverse event toxicity will be detected during weekly telephone assessment calls and during the Follow up appointment.

3.1.2 Data and Safety Monitoring Procedures

This study does not involve the testing of pharmacologic agents nor any "therapeutic" (at least as classified under the clinical trials rubric) treatments. Thus, it is classified as a Type 3 Study (Non-therapeutic, non-physical intervention), a minimal risk level study that dictates annual review for IRB compliance. Note that research staff will be capturing adverse events that are called into the study number, as well as captured from follow-up surveys.

3.1.3 Adverse Event Reporting

Study participants will be encouraged to report any "emergencies or events" by calling the toll-free study number. These instructions will be included within the intervention materials and the toll-free number will also be affixed to the front inside cover of the study workbook. The Auburn University project manager will record all reported events in the adverse event log (including the subject's name, date, and event description – see attached log entry form). The project manager will inform the principal investigator, Andrew Frugé, PhD, immediately who will consult with Dr. Fred Kam, the Director of the Auburn University Medical Center, on the action that should be taken. This action and date of implementation also will be recorded in the adverse event log. The entire investigative team will participate quarterly in classifying any reported events as "serious" or "non-serious (see listing as follows)," as well as "non-attributable," "possibly attributable" or "attributable" to the intervention (unlike a pharmaceutical trial where known side effects exist, the classification of "expected" vs. "unexpected" is inappropriate for this behavioral intervention).

Serious—any event or condition that is life threatening, results in overnight hospitalization, cancer or a physical or cardiac event serious enough to require medical attention. A brief listing follows: Fatal, Life threatening, Permanently disabling, Required or prolonged hospitalization (Admission—not ER visit), Overdose, Significant hazard to patient, Non-Serious—all other events.

In addition, during the follow-up assessments conducted at study completion, we will query subjects whether they had "any serious health events that caused them to seek medical attention" within the reference time frame, and if any of these resulted in "hospitalizations overnight." Details of these events will be recorded. All adverse events will be reviewed on a quarterly basis by the research team. Also, in keeping with NIH guidelines, minority status and gender also will be included in these reports to allow for detection of differential effects. Because this is an exploratory study, no formal interim analyses, other than that performed for the NIH will be conducted.