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Role of Gut Microbe Composition in Psychosocial Symptom Response to Exercise Training in Breast Cancer Survivors (ROME) Study - protocol for a randomised controlled trial

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ABSTRACT

Introduction Breast cancer survivors are at increased risk for chronic fatigue and altered gut microbiota composition, both of which negatively affect health and quality of life. Exercise has been shown to modestly improve fatigue and is linked to gut microbial diversity and production of beneficial metabolites. Previous studies suggest the gut microbiota composition is a potential mechanism underlying fatigue response to exercise. Randomised controlled trials testing the effects of exercise on the gut microbiome are limited and there is a scarcity of findings specific to breast cancer survivors. The objective of this study is to determine if fitness-related modifications to gut microbiota occur and, if so, mediate the effects of aerobic exercise on fatigue response.

Methods and Analysis The research is a randomised controlled trial of 10-weeks of aerobic exercise training vs. flexibility/toning standard attention control testing aerobic exercise effects on gut microbiota composition among breast cancer survivors aged 18-74 with fatigue. All participants receive a standardized controlled feeding diet. Assessments occur at baseline, 5 weeks, 10 weeks, and 15 weeks (5 weeks after completion of exercise intervention and controlled feeding). The gut microbiota is collected by fecal samples and 16S gene sequencing will identify the microbiome. Fatigue is measured by a 13-item multi-dimensional fatigue scale. Serum cytokines, heart rate variability, and hair cortisol assays will also be obtained.

Ethics and Dissemination The University of Alabama at Birmingham Institutional Review Board (IRB) approved this study, 15 May 2019, UAB IRB#30000320. A Data and Safety Monitoring Board (DSMB) convenes annually or more often if indicated. Findings will be disseminated in peer-reviewed journals and conference presentations.

Trial registration number NCT04088708, posted 13 September 2019.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study focuses on a prevalent symptom suffered by breast cancer survivors (fatigue) and the potential role of the gut microbiome in exercise effects on fatigue.
- This study is one of the very few randomized controlled trials testing the effects of exercise on the gut microbiome, especially in cancer survivors.
- A standardized, energy balanced diet reduces diet and body weight induced variance on gut microbiota yet no prior randomized exercise and gut microbiome study has provided the same diet for all participants, as being done in our study.
- More fully understanding the mechanistic links between exercise and the gut microbiome, as proposed here, can inform future strategies to enhance the benefits of exercise on fatigue.
- Although assessors are masked to study group allocation and a standard attention control condition is used, the intervention precludes participant masking to exercise type.

INTRODUCTION

Nearly 8 million individuals worldwide are living with a history of breast cancer.[1,2] Breast cancer survivors are at increased risk of altered gut microbiota composition (i.e., dysbiosis) that may worsen future cancer risk, comorbidities, and quality of life.[3] Factors that may contribute to the persistent gut microbiota composition changes include reduced physical activity and aerobic fitness, and detrimental changes in body composition after breast cancer diagnosis.[4-7] Given its importance on health and well-being,[8-12] strategies for reversing gut microbiota dysbiosis are needed, especially in breast cancer survivors.

While elucidating gut microbiota dysbiosis in breast cancer survivors remains imperative, it is relevant that the gut microbiome is associated with fatigue in breast cancer survivors[13] and survivors rank fatigue as the number one priority related to quality of life.[14] Additionally, breast cancer survivors are more likely to report fatigue than their age matched controls[15] and one in four suffer persistent fatigue years after their cancer diagnosis,[16] which exacerbates post-cancer disability and reduces quality of life.[17,18] Furthermore, fatigue is associated with greater risk of cancer recurrence and mortality.[19] Interestingly, the benefits of supervised exercise for breast cancer survivors extend beyond the expected improvements in cardio-metabolic parameters to include improvements in fatigue and other domains of quality of life.[20] As we (and others) have reported, exercise is a well-established non-pharmacologic therapy for fatigue, yet effects are somewhat modest (weighted effect size of 0.30 in a recent meta-analysis).[21-24] Hence, elucidating mechanisms underlying fatigue response is needed to optimize fatigue reductions for non-responders and increase effect sizes achievable with exercise.[24-27] Moreover, our prior work and that of others suggest the gut microbiota composition is one such mechanism, but further research is needed.[13,28]

Exercise training also presents as a promising strategy for reversing dysbiosis as it is linked to gut microbial diversity, abundance of select microbes, and production of beneficial metabolites (e.g., acetate, butyrate, propionate), albeit, these phenomena are currently limited to animal models or cross-sectional[29-36] and non-randomized prospective human studies.[37] Randomized controlled trials testing the effects of exercise on the gut microbiome are limited[38] and there is a scarcity of findings specific to breast cancer survivors.[7] One randomized controlled trial in healthy overweight and obese individuals found vigorous-intensity exercise training was associated with increased microbe diversity.[38] To support the importance of intensity in exercise training, we recently showed in breast cancer survivors, cardiorespiratory fitness was a better correlate of gut microbe diversity compared to free-living activity energy expenditure.[7] It is unknown if the modulation of the microbiota by exercise occurs solely through direct means such as alterations to colonic transit time, [39,40] or indirectly through inflammation,[41-43] autonomic nervous system,[44,45] or hypothalamic-pituitary-adrenal (HPA) axis.[46-48] Additionally other lifestyle interventions such diet[49] and body weight changes[50] independently affect gut microbiota, making controls for these variables critical in exercise trials. Rigorously testing the dysbiosis-exercise link while also exploring the bidirectional gut-brain axis pathways responsible for exercise effects[51,52] can inform future exercise recommendations and multimodal interventions to counter the adverse effects of gut dysbiosis.

Given the potential benefits of exercise training on the gut microbiome and fatigue, a better understanding of their relationships in response to an exercise intervention among breast cancer survivors is warranted. Herein, we describe our ongoing randomized controlled trial testing aerobic exercise training as a potential strategy to attenuate dysbiosis in breast cancer survivors with fatigue while also standardizing diet intake and maintaining energy balance. We

further propose to determine if fitness-related modifications to gut microbiota mediate the effects of aerobic exercise on fatigue response. This is a critical next step for several reasons. First, to our knowledge there are currently no completed randomized controlled trials utilizing exercise training as a potential modifier for dysbiosis in breast cancer survivors.[53] Additionally, no other trials exploring these variables have been performed with a standardized diet to: 1) mitigate the underlying variance on gut microbiota and 2) promote weight maintenance.[54,55] Therefore, we describe our methods to facilitate future replicability.

METHODS

Aims and hypotheses

The primary study aim is to determine the effects of a 10-week aerobic exercise training intervention compared to a flexibility/toning standard attention control on gut microbiota composition among breast cancer survivors with fatigue. All participants are following an energy balanced controlled feeding diet. The gut microbiome is being collected by fecal sample and assessed by 16S rRNA at baseline, week 5 to explore interim changes, week 10 as our primary time point, and week 15 to explore durability of effects. We hypothesize that compared to the control, the exercise training group will demonstrate significant differences in gut microbial diversity with increased Firmicutes (p), *Bacteroides (g)*,[7,56] and *Bifidobacterium (g),*[57] and decreased Actinobacteria (p) and Proteobacteria (p).[7]

A secondary study aim is to test if exercise training affects the gut microbiota composition directly and/or indirectly through inflammation, autonomic nervous system, or hypothalamic-pituitary-adrenal (HPA) axis mediators. We hypothesize that exercise training will have direct and indirect effects on gut microbiota composition through markers of the

hypothesized mechanisms (interleukin [IL]-6, IL-10,[41-43] heart rate variability,[44-46] cortisol[46-48]). Another secondary study aim is to test if the exercise training effect on fatigue is direct and/or indirect through changes in the gut microbiota composition. We hypothesize that exercise effects on fatigue will be mediated by changes in beta diversity,[13,58] specifically frequency of Firmicutes (p),[7] Actinobacteria (p),[13] and *Bacteroides (g)*.[13,41,59]

Overall mechanistic framework

Given the relationships between cardiorespiratory fitness and gut microbiota composition,[7] we have chosen an exercise intervention applying the principles of exercise prescription required to achieve an increase in cardiorespiratory fitness.[60] The biological plausibility of a dysbiosis-exercise link also common to fatigue (e.g., inflammation, autonomic nervous system, and HPA axis)[48,61-66] supports testing these potential mechanistic links in breast cancer survivors with fatigue. Thus, the overall mechanistic framework for our trial depicted in Figure 1 can be applied to potentially optimizing exercise interventions for treatment of fatigue.

Study overview and eligibility criteria

This 2-arm, parallel group-controlled trial is randomising breast cancer survivors to 10 weeks of supervised aerobic exercise training or standard attention control (flexibility/toning) while on a controlled feeding diet. The trial is taking place at the University of Alabama at Birmingham (UAB) in Birmingham, AL. Participant enrollment commenced 1 January 2020, was paused between March 2020 and August of 2020 due to the COVID-19 pandemic, and is projected to end 1 January 2025. Institutional Review Board (IRB) approval has been obtained and all participants provide informed consent prior to participation. Assessments occur at baseline and then at 5, 10, and 15 weeks. A study schema is provided in Figure 2 and an overview of participants' activities is provided in Table 1. An electronic study manual of procedures is kept

on a shared, HIPAA compliant cloud server accessible to all study staff.

Inclusion criteria are as followed: 1) female breast cancer survivors ages 18 to 74 years with a history of ductal carcinoma in situ (DCIS) or stage 0, I, II, III breast cancer, 2) who are ≥1 year post-primary cancer treatment completion (chemotherapy and/or radiation), 3) average fatigue over the past week rated as ≥3 on a 1 to 10 Likert scales,[67] 4) English speaking, 5) physician medical clearance for study participation, 6) able to ambulate without assistance, 7) no antibiotics for the past 90 days, 8) willing to avoid taking probiotics for the duration of the study, and 9) after all other criteria are met, lab-based screening is used to confirm low fitness level ($\dot{V}O_{2peak}$ < 30 mL/kg/min). Exclusion criteria are as follows: 1) metastatic or recurrent cancer, 2) another diagnosis of cancer in the past 5 years (not including skin or cervical cancer *in situ)*, 3) unstable angina, 4) New York Heart Association class II, III, IV congestive heart failure, 5) uncontrolled asthma, 6) interstitial lung disease, 7) current steroid use, 8) having been told by a physician to only do exercise prescribed by a physician, 9) dementia or organic brain syndrome, 10) schizophrenia or active psychosis, 11) connective tissue or rheumatologic disease, 12) anticipate elective surgery during the study period, 13) anticipate changes in usual medications during the study period, 14) plan to move residence out of the local area during the study period, 15) plan to travel out of the local area >1 week during study participation, 16) contraindication to engaging in moderate-to-vigorous intensity aerobic exercise, 17) current or anticipated pregnancy during study participation, 18) live or work >50 miles from study site or do not have transportation to study site, 19) body mass index (BMI) >50 (confirmed during lab-based screening), 20) anticipate needing antibiotics during the study period, or 21) any social, psychological, or physical condition that interferes with the participant's ability to complete study activities or unduly increases study risk.

Recruitment and screening

Participants are being recruited through multiple recruitment strategies (e.g., recruitment letters mailed to breast cancer survivors identified through the UAB O'Neal Comprehensive Cancer Center registry, UAB investigators' waiting lists of cancer survivors inquiring about exercise and weight loss studies, newspaper advertising, cancer support groups, institutional websites and group emails, relevant non-institutional websites, flyers in waiting areas [hospitals, physicians' offices]). Referrals from oncologists and other relevant health care providers are being requested using messaging (i.e., electronic health records or institutional email) and face-to-face meetings; recruitment materials such as patient flyers are provided, as appropriate. Potential participants are given a description of the study and screened for eligibility based on a pre-determined telephone script. In addition to questions related to the above eligibility criteria, participants are asked the following diet questions in the prescreening telephone screen to assess potential controlled feeding adherence and safety issues: 1) *do you have any food allergies, restrictions, preferences or special diet (vegetarian, gluten-free, etc.)*, 2) *are you willing to eat the meals we provide*, 3) *do you drink alcohol? If yes, are you willing to refrain from alcohol during your participation in this study*, and 4) *do you foresee any barriers to picking up the food, storing food, or doing minimal meal preparation*?

Enrollment and randomization

Interested potential participants who pass the pre-screening telephone interview are invited to an orientation visit (in person or by videoconference) to complete administrative forms, sign lab-based screening consent, and complete release forms for obtaining medical clearance with the study coordinator. Once medical clearance is received, the participant is scheduled for a lab-based screening visit which includes VO_{2peak} to confirm cardiorespiratory fitness < 30 ml/kg/min

and BMI ≤ 50 (see Section 3.5.3 for methods). If deemed eligible at the lab-based screening visit, informed consent for full study participation is obtained, including optional permission to retain health information and biospecimens for future research. The participant is scheduled for initiation of controlled feeding and baseline assessment visits #1 and #2 (Figure 3).

Participant randomization is based on computer generated random numbers and performed in blocks of 4 to facilitate an equal distribution between the two study groups. BMI is an important biological variable associated with gut microbiota composition,[18,68] hence randomization is stratified by BMI (< 30 *vs.* ≥ 30). The study statistician performed the computer generation of random numbers which were placed in sealed, opaque envelopes and delivered to the recruiting staff with written protocol for use. Assignments are made in the order in which participants complete baseline testing and are kept in the sealed envelope until the participant has completed all baseline testing. Once the study coordinator confirms completion of baseline testing, the coordinator chooses the next envelope with group allocation. Participants remain partially blinded to study condition (e.g., will not be told which study condition [exercise training or flexibility/toning intervention] is expected to yield more benefits and all receive controlled diet which is potentially perceived as a "treatment".) Assessments, assays, and data entry are conducted using objective and validated measures by staff who will remain blinded to study arm status.

Assessments

Schedule and masking

Assessments occur at baseline (pre-intervention), 5 weeks (mid-point intervention), 10 weeks (immediately post intervention), and 15 weeks (5 weeks post intervention) and are

performed by staff who are masked to participant study group allocation. Table 1 presents the timeline of data and measures collected at each assessment visit. If eligible based on lab-based screening and participant consents to full study participation, then controlled feeding preparations are made and the baseline visit #1 is scheduled for one week after controlled feeding begins (Figure 3). For each assessment, the participant completes two visits to the exercise testing laboratory. In preparation for assessment visit #1, participants are provided instructions for the lab-based measurements (location, parking, 12-hour fast, appropriate clothing, etc.). During assessment visit #1, the participant provides a hair sample, completes the fasted blood draw, resting energy expenditure by indirect calorimeter, resting heart rate variability (Actiheart), dual-energy X-ray absorptiometry (DXA), and walking economy (i.e., net $\dot{V}O_2$). Because the $\dot{V}O_{2peak}$ and BMI measurements are taken at the screening visit, these are not repeated at baseline but are repeated at the follow-up assessments. During assessment visit #1, study staff provide the participant with the additional assessment materials (survey, accelerometer with log, 3-day diet record, medication log, fecal sample kit, etc.) and related instructions. The participant ships the fecal sample back to the UAB microbiome laboratory within 7 days of visit #1 and returns the remaining assessment materials at assessment visit #2. To better align the temporal relationship between the gut microbiome and fatigue, the fatigue scale is collected at assessment visit #2 (i.e., several days after fecal sample collection).

Gut microbiota composition

Participants are provided with a stool collection kit at each baseline and follow up assessment visit #1 to self-collect the stool sample at home according to provided instructions. Briefly, the instructions are to collect the sample in a clean dry study-provided collection hat and scoop a small amount into the provided ParaPak vials (Meridian Biosciences, Inc; Cincinnati, OH) pre-

labelled with participant identification and assessment timepoint, and then ship the sample back to our site via pre-paid overnight shipping materials. Once received by the microbiome lab, each sample is aliquoted into labelled cryovials and stored at -80°C until time for DNA extraction and 16S rRNA processing. One cryovial of precisely 100 uL is retained and labeled for future metabolomics assays (if indicated and funds can be obtained).

With each sample collection, the participant completes a fecal sample questionnaire[69] and returns it to the research staff. The questionnaire asks the participant to report changes in normal diet and vitamin supplements; recent gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, and constipation); and usual frequency or changes in probiotic supplements, yogurt intake, and high fiber foods or fiber supplements. Participants also report recent medical treatments such as antibiotics, chemotherapy, or radiation therapy, and if they have ever had a major bowel resection, gastric bypass surgery, an inflammatory bowel disease (such as Crohn's disease, ulcerative colitis, indeterminate colitis), or irritable bowel syndrome. The participant is also asked to complete a 3-day diet record capturing dietary intake 2 days prior to and the day of fecal sample.

Cardiorespiratory fitness ($\dot{V}O_{2peak}$)

Participants perform a graded treadmill (TrackMaster TMX428CP; Full Vision, Inc.; Newton, KS) test in accordance with the modified-Balke protocol to elicit $\dot{V}O_{2peak}$ (i.e., the highest measured rate of oxygen uptake expressed in mL/kg/min. Initially, $\dot{V}O_2$ is stabilized over a 3-minute period of standing rest, after which, participants begin walking at 2.0 mph at 0% grade for 2 minutes. Grade is then increased 3.5% every 2 minutes until the 12th minute, at which point, grade is decreased to 12% and speed increased to 3.0 mph. Grade is increased by 2.5% each minute (as needed) until volitional exhaustion. $\dot{V}O_2$ and related gas exchange measures are

aggregated in 30-s bins and determined by open-circuit spirometry (True One 2400 system; ParvoMedics, Salt Lake City, UT). Gas analyzers and flowmeter are calibrated prior to each test using standard gases and 3-L syringe, respectively. Heart rate and Rating-of-Perceived Exertion (RPE; Borg 6-20, 6 = *no exertion at all, relaxed* and 20 = *maximal exertion*)[70] are recorded in the final 30 seconds of each stage. Blood pressure is measured via auscultation at minutes 6, 10, 14, 16 and/or final stage of the graded treadmill test.

Serum cytokines

Inflammatory cytokines, interleukin (IL)-6 and IL-10, are collected by blood samples. Participants are instructed to abstain from vigorous exercise, smoking, and alcohol for 24 hours prior and fast for 12 hours prior to the blood draw. Blood samples are collected, processed and stored (-80°C) using standard operating procedure consistent with expert consensus recommendations[71] and batch analyzed according to manufacturer's instructions by staff who are blinded to the participant's group allocation.[64] Serum cytokine assays will be analyzed by the UAB Metabolism Core using a MSD imager (MesoScale Discovery, Gaithersburg, MD; chemiluminescence technology; multiplex platform). Blood and serum samples are being processed and stored so that future metabolomic/functional metabolic studies can be done if indicated and funds can be obtained. A 7-day medication log is collected with each blood sample for medication changes between assessments that may influence study outcomes (e.g., anti-inflammatory agents, etc.).

Heart rate variability

Heart rate variability is evaluated with the Actiheart 5 (CamNtech Ltd., Cambridgeshire, UK) device. First, a urine sample is collected from participants to measure urine specific gravity – an indicator of hydration status. In accordance with manufacturer guidelines, skin is prepped with a

70% isopropyl alcohol wipe before positioning a two-lead electrode arrangement in the upper left quadrant across the participant's chest. Measurements are collected during 5-minutes of quiet rest in the seated position. High-frequency sampling is used to measure inter-beat intervals wherein Actiheart® software is used to perform offline analyses. The primary variables of interest include heart rate and root mean square of successive RR interval differences (RMSSD) as well as the low-frequency (LF), high-frequency (HF) components derived from the fast-Fourier transform. Procedures are performed in the morning hours in a dimly-lit, temperature-controlled room.

Hair cortisol

Hair specimens are collected by trained study staff. For participants whose hair is longer than 1.5 to 3 cm, a thin layer of hair (1-2 hairs thick) parallel to the floor is cut from a point close to the scalp across a 4-5 cm length (laterally), to obtain a minimum of 50 strands of hair. For participants with shorter hair, the lateral cut is 6-8 cm (2 cm vertical x 5 cm lateral for long hair, > 2 cm vertical x 7 cm lateral for shorter hair). String is used to indicate the end of the hair closest to the scalp; hair specimens are folded tightly into aluminum foil and placed in a small labeled bag at room temperature until being sent for assay at the Department of Biopsychology at Technische Universität Dresden in Dresden, Germany.

Fatigue

Fatigue is measured by a 13-item multi-dimensional fatigue scale (i.e., Fatigue Symptom Inventory).[72] On a 1 to 10 scale (1 = *not at all fatigued*, 10 = *as fatigued as I could be*), participants are asked to rate their level of fatigue on the day they felt most and least fatigued in the last week, the average level of fatigue in the last week, and the level of fatigue at the time of survey. Participants report how much fatigue interferes (1 = *no interference*, 10 = *extreme*

interference) with their general level of activity, ability to bathe and dress, their normal work activity, ability to concentrate, relations with other people, enjoyment of life, and mood. Participants report how many days in the past week they felt fatigued for any part of the day and how much of the day on average the participant experienced fatigue (1 = *none of the day*, 10 = *the entire day*). Since our prior studies have demonstrated that exercise effects on fatigue may vary by dimension (i.e., intensity *vs.* interference; intensity = mean of 4 items; interference = mean of 6 items, 0 to 10 scale) our final analyses will focus on fatigue interference.

Potential covariates

Self-administered survey measures age, race/ethnicity, education level, annual household income, marital status, smoking history, alcohol intake, employment status and number of recent sick days, cancer-related factors (date of diagnosis, stage, subtypes [e.g., receptor status], current and past cancer treatment type [including, but not limited to, radiation, chemotherapy, and anti-estrogen therapy]), caffeine intake, dietary supplements (including prebiotic, probiotic, and vitamins), current medications (including over the counter medications), any antibiotic medications over the last 6 months, any steroid medications or injections over the last 6 months, current/past diagnosis of and treatment for anxiety or depression, treatment duration, time since treatment completion), medical comorbidities[73] (including but not limited to endocrine or hormone disorders), history of surgeries, menopausal status,[6] and history of COVID-19 diagnosis. If a participant is not able to recall medical-related information, a medical release form is completed allowing study staff to request this information from the participant's physician.

Because depression, anxiety, sleep quality, pain and fatigue may cluster and be associated with inflammation,[74-76] depression and anxiety is measured by 14-item Hospital Anxiety and

Depression Scale [HADS]),[77] sleep dysfunction is measured subjectively using the Pittsburgh Sleep Quality Index (PSQI)[78], and pain is measured by the Patient Reported Outcomes Measurement Information System (PROMIS®; http://www.nihpromis.org/default.aspx).[79] Because post-traumatic stress symptoms are associated with psychosocial outcomes and gut microbiota composition,[80,81] post-traumatic stress is measured using the Posttraumatic Stress Disorder Checklist (PCL).[82-85]

To assess free-living physical activity, participants are given the same ActiGraph accelerometer (ActiGraph LLC; Pensacola, FL) device for each assessment to be worn at the waist for seven consecutive days during waking hours (non-dominant hip; same side each time). Participants are instructed to remove the accelerometer while bathing, showering, or swimming and are asked to complete an accelerometer log (times device removed, exercise not detectable by device, sleep times, etc.). The accelerometer is set for 30 second epochs and monitoring is repeated if less than four valid days are recorded. Non-wear time is defined when no motion is detected for 60 minutes. A valid day is defined as at least 10 hours of valid wear time. The following cut points are planned: Sedentary: 0 – 99 counts/min; Inactive: 100 – 499 counts/min; Light: 500 – 1951 counts/min; Moderate: 1952 – 5724 counts/min; and Vigorous: 5725+ counts/min.[86,87] Leisure-time physical activity is measured using the Godin Leisure Time Exercise Questionnaire which asks for average weekly frequency of leisure-time exercise for periods exceeding 10 minutes over the past month per three activity intensity levels (light, moderate, or vigorous).[88,89]

Body mass index (BMI) is calculated from weight and height [weight (kg)/height (m^2)] obtained from a scale (in light clothing) and wall stadiometer (without shoes). Dual-energy X-ray absorptiometry (DXA) scans assess lean mass and fat mass using the Lunar Dual Energy X-ray

Absorptiometry Scanner (iDXA; Lunar Radiation Corp. Madison, WI). Pre-menopausal women at risk for pregnancy undergo a urine pregnancy test prior to each DXA scan.

Other relevant measurements

Resting energy expenditure measurement is required to more accurately assess participant's calorie needs for the controlled feeding which facilitates energy balance and resultant weight maintenance during the study. Hence, resting energy expenditure is measured by ventilated hood indirect calorimetry (True One 2400 system; ParvoMedics, Salt Lake City, UT) while lying quietly on an exam table. Participants must fast for at least 6 hours prior (4 hours if they are diabetic), avoid physical activity for 12 hours and avoid any caffeine or nicotine for at least 2 hours prior to this test.

Although not originally proposed, walking economy (i.e., net $\dot{V}O_2$) was added because it reflects oxygen uptake during ambulation, an important alternative measure of (mobility) independence in older women.[90] Participants wear a hip-worn accelerometer and complete a fixed-workload task by walking on a treadmill at 2.0 mph (0% grade) for six minutes during which steady-state $\dot{V}O_2$ is reached. RPE (Borg 6-20, 6 = *no exertion at all*, relaxed and 20 = *maximal exertion*)[70] is collected at minutes three and six. At minute 5, the participant reports perceived difficulty of the test using a visual analogue scale (100 mm line). Blood pressure is measured at rest and while standing. Blood pressure is also measured at the 1-, 2-, and 5-minute timepoints during walking. Participants remain quietly seated for at least 10 minutes between the walking economy and $\dot{V}O_{2peak}$ tests during the follow-up assessments.

Quality of life is measured with The Functional Assessment of Cancer Therapy-Breast (FACT-B)[91] because of its relation to fatigue, relevance for breast cancer populations, and repeated use in prior studies which allow for comparison of study results. The FACT-B is a 37-item

instrument using 5-point Likert scales and includes the subscales of physical well-being, social well-being, emotional well-being, functional well-being, and additional concerns.[91]

Since cognitive function is associated with the gut microbiome[92] and physical activity in breast cancer survivors,[93] cognitive function is measured with the 10-item Frequency of Forgetting scale.[94] The summed score will assess subjective memory impairment (Total score) along with 4 memory subscales (general memory, frequency of forgetting, frequency of forgetting when reading, and remembering past events).

To improve adherence to future, similar exercise training protocols, the self-administered survey assesses social cognitive theory constructs: exercise self-efficacy (barriers and walking), enjoyment, social support, barriers, and outcome expectations. Barriers self-efficacy (i.e., confidence in ability to overcome barriers) is measured utilizing a 9-item scale specifically designed for breast cancer patients.[95] The scale utilizes frequently reported barriers among breast cancer patients (e.g., "*How confident are you that you can exercise when you are tired?*"). Walking task self-efficacy scale is assessed with a 6-item scale asking participants to rate confidence in their ability to walk at a moderately fast pace for 5, 10, 15, 20, 25, and 30 minutes.[96] Analyses for barriers and walking task self-efficacy are using the mean score for the Likert scale (0% = *not at all confident* to 100% = *extremely confident*). Perceived exercise barriers (or barriers interference) are measured by asking participants to rate on a 5-point Likert scale (1 = *never* to 5 = *very often*) how often 21 different barriers (e.g., lack of time, weather) interfere with exercise. The items are summed for a perceived barriers score.[97-99] Physical activity enjoyment is measured with a single question (5-point Likert scale).[99] Social support is measured by asking for the frequency with which friends (two items) or family (two items) encourage or offer to exercise with the participant. Items are summed for a friends, family, and

total social support score.[100,101] For outcome expectations, participants are asked to rate their agreement on a 5-point Likert scale (1 = *strongly disagree* to 5 = *strongly agree*) with the statement that exercise would result in 17 potential benefits or risks. Fourteen positive benefits (e.g., feel less depressed) and 3 negative outcomes (e.g., increased joint pain) are included. Responses are summed for positive outcome expectations and negative outcome expectations.[99] The participants answer the outcome expectation questions twice: once considering stretching and light resistance exercises and again considering aerobic exercise.

Participant satisfaction

At the 15-week assessment, participants are asked to provide a written evaluation of the study staff and procedures. All participants are asked to report their agreement (Likert scale; 1 = *strongly disagree* to 5 = *strongly agree*) with 10 statements relating to the clarity of study information, helpfulness of staff interactions, palatability of the provided food and ease of following the menu, likelihood of recommending this study to others, and overall satisfaction with the study staff and activities. One open-ended question seeks any additional information they would like to share with the study team.

Data quality control

Multiple strategies are being used to minimize missing data (e.g., baseline testing and controlled feeding before randomization provides a *"run-in"* period, monetary and non-monetary incentives, up to date contact information, ongoing review of source documents by study coordinator for immediate rectification of missing data, etc.).[102] Study staff are trained by the investigator with the relevant expertise using electronic manual of procedures with regular review of source documents for quality. Multiple trained staff are present during in-person assessment activities increasing accountability and immediate identification of potential drift in

protocol adherence. All most recent IRB approved study forms are stored on a shared, HIPAA compliant cloud server.

Interventions

Supervised exercise sessions

Participants are randomized to 10 weeks of either an aerobic exercise intervention or a flexibility/toning attention control condition. Sessions occur on nonconsecutive days of the week at the study site and are supervised by experienced exercise specialists who are not involved in the collection of outcome assessments.

Aerobic exercise sessions

Aerobic exercise sessions, supervised by trained exercise specialists, are primarily performed using the treadmill. However, the cycle ergometer may be used if preferred by the participant. The training target heart rate zone for each session corresponds with the heart rate at a given percentage of $\dot{V}O_{2peak}$ measured at the most recent assessment. Training sessions commence with a 5-minute warm-up consisting of light treadmill walking and stretching. During the 1st week of training, after warm-up, participants perform 20 minutes of exercise at ≈60% maximum heart rate (equivalent to ≈45-50% $\dot{V}O_{2peak}$). Over the next 3 weeks, *exercise duration* is increased by 5-minute intervals, as tolerated, so that by the beginning of the 5th week participants are exercising for 40 minutes. This coincides with an elevation in *exercise intensity* equating to ≈75% of maximum heart rate (≈55-60% of $\dot{V}O_{2peak}$) by the 5th week. Following each exercise bout, participants cool down for 3-5 minutes. To mitigate stagnation, and facilitate continued improvement of $\dot{V}O_{2peak}$,[103] high-intensity interval exercise is added during weeks 5-10 as described in Table 2. Eight to ten work-intervals are performed at a workload to elicit ≈85-90%

maximum heart rate for 60 seconds with rest intervals of 3 minutes with the total exercise duration ranging from 20 to 40 minutes.

Standard attention controls

The non-aerobic exercise attention control condition controls for the effects of attention and social interaction through administration of flexibility/range-of-motion activities using light resistance bands delivered at the same frequency as the aerobic condition (i.e., 3 times per week). The sessions last about 40 minutes and target the head/neck, shoulder, elbow/forearm, hand/wrist, trunk/hip, and ankle/foot. The progression of activities over the 10-week period involves performing additional exercises and sets (i.e., Thera-bands) that provide minimal resistance (i.e. sham). The first 5 weeks of the control condition involve performing body stretches without resistance (20-30 seconds for 1-2 sets). In weeks 6-7, the light resistance Thera-band is used to perform the stretches for the upper-extremities once per week for 8-10 repetitions for 2 sets, and the other two sessions are body stretches without resistance. In weeks 8-10, the light resistance Thera-band is used twice per week for 8-10 repetitions for 2 sets, and one session will be body weight stretches without resistance. Such a progression is not expected to induce aerobic fitness adaptations and is designed to maintain participant interest and expectation of treatment benefit. Control condition participants are asked to not undertake additional exercise (e.g., not join a gym and begin exercising) during the 10-week intervention period.

Missed exercise and control sessions

Session attendance is tracked weekly and missed sessions are made up as soon as possible during the intervention period. No more than four supervised aerobic sessions will occur in one week. Exercise specialists encourage exercise adherence by discussing social cognitive theory based educational newsletters with participants at six time points during the 10 weeks of aerobic

exercise and standard attention control.[104]

Controlled feeding

Controlled feeding provided by the UAB Center for Clinical and Translational Science (CCTS) Metabolic Kitchen standardizes dietary intake across all participants. The menus are designed to provide 55% of energy as carbohydrate primarily through complex sources (fiber: 21-38 g/day), 23% as fat, and a minimum of 22% as protein (≈0.8 g/kg). Dietary sodium intake and the polyunsaturated:saturated (P:S) fat ratio are held constant (sodium <3500 mg/d, P:S fat ratio of 1, and saturated fat less than 30% of total fat intake).

Prior to initiating controlled feeding, the participant meets with a study registered dietitian to review the study menu and collect information about food allergies and intolerances. Changes to the menu based on dietary preferences are attempted if substitutions are accessible to the Metabolic Kitchen and maintain the standardized diet protocol. The participant and study dietitian meet a second time to review the final menus and discuss approved beverages and seasonings. Each participant starts weekly meal pick up from the Metabolic Kitchen at least one week before baseline assessment visit #1.

To allow the Metabolic Kitchen time to prepare the controlled feeding, the daily calorie need (total energy expenditure) is estimated pre-baseline using the Harris Benedict equation and an activity factor to promote weight maintenance. This estimate is then updated once resting energy expenditure data is available at the baseline assessment. The estimate of total energy expenditure is further updated for participants randomized to the aerobic exercise condition using the individual's $\dot{V}O_{2peak}$ and resting energy expenditure data based on prior work by the investigative team (equation provided in Supplemental Material 1).[105,106] The total energy expenditure estimates for all participants are updated, if appropriate, based on the week 5

assessment of VO_{2peak} and resting energy expenditure. A study registered dietitian monitors body weight weekly and uses these changes and participant dietary preferences to further refine the calorie content and menus.

Controlled feeding adherence

Menu checklists are included with each weekly food pick up and participants are asked to log how much of the provided foods they consume and report additional foods and beverages along with the amounts consumed. The menu checklists are returned at exercise and control sessions on a weekly basis and reviewed by the dietitian for adherence. Participants with potential adherence issues or missing or incomplete checklists are called by a study dietitian for reminders and instruction.

Staff training

Staff are trained using a variety of electronic manuals, protocols, and up-to-date IRB approved study forms and scripts. An electronic manual of procedures is maintained in a shared, HIPAA compliant cloud server for reference by staff. Given the range of staff responsibilities (i.e., exercise intervention, diet, etc.), additional supplemental role-specific protocols are also maintained (e.g., exercise progression prescription for exercise specialist and controlled feeding menu review scripts for dietitian).

Intervention fidelity plan

The exercise and controlled feeding intervention fidelity plans include the five domains recommended by NIH Behavior Change Consortium[107] (i.e., study design, provider training, treatment delivery, treatment receipt, and enactment of treatment skills). Fidelity is facilitated

with the electronic manual of procedures, standardized scripts, and participant education materials. Data sources for tracking exercise intervention include review of all exercise session record sheets (i.e., attendance, if exercise goals met, and if exercise progression administered according to protocol) and direct observation by each interventionist at least once a month. The main data source for tracking controlled feeding fidelity are menu checklists on which the participant reports the provided foods consumed and any additional foods/beverages consumed. The food included in each controlled feeding pick up is reviewed for accuracy and completeness by a trained research staff before the food is given to the participant. Further, study registered dietitians offer the same food substitutions for all participants requesting a change. Monthly reports are presented to the study team to monitor fidelity of both the exercise and controlled feeding so that fidelity concerns can be rectified in a timely manner.

Statistical analysis

Sample size and power considerations

Sample size is based on detecting alpha diversity and beta diversity taxa comparisons. The power calculation is based on two-tailed test at power of 0.8 using software G*Power version 3.1.9.2.[108,109] Our pre-COVID pandemic sample size was estimated at 126 (63 in each group) with 100 (50 per study group) remaining after drop outs. This sample size would have allowed us to detect a medium effect size (d = .57; power of 0.8, $p < 0.05$) in alpha diversity which is sufficient for detecting effects related to associations with fatigue and intervention effects falling midway between that found in our two pilot studies. Relevant to taxa comparisons, we have > 0.8 power to detect the effect of any of the taxa after multiple testing correction (q value < 0.05).[110-112] Due to the detrimental impact of the COVID-19 pandemic on recruitment into on-

site, supervised exercise trials, we provide revised contingency power calculations in Figure 4, where we can see that with sample size decreasing, the effect size we can detect changes from moderate to large. For example, for enrolling at 100%, 75% (74 samples with 37 per group), and 50%, the effect size that can be detected changes from 0.57, to 0.67, and to 0.81 (with power of 0.8 and alpha of 0.05). Of note, larger effect sizes are possible in this study (compared to our pilot studies) because the study will provide controlled feeding (reducing variability), select low fit individuals (greater chance of improvement), and manipulate the exercise exposure (standardize the exercise exposure). Also relevant, the sample sizes in our pilot studies (N = 12 and 37) were smaller than our proposed study even with dropped enrollment yet yielded statistically significant results (e.g., a significant association between alpha diversity and cardiorespiratory fitness in 37 breast cancer survivors).[7,13]

Data management and analysis considerations

Microbiome 16S gene sequence data is analyzed using the QIIME[113] analysis package, our in-house developed automated analysis pipeline QWRAP,[69] and DADA2[114] to provide a robust error model for sample filtering and clustering. Data quality is assessed using FASTQC, with low-quality data filtered out using the FASTX toolset. Filtering, denoising, and clustering of reads into Amplicon Sequence Variants (ASVs) is done using DADA2. Taxon assignment is performed using Mothur[115] and the SILVA 16S rDNA database.[116] Alignment and phylogenetic inference is then performed using PyNAST[117] and Fasttree.[118] Comparative analytical tools such as UniFrac[119] are used to assess differences between samples and sample groups using principal coordinates analysis. To expedite sample processing and reporting, QWRAP automates the running of these tools using a single command line argument on UAB's high-performance computing cluster, Cheaha.

Survey and other data entry and checking is conducted by trained research staff masked to study group allocation using password protected Research Electronic Data Capture (REDCap). Data analyses will be carried out on an intent-to-treat basis. A multiple imputation approach will be employed to handle any missing data that cannot be rectified and we will conduct sensitivity analysis to assess the robustness of our findings.[102,120] SAS software, Version 9.3 (SAS Institute Inc., Cary, NC) and R software, version 4.3.1[121] will be used for data analysis. Transformations and non-parametric procedures will be performed when needed. The false discovery rate (FDR) will be used for multiple testing correction and the statistical significance threshold will be FDR $q \leq 0.05$ (q value is a p value after FDR correction). Each element (i.e., alpha diversity, beta diversity, and taxa level comparisons) describes a different perspective on gut microbiota changes and are integrated for interpretation (e.g., does exercise change the relative abundance of organisms and, if so, which organisms). We will assess the microbiota composition change over time using mixed-effects models.[122] All mediation analyses will conduct indirect effects analysis with the bootstrap method developed by Hayes.[123] Week 10 is our primary time point yet we will also analyze week 5 to assess interim changes that occur and week 15 to assess durability.

Participant safety and withdrawal

Risk management and safety

Participant safety is facilitated by obtaining medical clearance, limiting to a BMI < 50, collecting a medical history and the PAR-Q (physical activity readiness questionnaire) before the lab-based screening, and consulting clinical investigators, if indicated. Exercise sessions are supervised by exercise specialists who have experience training cancer survivors or chronic disease populations. Additionally, physician supervision is provided during fitness testing when deemed

appropriate based on ACSM guidelines.[124] Information about food allergies and intolerances are screened for and collected before initiating controlled feeding and throughout participation and these are communicated to the Metabolic Kitchen to minimize allergen contamination.

Adverse event reporting

Adverse events are identified spontaneously (e.g., reported to research staff during contact time) or non-spontaneously (structured interview done at each assessment time-point). Reported adverse events are reviewed promptly by the PI and reported to the IRB according to local requirements. A Data and Safety Monitoring Board (DSMB) is convened annually or more often if indicated.

Handling of withdrawals

Participants are informed of their right to withdraw at any time without consequences in the informed consent forms and during the signing of consent forms. Participants will be withdrawn from the study if any social, psychological, or physical conditions arise that may unduly increase risk of participating in the study. Data will be analysed on an intention-to-treat basis.

Unexpected Required Antibiotics

Given the effect of antibiotics on the gut microbiota composition, participants unexpectedly requiring intensive antibiotic therapy while enrolled in the study will be withdrawn from the study. Intensive antibiotic therapy is defined as intravenous, extended use (i.e., ≥ 2 weeks), or combined therapy (multiple broad-spectrum agents). Less intensive antibiotic use will be tracked by self-administered survey and considered during the analyses.

Patient and public involvement

Patients and members of the public were not involved in the design of the trial.

Ethics and dissemination

The University of Alabama at Birmingham Institutional Review Board (IRB) approved this study, 15 May 2019, UAB IRB#30000320. The trial is registered with ClinicalTrials.gov: NCT04088708. A Data and Safety Monitoring Board (DSMB) convenes annually or more often if indicated. Any amendments will be submitted to the IRB and DSMB for approval. Research findings will be disseminated in peer-reviewed journals and conference presentations.

DISCUSSION

The ROME study is the first randomized controlled exercise training study in fatigued breast cancer survivors testing exercise effects on gut microbiota composition while standardizing dietary intake with rigorous attention to energy balance. Our careful attention to diet and energy balance is critical to more fully understanding the role that exercise can play in altering dysbiosis in breast cancer survivors, a group at increased risk for detrimental changes in gut microbiota composition. Also, understanding the potential mechanistic links between aerobic exercise training, gut microbiota composition, and fatigue in cancer survivors has great potential to improve the lives of the breast cancer survivors suffering fatigue.

Thus, we describe a highly rigorous trial that is especially appropriate for studying exercise, gut microbiome and fatigue in breast cancer survivors because it integrates a standard attention control condition and energy balanced controlled feeding. The standard attention control condition is critical to detecting exercise effects on this patient-reported outcome beyond staff attention alone.[125] Further, few randomized trials testing exercise effects on the gut microbiome have attempted to standardize diet intake with energy balanced controlled feeding, a critical

element given the strong association between diet, body weight, and the gut microbiome characteristics.[49,53,126]

Given the careful attention to the temporal relationships and randomized study design, this study will explore mechanistic pathways heretofore most frequently studied in animal models rather than humans. With regard to the potential mechanisms through which exercise influences the gut microbiome, we will explore exercise induced changes to inflammation, the autonomic nervous system, and the HPA axis. Exercise training in breast cancer survivors positively impacts inflammatory markers.[127] In particular we have previously observed beneficial changes in IL-10 and tumor necrosis factor (TNF)-α.[25] A better understanding of the bidirectional communication between the microbiome and inflammation, HPA, and autonomic nervous system is needed. Microbes influence cytokine production and T cell activation[33,128] and they and their metabolic by-products can also directly stimulate immune cells with a resultant influence on cytokine release.[33,129] Similarly, pro-inflammatory cytokines influence serotonin availability, serotonin and norepinephrine synaptic reuptake pumps, HPA axis, and regional brain activity.[42] Gut microbes also influence the autonomic nervous system through the vagus nerve,[48] as exemplified by reduced anxiety and depression-related behavior in mice given *Lactobacillus rhamnosus*, with this effect absent in vagotomized mice.[130] In a separate animal study, mice pre-treated with a probiotic formulation (*Lactobacillus* helveticus R0052 and *Bifidobacterium longum* R0175), then exposed to a water avoidance stressor, exhibited attenuated HPA axis and autonomic nervous system activity.[131] Given that exercise alters the microbiome, inflammation, HPA, and autonomic nervous system, a better understanding of the direct and/or indirect relationships are needed.

Recent interest related to our primary aim to test exercise effects on gut microbiome has

grown. Allen et al.[58] observed significant changes in gut microbiome beta diversity after 6 weeks of supervised exercise training in healthy adults (20 to 45 years old) and showed the changes reversed post-intervention. Additionally, positive changes to the gut microbiome have been observed in older adults participating in exercise interventions.[57,59] Yet, the literature in cancer populations connecting exercise to changes in the microbiome warrants additional scrutiny. Sampsell et al.[132] recently conducted a 12-week exercise intervention in 10 breast cancer survivors with reassessment after a 12-week washout period. No statistically significant pre-post differences in alpha or beta diversity were detected yet a follow-up mouse study yielded a trend toward lower tumor development in mice colonized with post-exercise microbiota vs. those colonized with pre-exercise microbiota.

Others report on the relationship between fatigue and gut microbiota composition in cancer survivors,[133,134] but we were the first to focus on breast cancer survivors and observe fatigue was associated with alpha diversity and differences in beta diversity representing shifts in taxa relative abundance.[13] Additionally, understanding the role of exercise on the gut microbiota composition in fatigue response can be leveraged to identify new therapeutic strategies warranting testing in larger trials. Further, exercise is a well-known therapy for alleviating fatigue[135] yet not all cancer survivors report fatigue improvements with exercise.[26] Thus, a better understanding of the potential mediating effects of the microbiome can lead to exercise recommendations that optimize fatigue reductions.

LIMITATIONS

As no research study is perfect, several limitations warrant discussion. Notably, the high scientific rigor made possible by the supervised exercise and controlled feeding may limit

translatability of the results to less controlled interventions. However, this is offset by the opportunities for exploring potential mechanistic links related to exercise, gut microbiome, and fatigue. Moreover, the study inclusion and exclusion criteria may limit generalizability of the results to other cancer types or individuals with higher baseline cardiorespiratory fitness or BMI over 50. Finally, the COVID-19 pandemic's detrimental impact on our anticipated sample size may preclude detecting smaller effect sizes and mediating factors. This is offset by several a priori design features that enhance study power: 1) controlled feeding (reduces variability), 2) selecting low fit and fatigued individuals (greater chance of improvement), 3) manipulating the exercise exposure (standardizes the exercise exposure), and 4) stratifying randomization by BMI (reduces type 1 error and improves study power in trials with < 200 participants per study condition[136]).

CONCLUSIONS

The ROME study is a novel randomized controlled aerobic exercise training study in fatigued breast cancer survivors that integrates energy balanced controlled feeding and tests exercise effects on gut microbiota composition. Identifying microbiota composition changes resulting from exercise will inform trials integrating other modalities (e.g., probiotics) for optimizing exercise benefits, especially in breast cancer survivors with blunted beneficial fatigue responses to exercise. Collectively, this and future trials building on this trial, target the substantial public health problem of fatigue, a persistent and prevalent concern for millions of breast cancer survivors worldwide.[2,14,16]

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Supplementary materials

Supplemental Material 1. Equations for calculating daily calorie needs for energy balanced controlled feeding used in the ROME study (R01CA235598)

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Patient consent for publication Not required.

Ethics approval Ethics approval was obtained from the Institutional Review Board (IRB) of the University of Alabama at Birmingham (UAB IRB#30000320).

Table 1. Participant timeline (Note: To facilitate temporal relationships, data collection is ordered within each assessment period as follows: #1 - outcomes other than fecal sample and fatigue survey, #2 - fecal sample 2 to 3 days after outcomes other than fatigue, and #3 - fatigue survey 2 to 3 days after fecal sample)

	Lab-based screening	**Baseline Assessment**		**Exercise training or control**	**Follow-up assessments**
Study week (preW = week leading up to randomization [0]; W = week after randomization)	preW3	preW2 – preW1	preW1 –0	W1 – W10	W5, W10, & W15
Lab-based screening consent, obtain medical clearance, complete lab-based screening (e.g., $\dot{V}O_{2peak}$)	X				
Enrollment (consent for full participation)	X				
Controlled feeding diet (both study groups)		X	X	X	
Self-administered questionnaire		X			X
Fatigue survey			X		X
Fecal sample collection for gut microbiota composition (with 3-day diet record)			X		X
Medication log (7 days prior to blood draw)		X			X
Fasted blood draw, heart rate variability, hair sample		X			X
Resting energy expenditure		X			X
Walking economy		X			X
$\dot{V}O_{2peak}$, weight, body mass index (BMI)	X				X
Accelerometer with log sheet (7 days)		X			X
Dual-energy X-ray absorptiometry (DXA)		X			X
Randomization			X		
Exercise training or standard attention control				X	

Table 2. Aerobic exercise progression (based on maximum heart rate; high intensity added in later weeks to facilitate continued cardiorespiratory fitness improvement)

Week	Intensity	Max Heart Rate (%)	Duration (mins)	Frequency per Week
1 – 4	Moderate-intensity, continuous	60-75	20 - 35	3
5 – 7	Moderate-intensity, continuous	75	40	2
	High-intensity interval	85-90	20-22	1
8 – 10	Moderate-intensity, continuous	75	40	1
	High-intensity interval	85-90	22-28	2

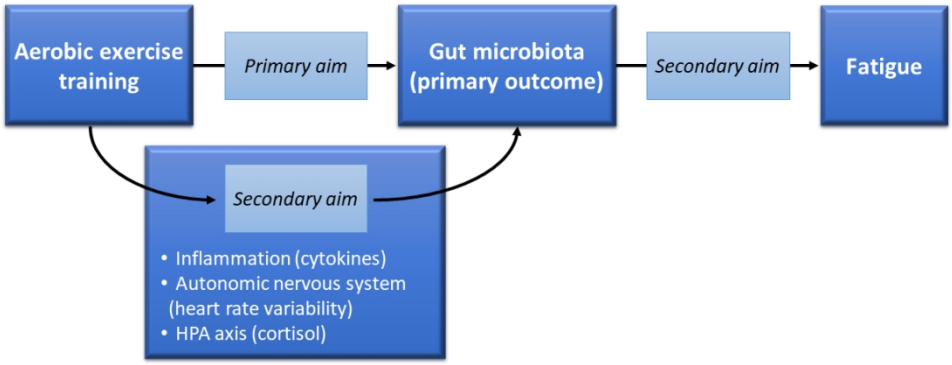

Figure 1. Framework for testing exercise effects on gut microbiota and mechanistic links between exercise, gut microbiota, and fatigue.

338x190mm (96 x 96 DPI)

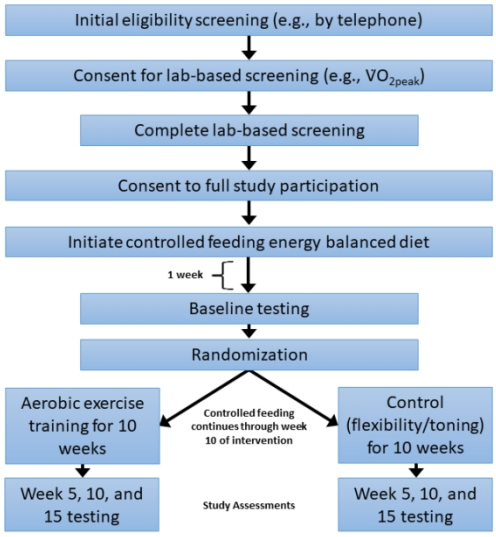

Figure 2. Study schema for testing aerobic exercise effects on gut microbiota composition and potential mechanistic links in breast cancer survivors.

338x190mm (96 x 96 DPI)

Figure 3. Participant screening, enrollment, and baseline assessment. A pre-screening telephone interview determines the potential eligibility of the participant. The orientation visit includes completion of administrative forms, lab-based screening informed consent, and release forms for obtaining medical clearance. Once medical clearance is received by the study team, the participant completes the lab-based screening visit, which includes collecting VO_2peak and BMI. If deemed eligible based on the screening visit, the individual will be invited to sign the consent for full study participation and be scheduled for controlled feeding initiation. Baseline assessment visit #1 is scheduled for at least one week after initiation of controlled feeding. Within seven days of visit #1, 1) the participant is asked to collect the fecal sample at home 2-3 days after visit #1 and promptly overnight ships it to the laboratory, and then 2) complete the remaining assessment materials (e.g., fatigue survey) 2-3 days after collecting the fecal sample and baseline visit #2 occurs to return these forms.

338x190mm (96 x 96 DPI)

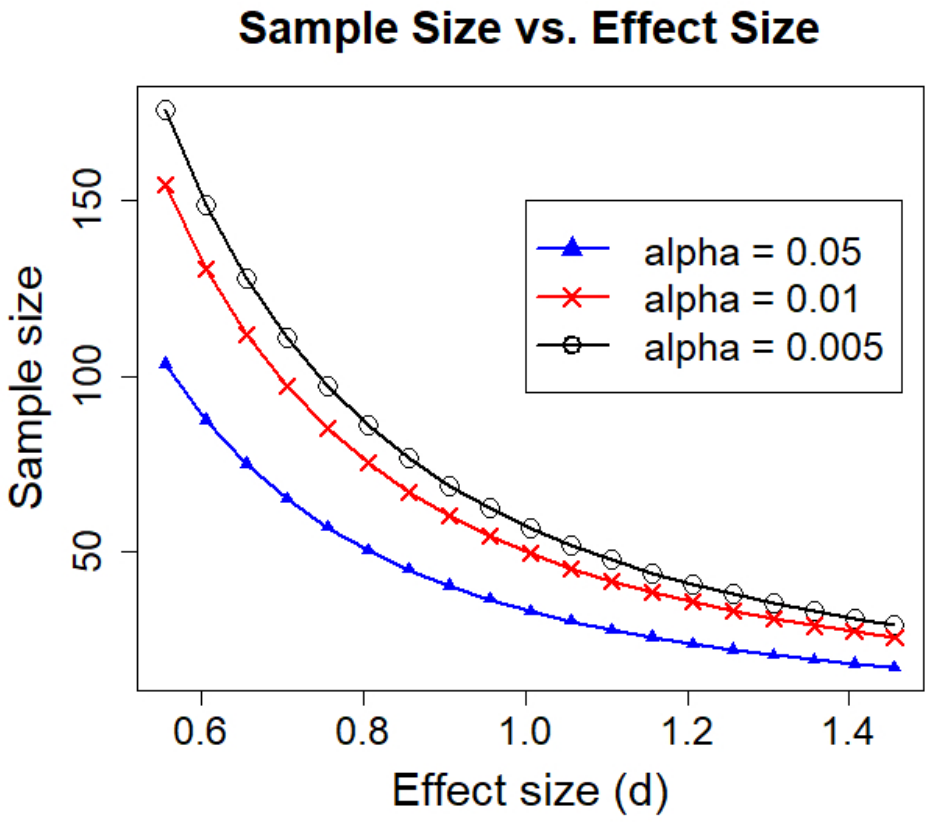

Figure 4. Revised contingency power curve.

226x198mm (72 x 72 DPI)

Supplemental Material 1

Equations for calculating daily calorie needs for energy balanced controlled feeding used in the ROME study (R01CA235598)

Step 1: Calculate a base equation (used for all participants with Step 2 adjusting it for participants randomized to the aerobic exercise condition)

To calculate total energy expenditure (TEE), insert resting energy expenditure (REE) measured by indirect calorimeter from the most recent study assessment into the following equations developed per race based on prior datasets generated in the laboratory of Dr. Gary Hunter.[105,106]

European Americans: TEE = 1124 + (.725 * REE)
African Americans: TEE = 1074 + (.725 * REE)

Note: The ROME study uses the European American equation for individuals of Asian descent.

Step 2: Refine base equation for participants randomized to the aerobic exercise condition

Exercise energy expenditure for each workout based on the a priori exercise progression protocol (VO_{2peak} in ml/kg/min and BDW [body weight] in kg) are entered into the equation with the weekly total averaged over 7 days (to get a daily average needed for the daily controlled feeding menu). This daily average is added to the base equation calculated under Step 1 to determine the daily calorie needs for participants randomized to the aerobic exercise condition.

	Continuous training		Interval training (added in later weeks per protocol)
WK1:	3 * 0.05 * VO_{2peak} * BDW/7		
WK2:	3 * 0.0597 * VO_{2peak} * BDW/7		
WK3:	3 * 0.08 * VO_{2peak} * BDW/7		
WK4:	3 * 0.103 * VO_{2peak} * BDW/7		
WK5:	2 * 0.13 * VO_{2peak} * BDW/7	+	1 * 0.0675 * VO_{2peak} * BDW/7
WK6:	2 * 0.13 * VO_{2peak} * BDW/7	+	1 * 0.0675 * VO_{2peak} * BDW/7
WK7:	2 * 0.13 * VO_{2peak} * BDW/7	+	1 * 0.0743 * VO_{2peak} * BDW/7
WK8:	1 * 0.13 * VO_{2peak} * BDW/7	+	2 * 0.078 * VO_{2peak} * BDW/7
WK9:	1 * 0.13 * VO_{2peak} * BDW/7	+	2 * 0.0844 * VO_{2peak} * BDW/7
WK10:	1 * 0.13 * VO_{2peak} * BDW/7	+	2 * 0.0911 * VO_{2peak} * BDW/7

Note: Exercise-related energy expenditure is greater during first 4 weeks (vs. later weeks) because interval training decreases volume and thus, decreases energy expenditure required.

Note: Rationale for coefficients used to estimate energy expenditure during exercise as follows:

- The week 1 coefficient of 0.05 is based on:
  - Subjects train at 50% VO_{2peak} (60% max heart rate is about 50% VO_{2peak}) or the proportion 0.5.
  - The VO_{2peak} is in ml/kg/min and must be converted to l/kg/min, therefore we must divide by 1000.

- There are 5 kcal burned for each liter of oxygen used and the subjects train for 20 minutes during the first week.

Therefore, the equation is 0.5 * 5 * 20/1000 = 0.05 for week 1. The same methods are used for subsequent weeks as the intensity (proportion $\dot{V}O_{2peak}$) and duration increase.

SPIRIT-Outcomes 2022 Checklist (for combined completion of SPIRIT 2013 and SPIRIT-Outcomes 2022 items)[a]

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Administrative information				
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	-	
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	-	
	2b	All items from the World Health Organization Trial Registration Data Set	-	
Protocol version	3	Date and version identifier	-	
Funding	4	Sources and types of financial, material, and other support	-	
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	-	
	5b	Name and contact information for the trial sponsor	-	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	-	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	-	
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	-	
	6b	Explanation for choice of comparators	-	
Objectives	7	Specific objectives or hypotheses	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	-	
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	-	
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	-	
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (for specific guidance see TIDieR checklist and guide)	-	
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	-	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	-	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-	
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
	12.1		Provide a rationale for the selection of the domain for the trial's primary outcome	
	12.2		If the analysis metric for the primary outcome represents within-participant change, define and justify the minimal important change in individuals	
	12.3		If the outcome data collected are continuous but will be analyzed as categorical (method of aggregation), specify the cutoff values to be used	
	12.4		If outcome assessments will be performed at several time points after randomization, state the time points that will be used for analysis	
	12.5		If a composite outcome is used, define all individual components of the composite outcome	
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	-	
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	-	
	14.1		Define and justify the target difference between treatment groups (eg, the minimal important difference)	
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-	
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	-	
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	-	
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	-	
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	-	
Methods: Data collection, management, and analysis				
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	-	
	18a.1		Describe what is known about the responsiveness of the study instruments in a population similar to the study sample	
	18a.2		Describe who will assess the outcome (eg, nurse, parent)	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	-	
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	-	
	20a.1		Describe any planned methods to account for multiplicity in the analysis or interpretation of the primary and secondary outcomes (eg, coprimary outcomes, same outcome assessed at multiple time points, or subgroup analyses of an outcome)	
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-	
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-	
Methods: Monitoring				
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	-	
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-	
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	-	
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	-	
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	-	
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	-	
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	-	
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-	
	31b	Authorship eligibility guidelines and any intended use of professional writers	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-	
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-	

[a]It is strongly recommended that this checklist be read in conjunction with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) Statement paper for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license and is reproduced with permission.

[b]Indicates page numbers and/or manuscript location: to be completed by authors.

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Role of Gut Microbe Composition in Psychosocial Symptom Response to Exercise Training in Breast Cancer Survivors (ROME) Study - protocol for a randomized controlled trial

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Role of Gut Microbe Composition in Psychosocial Symptom Response to Exercise Training in Breast Cancer Survivors (ROME) Study - protocol for a randomised controlled trial

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ABSTRACT

Introduction Breast cancer survivors have increased risk for chronic fatigue and altered gut microbiota composition, both with negative health and quality of life affects. Exercise modestly improves fatigue and is linked to gut microbial diversity and production of beneficial metabolites. Studies suggest the gut microbiota composition is a potential mechanism underlying fatigue response to exercise. Randomised controlled trials testing the effects of exercise on the gut microbiome are limited and there is a scarcity of findings specific to breast cancer survivors. The objective of this study is to determine if fitness-related modifications to gut microbiota occur and, if so, mediate the effects of aerobic exercise on fatigue response.

Methods and Analysis The research is a randomised controlled trial among breast cancer survivors aged 18-74 with fatigue. The primary aim is to determine the effects of aerobic exercise training compared to an attention control on gut microbiota composition. The secondary study aims are to test if exercise training a) affects the gut microbiota composition directly and/or indirectly through inflammation (serum cytokines), autonomic nervous system (heart rate variability), or hypothalamic-pituitary-adrenal (HPA) axis mediators (hair cortisol assays), and b) effects on fatigue are direct and/or indirect through changes in the gut microbiota composition. All participants receive a standardized controlled diet. Assessments occur at baseline, 5 weeks, 10 weeks, and 15 weeks (5 weeks post intervention completion). Fecal samples collect the gut microbiome and 16S gene sequencing will identify the microbiome. Fatigue is measured by a 13-item multi-dimensional fatigue scale.

Ethics and Dissemination The University of Alabama at Birmingham Institutional Review Board (IRB) approved this study, 15 May 2019, UAB IRB#30000320. A Data and Safety Monitoring Board (DSMB) convenes annually or more often if indicated. Findings will be disseminated in peer-reviewed journals and conference presentations.

Trial registration number NCT04088708, posted 13 September 2019.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study is one of the very few randomized controlled trials testing the effects of exercise on the gut microbiome, especially in cancer survivors experiencing fatigue.
- A standardized, energy balanced diet reduces diet and body weight induced variance on gut microbiota yet no prior randomized exercise and gut microbiome study has provided the same diet for all participants, as being done in our study.
- This study seeks to understand the mechanistic links (inflammation, autonomic nervous system, or hypothalamic-pituitary-adrenal (HPA) axis mediators) between exercise and the gut microbiome, and determine if the benefits of exercise on fatigue are directly and/or indirectly related to changes in the gut microbiota composition.
- Although assessors are masked to study group allocation and a standard attention control condition is used, the intervention precludes participant masking to exercise type.

INTRODUCTION

Nearly 8 million individuals worldwide are living with a history of breast cancer.[1,2] Breast cancer survivors are at increased risk of altered gut microbiota composition (i.e., dysbiosis) that may worsen future cancer risk, comorbidities, and quality of life.[3] Factors that may contribute to the persistent gut microbiota composition changes include reduced physical activity and aerobic fitness, and detrimental changes in body composition after breast cancer diagnosis.[4-7] Given its importance on health and well-being,[8-12] strategies for reversing gut microbiota dysbiosis are needed, especially in breast cancer survivors.

While elucidating gut microbiota dysbiosis in breast cancer survivors remains imperative, it is relevant that the gut microbiome is associated with fatigue in breast cancer survivors[13] and survivors rank fatigue as the number one priority related to quality of life.[14] Additionally, breast cancer survivors are more likely to report fatigue than their age matched controls[15] and one in four suffer persistent fatigue years after their cancer diagnosis,[16] which exacerbates post-cancer disability and reduces quality of life.[17,18] Furthermore, fatigue is associated with greater risk of cancer recurrence and mortality.[19] Interestingly, the benefits of supervised exercise for breast cancer survivors extend beyond the expected improvements in cardio-metabolic parameters to include improvements in fatigue and other domains of quality of life.[20] As we (and others) have reported, exercise is a well-established non-pharmacologic therapy for fatigue, yet effects are somewhat modest (weighted effect size of 0.30 in a recent meta-analysis).[21-24] Hence, elucidating mechanisms underlying fatigue response is needed to optimize fatigue reductions for non-responders and increase effect sizes achievable with exercise.[24-27] Moreover, our prior work and that of others suggest the gut microbiota composition is one such mechanism, but further research is needed.[13,28]

Exercise training also presents as a promising strategy for reversing dysbiosis as it is linked to gut microbial diversity, abundance of select microbes, and production of beneficial metabolites (e.g., acetate, butyrate, propionate), albeit, these phenomena are currently limited to animal models or cross-sectional[29-36] and non-randomized prospective human studies.[37] Randomized controlled trials testing the effects of exercise on the gut microbiome are limited[38] and there is a scarcity of findings specific to breast cancer survivors.[7] One randomized controlled trial in healthy overweight and obese individuals found vigorous-intensity exercise training was associated with increased microbe diversity.[38] To support the importance of intensity in exercise training, we recently showed in breast cancer survivors, cardiorespiratory fitness was a better correlate of gut microbe diversity compared to free-living activity energy expenditure.[7] It is unknown if the modulation of the microbiota by exercise occurs solely through direct means such as alterations to colonic transit time, [39,40] or indirectly through inflammation,[41-43] autonomic nervous system,[44,45] or hypothalamic-pituitary-adrenal (HPA) axis.[46-48] Additionally other lifestyle interventions such diet[49] and body weight changes[50] independently affect gut microbiota, making controls for these variables critical in exercise trials. Rigorously testing the dysbiosis-exercise link while also exploring the bidirectional gut-brain axis pathways responsible for exercise effects[51,52] can inform future exercise recommendations and multimodal interventions to counter the adverse effects of gut dysbiosis.

Given the potential benefits of exercise training on the gut microbiome and fatigue, a better understanding of their relationships in response to an exercise intervention among breast cancer survivors is warranted. Herein, we describe our ongoing randomized controlled trial testing aerobic exercise training as a potential strategy to attenuate dysbiosis in breast cancer survivors with fatigue while also standardizing diet intake and maintaining energy balance. We

further propose to determine if fitness-related modifications to gut microbiota mediate the effects of aerobic exercise on fatigue response. This is a critical next step for several reasons. First, to our knowledge there are currently no completed randomized controlled trials utilizing exercise training as a potential modifier for dysbiosis in breast cancer survivors.[53] Additionally, no other trials exploring these variables have been performed with a standardized diet to: 1) mitigate the underlying variance on gut microbiota and 2) promote weight maintenance.[54,55] Therefore, we describe our methods to facilitate future replicability.

METHODS

Aims and hypotheses

The primary study aim is to determine the effects of a 10-week aerobic exercise training intervention compared to a flexibility/toning standard attention control on gut microbiota composition among breast cancer survivors with fatigue. All participants are following an energy balanced controlled feeding diet. The gut microbiome is being collected by fecal sample and assessed by 16S rRNA at baseline, week 5 to explore interim changes, week 10 as our primary time point, and week 15 to explore durability of effects. The primary outcome measure will be the comparison of microbiome composition using standard diversity and taxa comparison metrics (Table 1). We hypothesize that compared to the control, the exercise training group will demonstrate significant differences in gut microbial diversity with increased Firmicutes (p), *Bacteroides (g)*,[7,56] and *Bifidobacterium (g),*[57] and decreased Actinobacteria (p) and Proteobacteria (p).[7]

A secondary study aim is to test if exercise training affects the gut microbiota composition directly and/or indirectly through inflammation, autonomic nervous system, or

hypothalamic-pituitary-adrenal (HPA) axis mediators (Table 1). We hypothesize that exercise training will have direct and indirect effects on gut microbiota composition through markers of the hypothesized mechanisms (interleukin [IL]-6, IL-10,[41-43] heart rate variability,[44-46] cortisol[46-48]). Another secondary study aim is to test if the exercise training effect on fatigue is direct and/or indirect through changes in the gut microbiota composition. We hypothesize that exercise effects on fatigue will be mediated by changes in beta diversity,[13,58] specifically frequency of Firmicutes (p),[7] Actinobacteria (p),[13] and *Bacteroides (g)*.[13,41,59]

Overall mechanistic framework

Given the relationships between cardiorespiratory fitness and gut microbiota composition,[7] we have chosen an exercise intervention applying the principles of exercise prescription required to achieve an increase in cardiorespiratory fitness.[60] The biological plausibility of a dysbiosis-exercise link also common to fatigue (e.g., inflammation, autonomic nervous system, and HPA axis)[48,61-66] supports testing these potential mechanistic links in breast cancer survivors with fatigue. Thus, the overall mechanistic framework for our trial depicted in Figure 1 can be applied to potentially optimizing exercise interventions for treatment of fatigue.

Study overview and eligibility criteria

This 2-arm, parallel group-controlled trial is randomising breast cancer survivors to 10 weeks of supervised aerobic exercise training or standard attention control (flexibility/toning) while on a controlled feeding diet. The trial is taking place at the University of Alabama at Birmingham (UAB) in Birmingham, AL. Participant enrollment commenced 1 January 2020, was paused between March 2020 and August of 2020 due to the COVID-19 pandemic, and is projected to end 1 January 2025. Institutional Review Board (IRB) approval has been obtained and all participants provide informed consent prior to participation (Supplemental Materials 1 and 2).

Assessments occur at baseline and then at 5, 10, and 15 weeks. A study schema is provided in Figure 2 and an overview of participants' activities is provided in Table 2. An electronic study manual of procedures is kept on a shared, HIPAA compliant cloud server accessible to all study staff.

Inclusion criteria are as followed: 1) female breast cancer survivors ages 18 to 74 years with a history of ductal carcinoma in situ (DCIS) or stage 0, I, II, III breast cancer, 2) who are ≥1 year post-primary cancer treatment completion (chemotherapy and/or radiation), 3) average fatigue over the past week rated as ≥3 on a 1 to 10 Likert scales,[67] 4) English speaking, 5) physician medical clearance for study participation, 6) able to ambulate without assistance, 7) no antibiotics for the past 90 days, 8) willing to avoid taking probiotics for the duration of the study, and 9) after all other criteria are met, lab-based screening is used to confirm low fitness level ($\dot{V}O_{2peak}$ < 30 mL/kg/min). Exclusion criteria are as follows: 1) metastatic or recurrent cancer, 2) another diagnosis of cancer in the past 5 years (not including skin or cervical cancer *in situ)*, 3) unstable angina, 4) New York Heart Association class II, III, IV congestive heart failure, 5) uncontrolled asthma, 6) interstitial lung disease, 7) current steroid use, 8) having been told by a physician to only do exercise prescribed by a physician, 9) dementia or organic brain syndrome, 10) schizophrenia or active psychosis, 11) connective tissue or rheumatologic disease, 12) anticipate elective surgery during the study period, 13) anticipate changes in usual medications during the study period, 14) plan to move residence out of the local area during the study period, 15) plan to travel out of the local area >1 week during study participation, 16) contraindication to engaging in moderate-to-vigorous intensity aerobic exercise, 17) current or anticipated pregnancy during study participation, 18) live or work >50 miles from study site or do not have transportation to study site, 19) body mass index (BMI) >50 (confirmed during lab-based

screening), or 20) anticipate needing antibiotics during the study period.

Recruitment and screening

Participants are being recruited through multiple recruitment strategies (e.g., recruitment letters mailed to breast cancer survivors identified through the UAB O'Neal Comprehensive Cancer Center registry, UAB investigators' waiting lists of cancer survivors inquiring about exercise and weight loss studies, newspaper advertising, cancer support groups, institutional websites and group emails, relevant non-institutional websites, flyers in waiting areas [hospitals, physicians' offices]). Referrals from oncologists and other relevant health care providers are being requested using messaging (i.e., electronic health records or institutional email) and face-to-face meetings; recruitment materials such as patient flyers are provided, as appropriate. Potential participants are given a description of the study and screened for eligibility based on a pre-determined telephone script. In addition to questions related to the above eligibility criteria, participants are asked the following diet questions in the prescreening telephone screen to assess potential controlled feeding adherence and safety issues: 1) *do you have any food allergies, restrictions, preferences or special diet (vegetarian, gluten-free, etc.)*, 2) *are you willing to eat the meals we provide*, 3) *do you drink alcohol? If yes, are you willing to refrain from alcohol during your participation in this study*, and 4) *do you foresee any barriers to picking up the food, storing food, or doing minimal meal preparation*?

Enrollment and randomization

Interested potential participants who pass the pre-screening telephone interview are invited to an orientation visit (in person or by videoconference) to complete administrative forms, sign lab-based screening consent (Supplemental Material 1), and complete release forms for obtaining medical clearance with the study coordinator. Once medical clearance is received, the participant

is scheduled for a lab-based screening visit which includes VO_{2peak} to confirm cardiorespiratory fitness < 30 ml/kg/min and BMI ≤ 50 (see Section 3.5.3 for methods). If deemed eligible at the lab-based screening visit, informed consent for full study participation is obtained (Supplemental Material 2), including optional permission to retain health information and biospecimens for future research. The participant is scheduled for initiation of controlled feeding and baseline assessment visits #1 and #2 (Figure 3).

Participant randomization is based on computer generated random numbers and performed in blocks of 4 to facilitate an equal distribution between the two study groups. BMI is an important biological variable associated with gut microbiota composition,[18,68] hence randomization is stratified by BMI (< 30 *vs.* ≥ 30). The study statistician performed the computer generation of random numbers which were placed in sealed, opaque envelopes and delivered to the recruiting staff with written protocol for use. Assignments are made in the order in which participants complete baseline testing and are kept in the sealed envelope until the participant has completed all baseline testing. Once the study coordinator confirms completion of baseline testing, the coordinator chooses the next envelope with group allocation. Participants remain partially blinded to study condition (e.g., will not be told which study condition [exercise training or flexibility/toning intervention] is expected to yield more benefits and all receive controlled diet which is potentially perceived as a "treatment".) Assessments, assays, and data entry are conducted using objective and validated measures by staff who will remain blinded to study arm status.

Assessments

Schedule and masking

Assessments occur at baseline (pre-intervention), 5 weeks (mid-point intervention), 10 weeks (immediately post intervention), and 15 weeks (5 weeks post intervention) and are performed by staff who are masked to participant study group allocation. Table 2 presents the timeline of data and measures collected at each assessment visit. If eligible based on lab-based screening and participant consents to full study participation (Supplemental Material 2), then controlled feeding preparations are made and the baseline visit #1 is scheduled for one week after controlled feeding begins (Figure 3). For each assessment, the participant completes two visits to the exercise testing laboratory. In preparation for assessment visit #1, participants are provided instructions for the lab-based measurements (location, parking, 12-hour fast, appropriate clothing, etc.). During assessment visit #1, the participant provides a hair sample, completes the fasted blood draw, resting energy expenditure by indirect calorimeter, resting heart rate variability (Actiheart), dual-energy X-ray absorptiometry (DXA), and walking economy (i.e., net $\dot{V}O_2$). Because the $\dot{V}O_{2peak}$ and BMI measurements are taken at the screening visit, these are not repeated at baseline but are repeated at the follow-up assessments. During assessment visit #1, study staff provide the participant with the additional assessment materials (survey, accelerometer with log, 3-day diet record, medication log, fecal sample kit, etc.) and related instructions. The participant ships the fecal sample back to the UAB microbiome laboratory within 7 days of visit #1 and returns the remaining assessment materials at assessment visit #2. To better align the temporal relationship between the gut microbiome and fatigue, the fatigue scale is collected at assessment visit #2 (i.e., several days after fecal sample collection).

Gut microbiota composition

Participants are provided with a stool collection kit at each baseline and follow up assessment visit #1 to self-collect the stool sample at home according to provided instructions. Briefly, the

instructions are to collect the sample in a clean dry study-provided collection hat and scoop a small amount into the provided ParaPak vials (Meridian Biosciences, Inc; Cincinnati, OH) pre-labelled with participant identification and assessment timepoint, and then ship the sample back to our site via pre-paid overnight shipping materials. Once received by the microbiome lab, each sample is aliquoted into labelled cryovials and stored at -80°C until time for DNA extraction and 16S rRNA processing. One cryovial of precisely 100 uL is retained and labeled for future metabolomics assays (if indicated and funds can be obtained).

With each sample collection, the participant completes a fecal sample questionnaire[69] and returns it to the research staff. The questionnaire asks the participant to report changes in normal diet and vitamin supplements; recent gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, and constipation); and usual frequency or changes in probiotic supplements, yogurt intake, and high fiber foods or fiber supplements. Participants also report recent medical treatments such as antibiotics, chemotherapy, or radiation therapy, and if they have ever had a major bowel resection, gastric bypass surgery, an inflammatory bowel disease (such as Crohn's disease, ulcerative colitis, indeterminate colitis), or irritable bowel syndrome. The participant is also asked to complete a 3-day diet record capturing dietary intake 2 days prior to and the day of fecal sample.

Cardiorespiratory fitness ($\dot{V}O_{2peak}$)

Participants perform a graded treadmill (TrackMaster TMX428CP; Full Vision, Inc.; Newton, KS) test in accordance with the modified-Balke protocol to elicit $\dot{V}O_{2peak}$ (i.e., the highest measured rate of oxygen uptake expressed in mL/kg/min. Initially, $\dot{V}O_2$ is stabilized over a 3-minute period of standing rest, after which, participants begin walking at 2.0 mph at 0% grade for 2 minutes. Grade is then increased 3.5% every 2 minutes until the 12th minute, at which point,

grade is decreased to 12% and speed increased to 3.0 mph. Grade is increased by 2.5% each minute (as needed) until volitional exhaustion. $\dot{V}O_2$ and related gas exchange measures are aggregated in 30-s bins and determined by open-circuit spirometry (True One 2400 system; ParvoMedics, Salt Lake City, UT). Gas analyzers and flowmeter are calibrated prior to each test using standard gases and 3-L syringe, respectively. Heart rate and Rating-of-Perceived Exertion (RPE; Borg 6-20, 6 = *no exertion at all, relaxed* and 20 = *maximal exertion*)[70] are recorded in the final 30 seconds of each stage. Blood pressure is measured via auscultation at minutes 6, 10, 14, 16 and/or final stage of the graded treadmill test.

Serum cytokines

Inflammatory cytokines, interleukin (IL)-6 and IL-10, are collected by blood samples. Participants are instructed to abstain from vigorous exercise, smoking, and alcohol for 24 hours prior and fast for 12 hours prior to the blood draw. Blood samples are collected, processed and stored (-80°C) using standard operating procedure consistent with expert consensus recommendations[71] and batch analyzed according to manufacturer's instructions by staff who are blinded to the participant's group allocation.[64] Serum cytokine assays will be analyzed by the UAB Metabolism Core using a MSD imager (MesoScale Discovery, Gaithersburg, MD; chemiluminescence technology; multiplex platform). Blood and serum samples are being processed and stored so that future metabolomic/functional metabolic studies can be done if indicated and funds can be obtained. A 7-day medication log is collected with each blood sample for medication changes between assessments that may influence study outcomes (e.g., anti-inflammatory agents, etc.).

Heart rate variability

Heart rate variability is evaluated with the Actiheart 5 (CamNtech Ltd., Cambridgeshire, UK)

device. First, a urine sample is collected from participants to measure urine specific gravity – an indicator of hydration status. In accordance with manufacturer guidelines, skin is prepped with a 70% isopropyl alcohol wipe before positioning a two-lead electrode arrangement in the upper left quadrant across the participant's chest. Measurements are collected during 5-minutes of quiet rest in the seated position. High-frequency sampling is used to measure inter-beat intervals wherein Actiheart® software is used to perform offline analyses. The primary variables of interest include heart rate and root mean square of successive RR interval differences (RMSSD) as well as the low-frequency (LF), high-frequency (HF) components derived from the fast-Fourier transform. Procedures are performed in the morning hours in a dimly-lit, temperature-controlled room.

Hair cortisol

Hair specimens are collected by trained study staff. For participants whose hair is longer than 1.5 to 3 cm, a thin layer of hair (1-2 hairs thick) parallel to the floor is cut from a point close to the scalp across a 4-5 cm length (laterally), to obtain a minimum of 50 strands of hair. For participants with shorter hair, the lateral cut is 6-8 cm (2 cm vertical x 5 cm lateral for long hair, > 2 cm vertical x 7 cm lateral for shorter hair). String is used to indicate the end of the hair closest to the scalp; hair specimens are folded tightly into aluminum foil and placed in a small labeled bag at room temperature until being sent for assay at the Department of Biopsychology at Technische Universität Dresden in Dresden, Germany.

Fatigue

Fatigue is measured by a 13-item multi-dimensional fatigue scale (i.e., Fatigue Symptom Inventory).[72] On a 1 to 10 scale (1 = *not at all fatigued*, 10 = *as fatigued as I could be*), participants are asked to rate their level of fatigue on the day they felt most and least fatigued in

the last week, the average level of fatigue in the last week, and the level of fatigue at the time of survey. Participants report how much fatigue interferes (1 = *no interference*, 10 = *extreme interference)* with their general level of activity, ability to bathe and dress, their normal work activity, ability to concentrate, relations with other people, enjoyment of life, and mood. Participants report how many days in the past week they felt fatigued for any part of the day and how much of the day on average the participant experienced fatigue (1 = *none of the day*, 10 = *the entire day*). Since our prior studies have demonstrated that exercise effects on fatigue may vary by dimension (i.e., intensity *vs.* interference; intensity = mean of 4 items; interference = mean of 6 items, 0 to 10 scale) our final analyses will focus on fatigue interference.

Potential covariates

Self-administered survey measures age, race/ethnicity, education level, annual household income, marital status, smoking history, alcohol intake, employment status and number of recent sick days, cancer-related factors (date of diagnosis, stage, subtypes [e.g., receptor status], current and past cancer treatment type [including, but not limited to, radiation, chemotherapy, and anti-estrogen therapy]), caffeine intake, dietary supplements (including prebiotic, probiotic, and vitamins), current medications (including over the counter medications), any antibiotic medications over the last 6 months, any steroid medications or injections over the last 6 months, current/past diagnosis of and treatment for anxiety or depression, treatment duration, time since treatment completion), medical comorbidities[73] (including but not limited to endocrine or hormone disorders), history of surgeries, menopausal status,[6] and history of COVID-19 diagnosis. If a participant is not able to recall medical-related information, a medical release form is completed allowing study staff to request this information from the participant's physician.

Because stress, depression, anxiety, sleep quality, pain and fatigue may cluster and be associated with inflammation,[74-76] stress is measured by Perceived Stress Scale-10 (PSS-10),[77] depression and anxiety is measured by 14-item Hospital Anxiety and Depression Scale [HADS]),[78] sleep dysfunction is measured subjectively using the Pittsburgh Sleep Quality Index (PSQI)[79], and pain is measured by the Patient Reported Outcomes Measurement Information System (PROMIS®; http://www.nihpromis.org/default.aspx).[80] Because post-traumatic stress symptoms are associated with psychosocial outcomes and gut microbiota composition,[81,82] post-traumatic stress is measured using the Posttraumatic Stress Disorder Checklist (PCL).[83-86]

To assess free-living physical activity, participants are given the same ActiGraph accelerometer (ActiGraph LLC; Pensacola, FL) device for each assessment to be worn at the waist for seven consecutive days during waking hours (non-dominant hip; same side each time). Participants are instructed to remove the accelerometer while bathing, showering, or swimming and are asked to complete an accelerometer log (times device removed, exercise not detectable by device, sleep times, etc.). The accelerometer is set for 30 second epochs and monitoring is repeated if less than four valid days are recorded. Non-wear time is defined when no motion is detected for 60 minutes. A valid day is defined as at least 10 hours of valid wear time. The following cut points are planned: Sedentary: 0 – 99 counts/min; Inactive: 100 – 499 counts/min; Light: 500 – 1951 counts/min; Moderate: 1952 – 5724 counts/min; and Vigorous: 5725+ counts/min.[87,88] Leisure-time physical activity is measured using the Godin Leisure Time Exercise Questionnaire which asks for average weekly frequency of leisure-time exercise for periods exceeding 10 minutes over the past month per three activity intensity levels (light, moderate, or vigorous).[89,90]

Body mass index (BMI) is calculated from weight and height [weight (kg)/height (m^2)]

obtained from a scale (in light clothing) and wall stadiometer (without shoes). Dual-energy X-ray absorptiometry (DXA) scans assess lean mass and fat mass using the Lunar Dual Energy X-ray Absorptiometry Scanner (iDXA; Lunar Radiation Corp. Madison, WI). Pre-menopausal women at risk for pregnancy undergo a urine pregnancy test prior to each DXA scan.

Other relevant measurements

Resting energy expenditure measurement is required to more accurately assess participant's calorie needs for the controlled feeding which facilitates energy balance and resultant weight maintenance during the study. Hence, resting energy expenditure is measured by ventilated hood indirect calorimetry (True One 2400 system; ParvoMedics, Salt Lake City, UT) while lying quietly on an exam table. Participants must fast for at least 6 hours prior (4 hours if they are diabetic), avoid physical activity for 12 hours and avoid any caffeine or nicotine for at least 2 hours prior to this test.

Although not originally proposed, walking economy (i.e., net $\dot{V}O_2$) was added because it reflects oxygen uptake during ambulation, an important alternative measure of (mobility) independence in older women.[91] Participants wear a hip-worn accelerometer and complete a fixed-workload task by walking on a treadmill at 2.0 mph (0% grade) for six minutes during which steady-state $\dot{V}O_2$ is reached. RPE (Borg 6-20, 6 = *no exertion at all*, relaxed and 20 = *maximal exertion*)[70] is collected at minutes three and six. At minute 5, the participant reports perceived difficulty of the test using a visual analogue scale (100 mm line). Blood pressure is measured at rest and while standing. Blood pressure is also measured at the 1-, 2-, and 5-minute timepoints during walking. Participants remain quietly seated for at least 10 minutes between the walking economy and $\dot{V}O_{2peak}$ tests during the follow-up assessments.

Quality of life is measured with The Functional Assessment of Cancer Therapy-Breast (FACT-

B)[92] because of its relation to fatigue, relevance for breast cancer populations, and repeated use in prior studies which allow for comparison of study results. The FACT-B is a 37-item instrument using 5-point Likert scales and includes the subscales of physical well-being, social well-being, emotional well-being, functional well-being, and additional concerns.[92]

Since cognitive function is associated with the gut microbiome[93] and physical activity in breast cancer survivors,[94] cognitive function is measured with the 10-item Frequency of Forgetting scale.[95] The summed score will assess subjective memory impairment (Total score) along with 4 memory subscales (general memory, frequency of forgetting, frequency of forgetting when reading, and remembering past events).

To improve adherence to future, similar exercise training protocols, the self-administered survey assesses social cognitive theory constructs: exercise self-efficacy (barriers and walking), enjoyment, social support, barriers, and outcome expectations. Barriers self-efficacy (i.e., confidence in ability to overcome barriers) is measured utilizing a 9-item scale specifically designed for breast cancer patients.[96] The scale utilizes frequently reported barriers among breast cancer patients (e.g., "*How confident are you that you can exercise when you are tired?*"). Walking task self-efficacy scale is assessed with a 6-item scale asking participants to rate confidence in their ability to walk at a moderately fast pace for 5, 10, 15, 20, 25, and 30 minutes.[97] Analyses for barriers and walking task self-efficacy are using the mean score for the Likert scale (0% = *not at all confident* to 100% = *extremely confident*). Perceived exercise barriers (or barriers interference) are measured by asking participants to rate on a 5-point Likert scale (1 = *never* to 5 = *very often*) how often 21 different barriers (e.g., lack of time, weather) interfere with exercise. The items are summed for a perceived barriers score.[98-100] Physical activity enjoyment is measured with a single question (5-point Likert scale).[100] Social support is

measured by asking for the frequency with which friends (two items) or family (two items) encourage or offer to exercise with the participant. Items are summed for a friends, family, and total social support score.[101,102] For outcome expectations, participants are asked to rate their agreement on a 5-point Likert scale (1 = *strongly disagree* to 5 = *strongly agree*) with the statement that exercise would result in 17 potential benefits or risks. Fourteen positive benefits (e.g., feel less depressed) and 3 negative outcomes (e.g., increased joint pain) are included. Responses are summed for positive outcome expectations and negative outcome expectations.[100] The participants answer the outcome expectation questions twice: once considering stretching and light resistance exercises and again considering aerobic exercise.

Participant satisfaction

At the 15-week assessment, participants are asked to provide a written evaluation of the study staff and procedures. All participants are asked to report their agreement (Likert scale; 1 = *strongly disagree* to 5 = *strongly agree*) with 10 statements relating to the clarity of study information, helpfulness of staff interactions, palatability of the provided food and ease of following the menu, likelihood of recommending this study to others, and overall satisfaction with the study staff and activities. One open-ended question seeks any additional information they would like to share with the study team.

Data quality control

Multiple strategies are being used to minimize missing data (e.g., baseline testing and controlled feeding before randomization provides a *"run-in"* period, monetary and non-monetary incentives, up to date contact information, ongoing review of source documents by study coordinator for immediate rectification of missing data, etc.).[103] Study staff are trained by the investigator with the relevant expertise using electronic manual of procedures with regular

review of source documents for quality. Multiple trained staff are present during in-person assessment activities increasing accountability and immediate identification of potential drift in protocol adherence. All most recent IRB approved study forms are stored on a shared, HIPAA compliant cloud server.

Interventions

Supervised exercise sessions

Participants are randomized to 10 weeks of either an aerobic exercise intervention or a flexibility/toning attention control condition. Sessions occur on nonconsecutive days of the week at the study site and are supervised by experienced exercise specialists who are not involved in the collection of outcome assessments.

Aerobic exercise sessions

Aerobic exercise sessions, supervised by trained exercise specialists, are primarily performed using the treadmill. However, the cycle ergometer may be used if preferred by the participant. The training target heart rate zone for each session corresponds with the heart rate at a given percentage of VO_{2peak} measured at the most recent assessment. Training sessions commence with a 5-minute warm-up consisting of light treadmill walking and stretching. During the 1st week of training, after warm-up, participants perform 20 minutes of exercise at ≈60% maximum heart rate (equivalent to ≈45-50% VO_{2peak}). Over the next 3 weeks, *exercise duration* is increased by 5-minute intervals, as tolerated, so that by the beginning of the 5th week participants are exercising for 40 minutes (up to a total of 60 minutes with warm-up and stretching time). This coincides with an elevation in *exercise intensity* equating to ≈75% of maximum heart rate (≈55-60% of VO_{2peak}) by the 5th week. Following each exercise bout, participants cool down for 3-5

minutes. To mitigate stagnation, and facilitate continued improvement of $\dot{V}O_{2peak}$,[104] high-intensity interval exercise is added during weeks 5-10 as described in Table 3. Eight to ten work-intervals are performed at a workload to elicit ≈85-90% maximum heart rate for 60 seconds with rest intervals of 3 minutes with the total exercise duration ranging from 20 to 40 minutes.

Standard attention controls

The non-aerobic exercise attention control condition controls for the effects of attention and social interaction through administration of flexibility/range-of-motion activities using light resistance bands delivered at the same frequency as the aerobic condition (i.e., 3 times per week). The sessions last about 40 minutes and target the head/neck, shoulder, elbow/forearm, hand/wrist, trunk/hip, and ankle/foot. The progression of activities over the 10-week period involves performing additional exercises and sets (i.e., Thera-bands) that provide minimal resistance (i.e. sham). The first 5 weeks of the control condition involve performing body stretches without resistance (20-30 seconds for 1-2 sets). In weeks 6-7, the light resistance Thera-band is used to perform the stretches for the upper-extremities once per week for 8-10 repetitions for 2 sets, and the other two sessions are body stretches without resistance. In weeks 8-10, the light resistance Thera-band is used twice per week for 8-10 repetitions for 2 sets, and one session will be body weight stretches without resistance. Such a progression is not expected to induce aerobic fitness adaptations and is designed to maintain participant interest and expectation of treatment benefit. Control condition participants are asked to not undertake additional exercise (e.g., not join a gym and begin exercising) during the 10-week intervention period.

Missed exercise and control sessions

Session attendance is tracked weekly and missed sessions are made up as soon as possible during the intervention period. No more than four supervised aerobic sessions will occur in one

week. Exercise specialists encourage exercise adherence by discussing social cognitive theory based educational newsletters with participants at six time points during the 10 weeks of aerobic exercise and standard attention control.[105]

Controlled feeding

Controlled feeding provided by the UAB Center for Clinical and Translational Science (CCTS) Metabolic Kitchen standardizes dietary intake across all participants. The menus are designed to provide 55% of energy as carbohydrate primarily through complex sources (fiber: 21-38 g/day), 23% as fat, and a minimum of 22% as protein (≈0.8 g/kg). Dietary sodium intake and the polyunsaturated:saturated (P:S) fat ratio are held constant (sodium <3500 mg/d, P:S fat ratio of 1, and saturated fat less than 30% of total fat intake).

Prior to initiating controlled feeding, the participant meets with a study registered dietitian to review the study menu and collect information about food allergies and intolerances. Changes to the menu based on dietary preferences are attempted if substitutions are accessible to the Metabolic Kitchen and maintain the standardized diet protocol. The participant and study dietitian meet a second time to review the final menus and discuss approved beverages and seasonings. Each participant starts weekly meal pick up from the Metabolic Kitchen at least one week before baseline assessment visit #1.

To allow the Metabolic Kitchen time to prepare the controlled feeding, the daily calorie need (total energy expenditure) is estimated pre-baseline using the Harris Benedict equation and an activity factor to promote weight maintenance. This estimate is then updated once resting energy expenditure data is available at the baseline assessment. The estimate of total energy expenditure is further updated for participants randomized to the aerobic exercise condition using the individual's $\dot{V}O_{2peak}$ and resting energy expenditure data based on prior work by the

investigative team (equation provided in Supplemental Material 3).[106,107] The total energy expenditure estimates for all participants are updated, if appropriate, based on the week 5 assessment of $\dot{V}O_{2peak}$ and resting energy expenditure. A study registered dietitian monitors body weight weekly and uses these changes and participant dietary preferences to further refine the calorie content and menus.

Controlled feeding adherence

Menu checklists are included with each weekly food pick up and participants are asked to log how much of the provided foods they consume and report additional foods and beverages along with the amounts consumed. The menu checklists are returned at exercise and control sessions on a weekly basis and reviewed by the dietitian for adherence. Participants with potential adherence issues or missing or incomplete checklists are called by a study dietitian for reminders and instruction.

Staff training

Staff are trained using a variety of electronic manuals, protocols, and up-to-date IRB approved study forms and scripts. An electronic manual of procedures is maintained in a shared, HIPAA compliant cloud server for reference by staff. Given the range of staff responsibilities (i.e., exercise intervention, diet, etc.), additional supplemental role-specific protocols are also maintained (e.g., exercise progression prescription for exercise specialist and controlled feeding menu review scripts for dietitian).

Intervention fidelity plan

The exercise and controlled feeding intervention fidelity plans include the five domains

recommended by NIH Behavior Change Consortium[108] (i.e., study design, provider training, treatment delivery, treatment receipt, and enactment of treatment skills). Fidelity is facilitated with the electronic manual of procedures, standardized scripts, and participant education materials. Data sources for tracking exercise intervention include review of all exercise session record sheets (i.e., attendance, if exercise goals met, and if exercise progression administered according to protocol) and direct observation by each interventionist at least once a month. The main data source for tracking controlled feeding fidelity are menu checklists on which the participant reports the provided foods consumed and any additional foods/beverages consumed. The food included in each controlled feeding pick up is reviewed for accuracy and completeness by a trained research staff before the food is given to the participant. Further, study registered dietitians offer the same food substitutions for all participants requesting a change. Monthly reports are presented to the study team to monitor fidelity of both the exercise and controlled feeding so that fidelity concerns can be rectified in a timely manner.

Statistical analysis

Sample size and power considerations

Sample size is based on detecting alpha diversity and beta diversity taxa comparisons. The power calculation is based on two-tailed test at power of 0.8 using software G*Power version 3.1.9.2.[109,110] Our pre-COVID pandemic sample size was estimated at 126 (63 in each group) with 100 (50 per study group) remaining after drop outs. This sample size would have allowed us to detect a medium effect size (d = .57; power of 0.8, $p < 0.05$) in alpha diversity which is sufficient for detecting effects related to associations with fatigue and intervention effects falling midway between that found in our two pilot studies. Relevant to taxa comparisons, we have >

0.8 power to detect the effect of any of the taxa after multiple testing correction (q value < 0.05).[111-113] Due to the detrimental impact of the COVID-19 pandemic on recruitment into on-site, supervised exercise trials, we provide revised contingency power calculations in Figure 4, where we can see that with sample size decreasing, the effect size we can detect changes from moderate to large. For example, for enrolling at 100%, 75% (74 samples with 37 per group), and 50%, the effect size that can be detected changes from 0.57, to 0.67, and to 0.81 (with power of 0.8 and alpha of 0.05). Of note, larger effect sizes are possible in this study (compared to our pilot studies) because the study will provide controlled feeding (reducing variability), select low fit individuals (greater chance of improvement), and manipulate the exercise exposure (standardize the exercise exposure). Also relevant, the sample sizes in our pilot studies (N = 12 and 37) were smaller than our proposed study even with dropped enrollment yet yielded statistically significant results (e.g., a significant association between alpha diversity and cardiorespiratory fitness in 37 breast cancer survivors).[7,13]

Data management and analysis considerations

Microbiome 16S gene sequence data is analyzed using the QIIME[114] analysis package, our in-house developed automated analysis pipeline QWRAP,[69] and DADA2[115] to provide a robust error model for sample filtering and clustering. Data quality is assessed using FASTQC, with low-quality data filtered out using the FASTX toolset. Filtering, denoising, and clustering of reads into Amplicon Sequence Variants (ASVs) is done using DADA2. Taxon assignment is performed using Mothur[116] and the SILVA 16S rDNA database.[117] Alignment and phylogenetic inference is then performed using PyNAST[118] and Fasttree.[119] Comparative analytical tools such as UniFrac[120] are used to assess differences between samples and sample groups using principal coordinates analysis. To expedite sample processing and reporting, QWRAP automates the

running of these tools using a single command line argument on UAB's high-performance computing cluster, Cheaha.

Survey and other data entry and checking is conducted by trained research staff masked to study group allocation using password protected Research Electronic Data Capture (REDCap). Data analyses will be carried out on an intent-to-treat basis. A multiple imputation approach will be employed to handle any missing data that cannot be rectified and we will conduct sensitivity analysis to assess the robustness of our findings.[103,121] SAS software, Version 9.3 (SAS Institute Inc., Cary, NC) and R software, version 4.3.1[122] will be used for data analysis. Transformations and non-parametric procedures will be performed when needed. The false discovery rate (FDR) will be used for multiple testing correction and the statistical significance threshold will be FDR $q \leq 0.05$ (q value is a p value after FDR correction). Each element (i.e., alpha diversity, beta diversity, and taxa level comparisons) describes a different perspective on gut microbiota changes and are integrated for interpretation (e.g., does exercise change the relative abundance of organisms and, if so, which organisms). We will assess the microbiota composition change over time using mixed-effects models.[123] All mediation analyses will conduct indirect effects analysis with the bootstrap method developed by Hayes.[124] Week 10 is our primary time point yet we will also analyze week 5 to assess interim changes that occur and week 15 to assess durability.

Participant safety and withdrawal

Risk management and safety

Participant safety is facilitated by obtaining medical clearance, limiting to a BMI < 50, collecting a medical history and the PAR-Q (physical activity readiness questionnaire) before the lab-based screening, and consulting clinical investigators, if indicated. Exercise sessions are supervised by

exercise specialists who have experience training cancer survivors or chronic disease populations. Additionally, physician supervision is provided during fitness testing when deemed appropriate based on ACSM guidelines.[125] Information about food allergies and intolerances are screened for and collected before initiating controlled feeding and throughout participation and these are communicated to the Metabolic Kitchen to minimize allergen contamination.

Adverse event reporting

Adverse events are identified spontaneously (e.g., reported to research staff during contact time) or non-spontaneously (structured interview done at each assessment time-point). Reported adverse events are reviewed promptly by the PI and reported to the IRB according to local requirements. A Data and Safety Monitoring Board (DSMB) is convened annually or more often if indicated.

Handling of withdrawals

Participants are informed of their right to withdraw at any time without consequences in the informed consent forms and during the signing of consent forms. Participants will be withdrawn from the study if any social, psychological, or physical conditions arise that may unduly increase risk of participating in the study. Data will be analysed on an intention-to-treat basis.

Unexpected Required Antibiotics

Given the effect of antibiotics on the gut microbiota composition, participants unexpectedly requiring intensive antibiotic therapy while enrolled in the study will be withdrawn from the study. Intensive antibiotic therapy is defined as intravenous, extended use (i.e., ≥ 2 weeks), or combined therapy (multiple broad-spectrum agents). Less intensive antibiotic use will be tracked by self-administered survey and considered during the analyses.

Patient and public involvement

Patients and members of the public were not involved in the design of the trial.

Ethics and dissemination

The University of Alabama at Birmingham Institutional Review Board (IRB) approved this study, 15 May 2019, UAB IRB#30000320. The trial is registered with ClinicalTrials.gov: NCT04088708. A Data and Safety Monitoring Board (DSMB) convenes annually or more often if indicated. Any amendments will be submitted to the IRB and DSMB for approval. Research findings will be disseminated in peer-reviewed journals and conference presentations.

DISCUSSION

The ROME study is the first randomized controlled exercise training study in fatigued breast cancer survivors testing exercise effects on gut microbiota composition while standardizing dietary intake with rigorous attention to energy balance. Our careful attention to diet and energy balance is critical to more fully understanding the role that exercise can play in altering dysbiosis in breast cancer survivors, a group at increased risk for detrimental changes in gut microbiota composition. Also, understanding the potential mechanistic links between aerobic exercise training, gut microbiota composition, and fatigue in cancer survivors has great potential to improve the lives of the breast cancer survivors suffering fatigue.

Thus, we describe a highly rigorous trial that is especially appropriate for studying exercise, gut microbiome and fatigue in breast cancer survivors because it integrates a standard attention control condition and energy balanced controlled feeding. The standard attention control condition is critical to detecting exercise effects on this patient-reported outcome beyond staff

attention alone.[126] Further, few randomized trials testing exercise effects on the gut microbiome have attempted to standardize diet intake with energy balanced controlled feeding, a critical element given the strong association between diet, body weight, and the gut microbiome characteristics.[49,53,127]

Given the careful attention to the temporal relationships and randomized study design, this study will explore mechanistic pathways heretofore most frequently studied in animal models rather than humans. With regard to the potential mechanisms through which exercise influences the gut microbiome, we will explore exercise induced changes to inflammation, the autonomic nervous system, and the HPA axis. Exercise training in breast cancer survivors positively impacts inflammatory markers.[128] In particular we have previously observed beneficial changes in IL-10 and tumor necrosis factor (TNF)-α.[25] A better understanding of the bidirectional communication between the microbiome and inflammation, HPA, and autonomic nervous system is needed. Microbes influence cytokine production and T cell activation[33,129] and they and their metabolic by-products can also directly stimulate immune cells with a resultant influence on cytokine release.[33,130] Similarly, pro-inflammatory cytokines influence serotonin availability, serotonin and norepinephrine synaptic reuptake pumps, HPA axis, and regional brain activity.[42] Gut microbes also influence the autonomic nervous system through the vagus nerve,[48] as exemplified by reduced anxiety and depression-related behavior in mice given *Lactobacillus rhamnosus*, with this effect absent in vagotomized mice.[131] In a separate animal study, mice pre-treated with a probiotic formulation (*Lactobacillus* helveticus R0052 and *Bifidobacterium longum* R0175), then exposed to a water avoidance stressor, exhibited attenuated HPA axis and autonomic nervous system activity.[132] Given that exercise alters the microbiome, inflammation, HPA, and autonomic nervous system, a better understanding of the

direct and/or indirect relationships are needed.

Recent interest related to our primary aim to test exercise effects on gut microbiome has grown. Allen et al.[58] observed significant changes in gut microbiome beta diversity after 6 weeks of supervised exercise training in healthy adults (20 to 45 years old) and showed the changes reversed post-intervention. Additionally, positive changes to the gut microbiome have been observed in older adults participating in exercise interventions.[57,59] Yet, the literature in cancer populations connecting exercise to changes in the microbiome warrants additional scrutiny. Sampsell et al.[133] recently conducted a 12-week exercise intervention in 10 breast cancer survivors with reassessment after a 12-week washout period. No statistically significant pre-post differences in alpha or beta diversity were detected yet a follow-up mouse study yielded a trend toward lower tumor development in mice colonized with post-exercise microbiota vs. those colonized with pre-exercise microbiota.

Others report on the relationship between fatigue and gut microbiota composition in cancer survivors,[134,135] but we were the first to focus on breast cancer survivors and observe fatigue was associated with alpha diversity and differences in beta diversity representing shifts in taxa relative abundance.[13] Additionally, understanding the role of exercise on the gut microbiota composition in fatigue response can be leveraged to identify new therapeutic strategies warranting testing in larger trials. Further, exercise is a well-known therapy for alleviating fatigue[136] yet not all cancer survivors report fatigue improvements with exercise.[26] Thus, a better understanding of the potential mediating effects of the microbiome can lead to exercise recommendations that optimize fatigue reductions.

As no research study is perfect, several limitations warrant discussion. Notably, the high

scientific rigor made possible by the supervised exercise and controlled feeding may limit translatability of the results to less controlled interventions. However, this is offset by the opportunities for exploring potential mechanistic links related to exercise, gut microbiome, and fatigue. Moreover, the study inclusion and exclusion criteria may limit generalizability of the results to other cancer types or individuals with higher baseline cardiorespiratory fitness or BMI over 50. Finally, the COVID-19 pandemic's detrimental impact on our anticipated sample size may preclude detecting smaller effect sizes and mediating factors. This is offset by several a priori design features that enhance study power: 1) controlled feeding (reduces variability), 2) selecting low fit and fatigued individuals (greater chance of improvement), 3) manipulating the exercise exposure (standardizes the exercise exposure), and 4) stratifying randomization by BMI (reduces type 1 error and improves study power in trials with < 200 participants per study condition[137]).

We will report findings in peer-reviewed journals and present them at conferences.

Figure captions

Figure 1. Framework for testing exercise effects on gut microbiota and mechanistic links between exercise, gut microbiota, and fatigue.

Figure 2. Study schema for testing aerobic exercise effects on gut microbiota composition and potential mechanistic links in breast cancer survivors.

Figure 3. Participant screening, enrollment, and baseline assessment. A pre-screening telephone interview determines the potential eligibility of the participant. The orientation visit includes completion of administrative forms, lab-based screening informed consent, and release forms for obtaining medical clearance. Once medical clearance is received by the study team, the

participant completes the lab-based screening visit, which includes collecting VO2peak and BMI. If deemed eligible based on the screening visit, the individual will be invited to sign the consent for full study participation and be scheduled for controlled feeding initiation. Baseline assessment visit #1 is scheduled for at least one week after initiation of controlled feeding. Within seven days of visit #1, 1) the participant is asked to collect the fecal sample at home 2-3 days after visit #1 and promptly overnight ships it to the laboratory, and then 2) complete the remaining assessment materials (e.g., fatigue survey) 2-3 days after collecting the fecal sample and baseline visit #2 occurs to return these forms.

Figure 4. Revised contingency power curve.

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Supplementary materials

Supplemental Material 1. Informed consent document to lab-based screening.
Supplemental Material 2. Informed consent document to full study.
Supplemental Material 3. Equations for calculating daily calorie needs for energy balanced controlled feeding used in the ROME study (R01CA235598)

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Contributors: Laura Q. Rogers conceived the study design, led and integrated the multidisciplinary input, and achieved funding through the National Cancer Institute as the lead investigator. Stephen J. Carter, Robert W. Motl, Gary R. Hunter, Nianjun Liu, Helen Krontiras, Elliot J. Lefkowitz, and Bulent Turan helped design the final study protocol and choice of outcome measures and provided intellectual contributions in their expert areas. Rebecca B. Little led development of sample process tracking and operationalizing controlled feeding implementation and fidelity monitoring; she also completed the initial draft of the manuscript. Abby Cook and Erica Schleicher assisted with exercise intervention fidelity and adherence tracking protocols. Abby Cook completed a literature review to guide data management related to current medication use. All authors assisted with drafting the manuscript and have read, edited and approved the final manuscript.

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Competing interests: None declared.

Patient consent for publication Included as Supplemental Material 1 and Supplemental Material 2.

Ethics approval Ethics approval was obtained from the Institutional Review Board (IRB) of the University of Alabama at Birmingham (UAB IRB#30000320).

Table 1. Outcome measures for the primary and secondary study aims.

Aim	Outcome of Interest	Outcome Measure
Primary Aim: To determine the effects of a 10-week aerobic exercise training intervention compared to a flexibility/toning standard attention control on gut microbiota composition among breast cancer survivors with fatigue	Gut microbiota composition assessed by 16S rRNA	Diversity comparisons: • α-diversity • β-diversity Taxa comparisons
Aim 2a: To test if exercise training affects the gut microbiota composition directly and/or indirectly through inflammation, autonomic nervous system, or hypothalamic-pituitary-adrenal (HPA) axis mediators.	Inflammation	Serum cytokines: • interleukin [IL]-6 • IL-10
	Autonomic nervous system	Heart rate variability: • Low frequency, high frequency, and low:high frequency ratio • Root mean square of successive RR interval differences (RMSSD)
	Hypothalamic-pituitary-adrenal (HPA) axis	Hair cortisol
Aim 2b: To test if the exercise training effect on fatigue is direct and/or indirect through changes in the gut microbiota composition	Gut microbiota composition assessed by 16S rRNA	Diversity comparisons: • α-diversity • β-diversity Taxa comparisons
	Fatigue	13-item multi-dimensional fatigue scale Fatigue Symptom Inventory (FSI)

Table 2. Participant timeline (Note: To facilitate temporal relationships, data collection is ordered within each assessment period as follows: #1 - outcomes other than fecal sample and fatigue survey, #2 - fecal sample 2 to 3 days after outcomes other than fatigue, and #3 - fatigue survey 2 to 3 days after fecal sample)

	Lab-based screening	**Baseline Assessment**		**Exercise training or control**	**Follow-up assessments**
Study week (preW = week leading up to randomization [0]; W = week after randomization)	preW3	preW2 – preW1	preW1 –0	W1 – W10	W5, W10, & W15
Lab-based screening consent, obtain medical clearance, complete lab-based screening (e.g., $\dot{V}O_{2peak}$)	X				
Enrollment (consent for full participation)	X				
Controlled feeding diet (both study groups)		X	X	X	
Self-administered questionnaire		X			X
Fatigue survey			X		X
Fecal sample collection for gut microbiota composition (with 3-day diet record)			X		X
Medication log (7 days prior to blood draw)		X			X
Fasted blood draw, heart rate variability, hair sample		X			X
Resting energy expenditure		X			X
Walking economy		X			X
$\dot{V}O_{2peak}$, weight, body mass index (BMI)	X				X
Accelerometer with log sheet (7 days)		X			X
Dual-energy X-ray absorptiometry (DXA)		X			X
Randomization			X		
Exercise training or standard attention control				X	

Table 3. Aerobic exercise progression (based on maximum heart rate; high intensity added in later weeks to facilitate continued cardiorespiratory fitness improvement)

Week	Intensity	Max Heart Rate (%)	Duration (mins)	Frequency per Week
1 – 4	Moderate-intensity, continuous	60-75	20 - 35	3
5 – 7	Moderate-intensity, continuous	75	40	2
	High-intensity interval	85-90	20-22	1
8 – 10	Moderate-intensity, continuous	75	40	1
	High-intensity interval	85-90	22-28	2

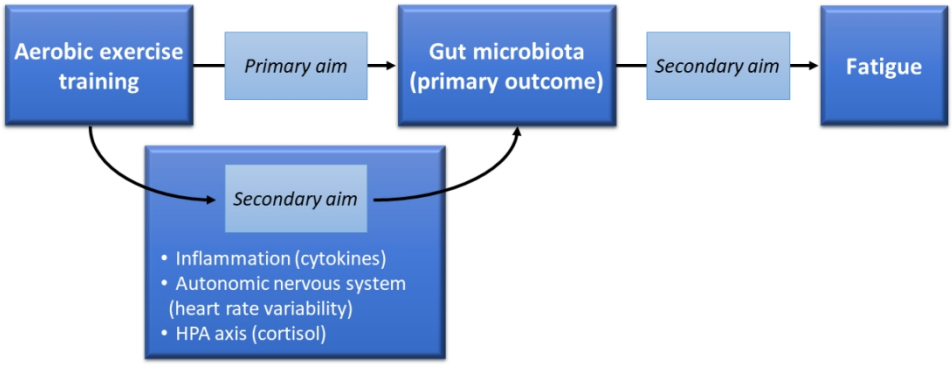

Figure 1. Framework for testing exercise effects on gut microbiota and mechanistic links between exercise, gut microbiota, and fatigue.

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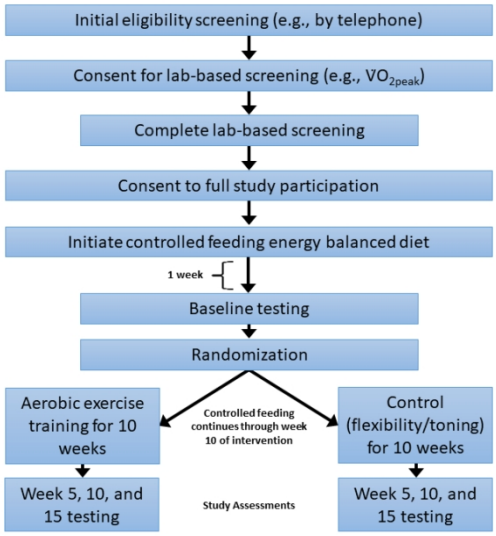

Figure 2. Study schema for testing aerobic exercise effects on gut microbiota composition and potential mechanistic links in breast cancer survivors.

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Figure 3. Participant screening, enrollment, and baseline assessment. A pre-screening telephone interview determines the potential eligibility of the participant. The orientation visit includes completion of administrative forms, lab-based screening informed consent, and release forms for obtaining medical clearance. Once medical clearance is received by the study team, the participant completes the lab-based screening visit, which includes collecting VO_2peak and BMI. If deemed eligible based on the screening visit, the individual will be invited to sign the consent for full study participation and be scheduled for controlled feeding initiation. Baseline assessment visit #1 is scheduled for at least one week after initiation of controlled feeding. Within seven days of visit #1, 1) the participant is asked to collect the fecal sample at home 2-3 days after visit #1 and promptly overnight ships it to the laboratory, and then 2) complete the remaining assessment materials (e.g., fatigue survey) 2-3 days after collecting the fecal sample and baseline visit #2 occurs to return these forms.

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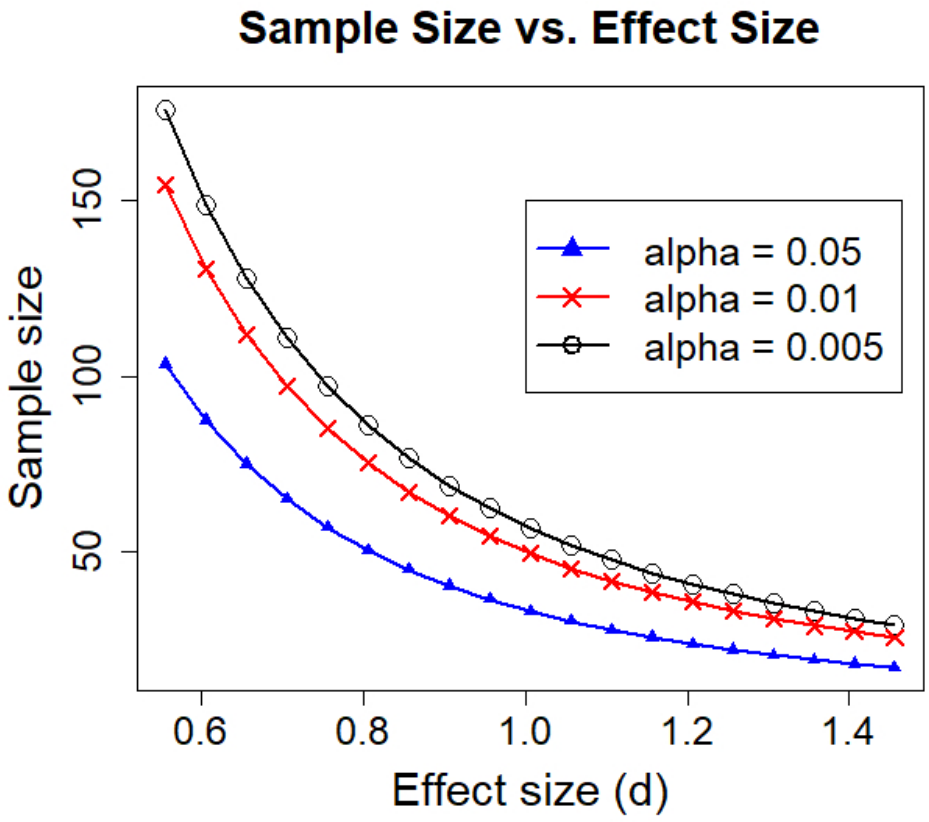

Figure 4. Revised contingency power curve.

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Title of Research: Role of Gut Microbe Composition in Psychosocial Symptom Response to Exercise Training in Breast Cancer Survivors

UAB IRB Protocol #: IRB-300003230

Principal Investigator: Laura Q. Rogers, M.D., M.P.H

Sponsor: National Cancer Institute (NCI)

Pre-Study Participant Consent

General Information	You are being asked to take part in a research study. This research study is voluntary, meaning you do not have to take part in it. The procedures, risks, and benefits are fully described further in the consent form.
Purpose	The purpose of this portion is to find out if you are able to participate in the study to determine the effects of diet and exercise on the number, distribution, and types of bacteria in the gut of breast cancer survivors.
Duration & Visits	You will be in this portion of the study for 1 hour. This portion is the first part of the screening visit for this study.
Overview of Procedures	This portion of the study will include the following procedures and assessments: • Peak VO_2 Testing You will be asked to perform a graded treadmill or stationary bicycle test. You will walk on the treadmill or pedal on the bike at increasing intensity until you feel you can no longer walk. We will measure you total aerobic fitness by measuring oxygen consumed when performing this test. • Weight • Height • Body mass index (BMI) • Blood pressure • Heart rate
Risks	The most common risks include: • Muscle soreness • Fatigue (tiredness) • Shortness of breath during and/or after performance of peak VO_2 • Dry mouth • Embarrassment during height and weight collection
Benefits	You will not benefit directly from taking part in this portion of the study.
Alternatives	The alternative to this study is to not take part in it.

Purpose of the Research Study

The purpose of this research study is to determine diet and exercise effects on the number, distribution, and types of bacteria in the gut of breast cancer survivors. This study will enroll 200 participants at UAB.

Eligibility

You are eligible to participate in this study if you(r):

- Are a woman aged 18 to 74 years with a history of breast cancer stage 0, I, II, or III

- Are one to five years post completion of primary treatment for breast cancer (chemotherapy and/or radiation)
- Average fatigue over the past week is rated as ≥3 on a 1 to 10 Likert scale
- Are English speaking
- Have physician medical clearance for study participation
- Are able to ambulate (walk) without assistance
- Have not taken antibiotics for the past 90 days
- Are willing to avoid taking probiotics for the duration of the study
- Peak VO2 is ≤30 ml/kg/min (note: will measure peak VO2 if you meet all other criteria and consent to lab-based screening)

You are not eligible to participate in this study if you(r):

- Have metastatic or recurrent cancer
- Have another diagnosis of cancer in the past 5 years (not including skin or cervical cancer in situ)
- Unstable angina
- Have New York Heart Association class II, III, or IV congestive heart failure
- Have uncontrolled asthma
- Have interstitial lung disease
- Have current steroid use
- Have been told by a physician to only do exercise prescribed by a physician
- Have Dementia or organic brain syndrome
- Have Schizophrenia or active psychosis
- Have connective tissue or rheumatologic disease (i.e., systemic lupus erythematosus, rheumatoid arthritis, amyloidosis, Reiter's syndrome, psoriatic arthritis, mixed connective tissue disease, Sjogren's syndrome, CREST syndrome, polymyositis, dermatomyositis, progressive systemic sclerosis, vasculitis, polymyalgia rheumatic, temporal arteritis)
- Anticipate elective surgery during the study period
- Anticipate changes in usual medications during the study period
- Plan to move residence out of the local area during the study period
- Plan to travel out of the local area for >1 week during study participation
- Have contraindications to engaging in moderate-to-vigorous intensity aerobic exercise
- Are currently pregnant or anticipate pregnancy during study participation
- Live or work >50 miles from study site or do not have transportation to study site
- Have a BMI >50
- Anticipate needing antibiotics during the study period

Study Participation & Procedures

If you agree to join the screening portion of the study, you will undergo Peak VO_2 testing, have your height and weight measured to calculate your body mass index (BMI), and have your blood pressure and heart rate measured. If the results from the Peak VO_2 test and the BMI results are within the eligibility criteria, and you sign the Consent Form for full study participation, you will be started on the controlled feeding diet.

Procedures

Height, Weight, Blood Pressure, and Heart Rate: We will measure your height, weight, blood pressure, and heart rate, similar to how they are measured in a doctor's office.

Peak VO_2 Testing: Peak VO_2 will be measured while you perform a graded treadmill or stationary bicycle test. During this test, we will also measure your heart rate and blood pressure. This test requires you exercise until exhaustion.

Risks and Discomforts

You may have some risks from taking part in this study.

The risks are:

Moderate likelihood:
- Muscle soreness
- Fatigue
- Shortness of breath during and/or after performance of peak VO_2
- Dry mouth
- Embarrassment during height and weight collection.

Low likelihood:
- Injury to muscle, joint, ligaments, tendons, or bones
- Inconvenience
- Exacerbation of musculoskeletal condition
- Lightheadedness
- Dizziness
- Difficulty swallowing, coughing, or nausea when performing peak VO_2 test

Very low likelihood
- Cardia ischemia or arrest during peak VO_2 test or exercise training

There may also be risks that are unknown at this time. You will be given more information if other risks are found.

Information for Women of Childbearing Potential

Women who are pregnant or breastfeeding are not permitted to participate in this study. During the study, you may use any form of birth control that you wish; we simply ask that you not change the type of birth control that you use or its dose during the study.

Benefits

You will not benefit directly from taking part in this portion of the study. Your participation may qualify you to screen for the entire research study.

Alternatives

Your alternative is to not participate in the study.

Confidentiality and Authorization to Use and Disclose Information for Research Purposes

Federal regulations give you certain rights related to your health information. These include the right to know who will be able to get the information and why they may be able to get it. The study doctor must get your authorization (permission) to use or give out any health information that might identify you.

What protected health information may be used and/or given to others?

All medical information, including but not limited to information and/or records of any diagnosis or treatment of disease or condition, which may include sexually transmitted diseases (e.g., HIV, etc.) or communicable diseases, drug/alcohol dependency, etc.; all personal identifiers, including but not limited to your name, social security number, medical record number, date of birth, dates of service, etc.; any past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of any kind, including but not limited to drug/alcohol treatment, psychiatric/psychological treatment; financial/billing information, including but not limited to copies of your medical bills; any other information related to or collected for use in the research study, regardless of whether the information was collected for research or non-research (e.g., treatment) purposes; records about any study drug you received or about study devices used; and consent forms from past studies that might be in your medical record.

Your consent form will be placed in your medical record at UAB Health System or Children's of Alabama. This may include either a paper medical record or electronic medical record (EMR). An EMR is an electronic version of a paper medical record of your care within this health system. Your EMR may indicate that you are on a clinical trial and provide the name and contact information for the principal investigator.

If you are receiving care or have received care within this health system (outpatient or inpatient), results of research tests or procedures (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in your existing medical record.

If you have never received care within this health system (outpatient or inpatient), a medical record will be created for you to maintain results of research tests or procedures.

Results of research tests or procedures may be placed in your medical record. All information within your medical record can be viewed by individuals authorized to access the record.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who may use and give out information about you?

Information about your health may be used and given to others by the study doctor and staff. They might see the research information during and after the study.

Who might get this information?

All Individuals/entities listed in the informed consent document(s), including but not limited to, the physicians, nurses and staff and others performing services related to the research (whether at UAB or elsewhere). Your information may also be given to the sponsor of this research. "Sponsor" includes any persons or companies that are working for or with the sponsor, or are owned by the sponsor, or are providing support to the sponsor (e.g., contract research organization).

Information about you and your health which might identify you may be given to:

- the Office for Human Research Protections(OHRP)
- the U.S. Food and Drug Administration(FDA)
- Department of Health and Human Services (DHHS)agencies
- Governmental agencies in othercountries
- Governmental agencies to whom certain diseases (reportable diseases) must bereported
- the University of Alabama at Birmingham - the physicians, nurses and staff working on the research study (whether at UAB or elsewhere); other operating units of UAB, UAB Hospital, UAB Highlands Hospital, University of Alabama Health Services Foundation, Children's of Alabama, Eye Foundation Hospital, and the Jefferson County Department of Health, as necessary for their operations; the UAB IRB and its staff
- the billing offices of UAB and UAB Health Systems affiliates and/or Children's of Alabama and its billing agents

Why will this information be used and/or given to others?
Information about you and your health that might identify you may be given to others to carry out the research study. The sponsor will analyze and evaluate the results of the study. In addition, people from the sponsor and its consultants will be visiting the research site. They will follow how the study is done, and they will be reviewing your information for this purpose.

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if you have consented to the disclosure, including for your medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the National Cancer Institute which is funding this project or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

The Certificate of Confidentiality will not be used to prevent disclosure as required by federal, state, or local law of child abuse and neglect, or harm to self or others.

The Certificate of Confidentiality will not be used to prevent disclosure for any purpose you have consented to in this informed consent document.

A federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and some employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

Be aware that this federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance, nor does it protect you against genetic discrimination by all employers.

What if I decide not to give permission to use and give out my health information?
By signing this consent form, you are giving permission to use and give out the health information listed above for the purposes described above. If you refuse to give permission, you will not be able to be in this research.

May I review or copy the information obtained from me or created about me?
You have the right to review and copy your health information. However, if you decide to be in this study and sign this permission form, you will not be allowed to look at or copy your information until after the research is completed.

May I withdraw or revoke (cancel) my permission?
Yes, but this permission will not stop automatically. The use of your personal health information will continue until you cancel your permission.

You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the study doctor. If you withdraw your permission, you will not be able to continue being in this study.

When you withdraw your permission, no new health information which might identify you will be gathered after that date. Information that has already been gathered may still be used and given to others. This would be done if it were necessary for the research to be reliable.

Is my health information protected after it has been given to others?
If you give permission to give your identifiable health information to a person or business, the information may no longer be protected. There is a risk that your information will be released to others. Including others outside of UAB, without your permission.

<u>Voluntary Participation and Withdrawal</u>

Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in it. If you decide not to be in the study, you will not lose any benefits you are otherwise owed.

If you are a UAB student or employee, taking part in this research is not a part of your UAB class work or duties. You can refuse to enroll, or withdraw after enrolling at any time before the study is over, with no effect on your class standing, grades, or job at UAB. You will not be offered or receive any special consideration if you take part in this research.

Cost of Participation

There will be no cost to you for taking part in this study.

Payment for Participation

There will be no payment for this portion of the study.

Payment for Research-Related Injuries

UAB, UAB-Lakeshore Research Collaborative Exercise Center, and NCI have not provided for any payment if you are harmed as a result of taking part in this study. If such harm occurs, treatment will be provided. However, this treatment will not be provided free of charge.

New Findings

You will be told by the study doctor or the study staff if new information becomes available that might affect your choice to stay in the study.

Questions

If you have any questions, concerns, or complaints about the research or a research-related injury including available treatments, please contact the study doctor. You may contact Dr. Laura Rogers at 205-934-9735.

If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the UAB Office of the IRB (OIRB) at (205) 934-3789 or toll free at 1-855-860-3789. Regular hours for the OIRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday.

Legal Rights

You are not waiving any of your legal rights by signing this consent form.

Signatures

Your signature below indicates that you have read (or been read) the information provided above and agree to participate in this portion of the study. You will receive a copy of this signed consent form.

_____

Signature of Participant Date

_____

Signature of Person Obtaining Consent Date

Title of Research: Role of Gut Microbe Composition in Psychosocial Symptom Response to Exercise Training in Breast Cancer Survivors

UAB IRB Protocol #: IRB-300003230

Principal Investigator: Laura Q. Rogers, M.D., M.P.H

Sponsor: National Cancer Institute (NCI)

Full Study Participation Consent

General Information	You are being asked to take part in a research study. This research study is voluntary, meaning you do not have to take part in it. The procedures, risks, and benefits are fully described further in the consent form.
Purpose	The purpose of the study is to determine the effects of diet and exercise on the number, distribution, and types of bacteria in the gut of breast cancer survivors.
Duration & Visits	• You will be in this study for up to 21 weeks. There will be a total of 57 study visits: • 1 Screening visit (2 hours) • 2 Baseline visits (Baseline Visit 1: 3 hours, Baseline Visit 2: 1 hour) • 2 Mid-intervention visits (Mid-intervention Visit 1: 3.5 hours; Mid-intervention Visit 2: 1 hour) • 2 Post-intervention visits (Post-intervention Visit 1: 3.5 hours; Post-intervention Visit 2: 1 hour) • 2 Week-5 Post-intervention visits (Week-5 Post-intervention Visit 1: 3.5 hours; Week-5 Post-intervention Visit 2: 1 hour) • 4 Between Visits (All take about 30 minutes, and may be completed at your home) • Up to 30 Aerobic exercise training sessions or 30 flexibility/toning sessions (depending on the group you are assigned to). (Aerobic exercise training sessions: 20 – 60 minutes each, depending on your level of progression; Flexibility/toning sessions: 40 minutes each) • 11-13 Food Pick-up Visits (30 minutes per pick-up)
Overview of Procedures	• This study will include the following procedures and assessments: • Controlled feeding diet: You will be required to pick-up your food once a week from the Bionutrition Core at the University of Alabama at Birmingham (UAB) or the UAB-Lakeshore Research Collaborative Exercise Center. No outside food may be eaten during the 11 to 13 weeks you are on the controlled feeding diet. • Stool sample collection • Diet log for 3 days (diary of what you eat for 3 days) • Medication log (listing your medicines) • Fasted blood draw • Heart rate variability with impedance cardiography - non-invasive test using ECG leads • Peak VO_2 Testing – You will be asked to perform a graded treadmill or stationary bicycle test. You will walk on the treadmill or pedal on the bike at increasing intensity until you feel you cannot longer walk or pedal. We will measure your total aerobic fitness by measuring oxygen consumed when performing this test.

	• Resting Energy Expenditure - You will be asked to lie on your back for 30 minutes in a bed with a canopy system over your head. We will ask you to breathe normally during this time while we collect the exhaled air. • Walking Economy – You will be asked to perform a walking economy test. You will wear a hip-worn accelerometer while walking on a treadmill at slow pace for six minutes. • Hair sample collection • Body mass index, weight and height measurement • Blood pressure and heart rate • Wear an accelerometer for 7 days and keep accelerometer wear log - The accelerometer is a small monitor that will be worn around your waist. This monitor will collect data on how much you move during your daily living activities. • Dual Energy X-Ray Absorptiometry (DXA) scan • Randomized into aerobic or flexibility/toning exercise group • Self-administered survey (questions about how you feel and your medical history)
Risks	• The most common risks include: • Muscle soreness • Fatigue (tiredness) • Shortness of breath during and/or after exercise or performance of Peak VO_2 • Dry mouth • Skin irritation from impedance cardiography and heart rate variability non-invasive chest electrode (similar to ECG) preparation • Feelings of claustrophobia during resting energy expenditure testing • Embarrassment during height and weight collection • Change in diet might cause gastrointestinal discomfort including, but not limited to general discomfort, bloating, gas, reflux, diarrhea and constipation.
Benefits	You may or may not benefit directly from taking part in this study. However, this study may help us better understand how to reduce the great burden of suffering caused by fatigue after a cancer diagnosis in the future.
Alternatives	The alternative to this study is to not take part in it.

Purpose of the Research Study

The purpose of this research study is to determine diet and exercise effects on the number, distribution, and types of bacteria in the gut of breast cancer survivors. This study will enroll 200 participants at UAB.

Study Participation & Procedures

If you agree to join the study, you will be involved in the following procedures:

Screening Visit (2 hours)
During the screening visit, you will come to the Webb building in the morning time as you will need to come in a fasting state. At this visit you will undergo Peak VO_2 testing and have your weight and height taken to calculate your body mass index (BMI). If the results from the Peak VO_2 test and the BMI results are within the eligibility criteria, you will be started on the controlled feeding diet and be given a 7-day medication log sheet to complete. Participants will be shown a menu with all meals that will be delivered throughout the baseline testing and 10 weeks of exercise. Participants will have the ability to ask questions regarding food choices, and controlled feeding.

Baseline Testing Visit 1 (3 hours)
Once you have completed at least 1 full week of the controlled feeding diet, you will come in to the Webb building in a fasted state for Baseline Testing Visit 1. At this visit, you will be asked to have your weight measured, provide your medication log for the prior 7 days and will have a fasted blood draw, heart rate variability with impedance cardiography test, resting energy expenditure test, walking economy test, and a hair sample collected. You will complete the DXA scan and a pregnancy test (if pre-menopausal). You will be asked to complete a survey about your medical history, mood, attitudes, sleep, etc. You will also start wearing an accelerometer, and will be required to wear it for 7 days while also keeping an accelerometer wear log.

Between Baseline Testing Visit 1 and 2 (30 minutes)
You will be asked to provide a fecal sample 2 to 3 days after Baseline Testing Visit 1, along with a 3-day diet record for the 2 days prior to and day of fecal sample collection. You will provide this fecal sample from the comfort of your own home, and mail the sample to us via pre-paid shipping materials.

Baseline Testing Visit 2 (1 hour)
You will come to the Webb building, Medical Towers, or UAB-Lakeshore Research Collaborative Exercise Center 2 to 3 days after the fecal sample collection to return the accelerometer (and accelerometer log) and complete a brief self-administered survey about your energy level. These activities may be done through the mail if preferred. We will provide pre-paid shipping materials.

Mid-intervention Testing Visit 1 (3.5 hours)
At week 5 of the intervention, you will come to the Webb building for the mid-intervention visit. This visit will mirror that of the baseline testing visit 1 and the Peak VO_2 testing done during the screening visit.

Between Mid-intervention Testing Visit 1 and 2 (30 minutes)
You will be asked to provide a fecal sample 2 to 3 days after Mid-intervention Testing Visit 1, along with a 3-day diet record for the 2 days prior to and day of fecal sample collection. You will provide this fecal sample from the comfort of your own home, and mail the sample to us via pre-paid shipping materials.

Mid-intervention Testing Visit 2 (1 hour)
You will come to the Webb building, Medical Towers, or UAB-Lakeshore Research Collaborative Exercise Center 2 to 3 days after the fecal sample collection to return the accelerometer (and accelerometer log) and complete a brief self-administered survey about your energy level. These activities may be done through the mail if preferred. We will provide pre-paid shipping materials.

Post-intervention and 5 Weeks Post-intervention Testing Visit 1 (3.5 hours)
At week 10 of the intervention and week 15 of the study (5 weeks post-intervention), you will come to the Webb building for the post-intervention and 5 week post-intervention testing. We will ask you to request/collect medical information (i.e., tumor characteristics) as well as medical clearance from your treating physician before this visit. These visits will mirror the Mid-intervention Testing Visit 1.

Between Post-intervention and 5 Weeks Post-intervention Testing Visit 1 and 2 (30 minutes)
You will be asked to provide a fecal sample 2 to 3 days after Post-intervention and 5 weeks Post-intervention Testing Visit 1, along with a 3-day diet record for the 2 days prior to and day of fecal sample collection. You will provide this fecal sample from the comfort of your own home, and mail the sample to us via pre-paid shipping materials.

Post-intervention and 5 Week Post-intervention Testing Visit 2 (1 hour)
You will come to the Webb building, Medical Towers, or UAB-Lakeshore Research Collaborative Exercise Center 2 to 3 days after the fecal sample collection to return the accelerometer (and accelerometer log) and complete a

brief self-administered survey about your energy level. These activities may be done through the mail if preferred. We will provide pre-paid shipping materials.

Aerobic Exercise Training Sessions (if you are randomized to this group)

These sessions will take place at the UAB-Lakeshore Research Collaborative Exercise Center or at the UAB campus, and will be supervised by exercise specialists who have experience training cancer survivors. Each session will last 20 to 60 minutes depending on your level of progression (shorter duration in the first few weeks). These sessions will occur on nonconsecutive days of the week, and will be held 3 times per week.

Flexibility/toning exercise Group (if you are randomized to this group)

These sessions will take place at the UAB-Lakeshore Research Collaborative Exercise Center or at the UAB campus, and will be led by exercise specialists who have experience training cancer survivors. Each session will last about 40 minutes, and will be held 3 times per week.

Weekly Food Pick Up

Once a week, you will be required to come to UAB's campus or UAB-Lakeshore Research Collaborative Exercise Center to pick up your food. During the baseline assessment period and the 10-week intervention, and the post-intervention assessment period, you will consume only food prepared by the UAB Bionutrition Core as no "outside" foods are allowed. However, you may consume calorie-free beverages, such as water and black tea, or chew sugarless gum. You will not be given food after the post-intervention assessment or during the 5 weeks after finishing the post-intervention assessments.

We ask that you do not change your usual physical activity or engage in additional exercise sessions outside of the study appointments while you are participating in this study.

If you agree to join the study, you will be in the study for up to 21 weeks.

Explanation of the Procedures

Weight, Height, Blood Pressure, and Heart Rate: We will measure your weight, height, blood pressure, and heart rate, similar to how they are measured in a doctor's office.

Fecal and Hair Sample Collection: To collect your stool at home, we will give you a collection kit at each visit prior to the collection. You will collect the sample per instructions provided to you, and ship it back to our site via pre-paid shipping materials. We will collect the hair sample when you come in for each follow-up visit. For the hair sample collection, we will cut a thin layer of hair from a point close to the scalp across a 4-5 centimeter length, and 6-8 centimeter length for shorter hair. We will obtain a minimum of 50 strands of hair.

Diet Record: You will complete a diet record for the 2 days prior to and day of fecal sample collection.

7-day Medication Log: You will complete a 7-day medication log listing all medications taken within the 7 days prior to the fasted blood draw. This will be completed for the Baseline Testing Visit 1, Mid-intervention Testing Visit 1, and Post-intervention and 5-weeks Post-Intervention Testing Visits 1. We do ask that you refrain from taking sporadic or "as-needed" medications during these 7 days.

Blood Draw: Fasting blood draws will be conducted to measure systemic markers of inflammation and health. You will need to be in a fasting state for 12 hours prior to the blood draw. We will take no more than 25 ml (5 teaspoons) of blood.

Heart Rate Variability, Impedance Cardiography, Resting Energy Expenditure, Walking Economy, and Peak VO_2 Testing: Heart rate variability and impedance cardiography will be measured using non-invasive chest electrodes. You will be asked to provide a urine sample prior to the heart rate variability test to measure your hydration. We will assess your resting metabolic rate (or energy expenditure) using a ventilated hood while lying quietly on a table (approximately 30 minutes). You must fast for at least 12 hours prior, complete no physical activity for 24 hours and avoid any caffeine or nicotine for at least 2 hours prior to this test. Please notify the staff if you have diabetes so special precautions can be taken to ensure your safety. Walking economy will be tested by having you walk for six minutes on a treadmill while wearing a motion sensor and breathing through a mouthpiece that measures your metabolism. Peak VO_2 will be measured by you walking on a treadmill or pedaling on a stationary bicycle while wearing a motion sensor and breathing through a mouthpiece that measures your metabolism. You will perform a graded treadmill or a stationary bicycle test while we measure your heart rate and blood pressure and you will walk or pedal until exhaustion.

Accelerometer activity: You will be asked to wear an accelerometer (motion sensor) at the waist for 7 consecutive days while also keeping an accelerometer wear log.

Dual X-Ray Absorptiometry: DXA is a method to test body composition as well as bone density. In this procedure you will lie on your back on a padded table while a measuring device moves back and forth over your body from head to foot, taking about 30 minutes. You will be asked to lie still, but there is no discomfort in this procedure. DXA involves extremely low levels of radiation (x-ray) exposure. The amount of radiation involved is equivalent to one to two days of natural background radiation. Natural background radiation is radiation normally received from sources such as cosmic rays and natural radioactivity in building materials and the ground.

Urine Pregnancy Test: For pre-menopausal females, a urine pregnancy test will be required before each DXA scan.

Self-administered Surveys: You will be asked to complete a questionnaire regarding your medical history, demographics, mood, attitudes, sleep, physical activity, etc.

You will be randomly picked (like the flip of a coin) by a computer to receive either aerobic or flexibility/toning exercise. The exercise schedules are as mentioned above. This is a single-blind study. This means the person performing the testing visits on you will not know which group you have been randomized to. We will ask you not to tell this person your group assignment.

Incidental Findings

We are performing imaging solely for the research purposes described above. It is not a clinical scan intended for diagnostic or therapeutic purposes. Under no circumstance will the investigator, research staff, or imaging staff interpret the scan as normal or abnormal. They are unable to make any medical comments about your scan. The scan will not be looked at or read for any healthcare treatment or diagnostic purpose. If you want your scan to be reviewed by a physician so the physician can look for medical issues, you can request a copy of your scan. We will provide an electronic copy at no charge.

Additional Information

Your de-identified private information and de-identified biospecimens (private information and biospecimens with all identifiers removed) may be used for future research studies or distributed to another researcher for future research studies without additional informed consent. **This is only when there are no identifiers associated with the data or biospecimens.**

The biospecimens obtained from you in this research, which may or may not include your identifiable private information, may be used for commercial profit. There are no plans to provide financial compensation to you should this occur.

The clinical results (including individual research results) will only be given to you upon request and after completion of the study.

Risks and Discomforts

You may have some risks from taking part in this study.

The risks are:

Moderate likelihood:

- Muscle soreness
- Fatigue (tiredness)
- Shortness of breath during and/or after exercise or performance of Peak VO_2
- Dry mouth
- Feelings of claustrophobia during resting energy expenditure testing
- Embarrassment during height and weight collection.
- Low levels of radiation (X-ray) exposure

Low likelihood:

- Injury to muscle, joint, ligaments, tendons, or bones
- Tripping or falling during exercise or while completing the fitness test
- Inconvenience
- Emotional stress while completing surveys or having blood drawn
- Exacerbation of musculoskeletal condition
- Mild bruising or soreness at the site of the blood draw
- Passing out during the blood draw
- Lightheadedness
- Dizziness
- Hypoglycemia when fasting (low blood sugar)
- Difficulty swallowing, coughing, or nausea when performing Peak VO_2 test
- Gastrointestinal discomfort due to a higher fiber content of the diet

Very low likelihood:

- Cardia ischemia or cardiac arrest during peak VO_2 test or exercise training

There may also be risks that are unknown at this time. You will be given more information if other risks are found.
You will be assigned to a group by chance, which may prove to be less effective or to have more side effects than the other study group or alternatives.

Information for Women of Childbearing Potential

Women who are pregnant or breastfeeding are not permitted to participate in this study. During the study, you may use any form of birth control that you wish; we simply ask that you not change the type of birth control that you use or its dose during the study.

Benefits

You may or may not benefit directly from taking part in this study. However, this study may help us better understand how to reduce the great burden of suffering caused by fatigue after a cancer diagnosis in the future. You will be assigned to a group by chance, which may prove to have more or less benefits than the other study group.

Alternatives

Your alternative is to not participate in the study.

Confidentiality and Authorization to Use and Disclose Information for Research Purposes

Federal regulations give you certain rights related to your health information. These include the right to know who will be able to get the information and why they may be able to get it. The study doctor must get your authorization (permission) to use or give out any health information that might identify you.

What protected health information may be used and/or given to others?
All medical information, including but not limited to information and/or records of any diagnosis or treatment of disease or condition, which may include sexually transmitted diseases (e.g., HIV, etc.) or communicable diseases, drug/alcohol dependency, etc.; all personal identifiers, including but not limited to your name, social security number, medical record number, date of birth, dates of service, etc.; any past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of any kind, including but not limited to drug/alcohol treatment, psychiatric/psychological treatment; financial/billing information, including but not limited to copies of your medical bills; any other information related to or collected for use in the research study, regardless of whether the information was collected for research or non-research (e.g., treatment) purposes; records about any study drug you received or about study devices used; and consent forms from past studies that might be in your medical record.

Your consent form will be placed in your medical record at UAB Health System or Children's of Alabama. This may include either a paper medical record or electronic medical record (EMR). An EMR is an electronic version of a paper medical record of your care within this health system. Your EMR may indicate that you are on a clinical trial and provide the name and contact information for the principal investigator.

If you are receiving care or have received care within this health system (outpatient or inpatient), results of research tests or procedures (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in your existing medical record.

If you have never received care within this health system (outpatient or inpatient), a medical record will be created for you to maintain results of research tests or procedures.

Results of research tests or procedures may be placed in your medical record. All information within your medical record can be viewed by individuals authorized to access the record.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who may use and give out information about you?
Information about your health may be used and given to others by the study doctor and staff. They might see the research information during and after the study.

Who might get this information?
All Individuals/entities listed in the informed consent document(s), including but not limited to, the physicians, nurses and staff and others performing services related to the research (whether at UAB or elsewhere). Your information may also be given to the sponsor of this research. "Sponsor" includes any persons or companies that are working for or with the sponsor, or are owned by the sponsor, or are providing support to the sponsor (e.g., contract research organization).

Information about you and your health which might identify you may be given to:

- the Office for Human Research Protections (OHRP)
- the U.S. Food and Drug Administration (FDA)
- Department of Health and Human Services (DHHS) agencies
- Governmental agencies in other countries
- Governmental agencies to whom certain diseases (reportable diseases) must be reported
- the University of Alabama at Birmingham - the physicians, nurses and staff working on the research study (whether at UAB or elsewhere); other operating units of UAB, UAB Hospital, UAB Highlands Hospital, University of Alabama Health Services Foundation, Children's of Alabama, Eye Foundation Hospital, and the Jefferson County Department of Health, as necessary for their operations; the UAB IRB and its staff
- the billing offices of UAB and UAB Health Systems affiliates and/or Children's of Alabama and its billing agents

Why will this information be used and/or given to others?
Information about you and your health that might identify you may be given to others to carry out the research study. The sponsor will analyze and evaluate the results of the study. In addition, people from the sponsor and its consultants will be visiting the research site. They will follow how the study is done, and they will be reviewing your information for this purpose.

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if you have consented to the disclosure, including for your medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the National Cancer Institute which is funding this project or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

The Certificate of Confidentiality will not be used to prevent disclosure as required by federal, state, or local law of child abuse and neglect, or harm to self or others.

The Certificate of Confidentiality will not be used to prevent disclosure for any purpose you have consented to in this informed consent document.

A federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and some employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

Be aware that this federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance, nor does it protect you against genetic discrimination by all employers.

What if I decide not to give permission to use and give out my health information?

By signing this consent form, you are giving permission to use and give out the health information listed above for the purposes described above. If you refuse to give permission, you will not be able to be in this research.

May I review or copy the information obtained from me or created about me?

You have the right to review and copy your health information. However, if you decide to be in this study and sign this permission form, you will not be allowed to look at or copy your information until after the research is completed.

May I withdraw or revoke (cancel) my permission?

Yes, but this permission will not stop automatically. The use of your personal health information will continue until you cancel your permission.

You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the study doctor. If you withdraw your permission, you will not be able to continue being in this study.

When you withdraw your permission, no new health information which might identify you will be gathered after that date. Information that has already been gathered may still be used and given to others. This would be done if it were necessary for the research to be reliable.

Is my health information protected after it has been given to others?

If you give permission to give your identifiable health information to a person or business, the information may no longer be protected. There is a risk that your information will be released to others. Including others outside of UAB, without your permission.

<u>Voluntary Participation and Withdrawal</u>

Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in it.

If you decide not to be in the study, you will not lose any benefits you are otherwise owed.

You are free to withdraw from this study at any time. Your choice to leave the study will not affect your relationship with this institution. Contact the study doctor if you want to withdraw from the study.

You may be removed from the study without your consent if the sponsor ends the study, if the study doctor decides it is not in the best interest of your health, or if you are not following the study rules.

If you are a UAB student or employee, taking part in this research is not a part of your UAB class work or duties. You can refuse to enroll, or withdraw after enrolling at any time before the study is over, with no effect on your class standing, grades, or job at UAB. You will not be offered or receive any special consideration if you take part in this research.

Cost of Participation

There will be no cost to you for taking part in this study. All exams, medical care, food, and exercise or toning/flexing training related to this study will be provided to you at no cost during the 21 week study period.

Payment for Participation

The total payment you may receive is $600. You will be paid:

$150 for each assessment period for a possible total of $600 (all testing visits within an assessment must be completed; assessment periods include Baseline, Mid-intervention, Post-intervention, and 5-weeks Post-intervention).

In addition, you will receive 11 to 13 weeks of meals at no cost to you.

After completing the final assessment, you will be offered counseling from a registered dietitian regarding a diet plan that will help you maintain or lose weight, as appropriate. Similarly, you will be offered three free sessions (after completing the final assessment) with one of the ACSM certified Cancer Exercise Trainers, during which, you will receive instruction for continuing exercises at home.

Ask the study staff about the method of payment that will be used for this study (e.g., check, cash, gift card, direct deposit).

You are responsible for paying any state, federal, Social Security or other taxes on the payments you receive. You will receive a form 1099 in January of the year following your participation in this study. This form is also sent to the IRS to report any money paid to you. No taxes are kept from your payment.

Payment for Research-Related Injuries

UAB, UAB-Lakeshore Research Collaborative Exercise Center, and NCI have not provided for any payment if you are harmed as a result of taking part in this study. If such harm occurs, treatment will be provided. However, this treatment will not be provided free of charge.

New Findings

You will be told by the study doctor or the study staff if new information becomes available that might affect your choice to stay in the study.

Optional Research

Future Research Use of Private Information and/or Biospecimens

We would like your permission to keep your private information (data containing personal information) and biospecimens (blood) collected in this study for future research. The future research may be similar to this study or may be completely different. Your private information and biospecimens will be stored indefinitely or until used.

Your private information and biospecimens will be labeled with a code that only the study doctor can link back to you. Results of any future research will not be given to you or your doctor.

You can take part in this study even if you decide not to let us keep your private information and biospecimens for future research.

If you give us permission now to keep your private information and biospecimens, you can change your mind later and ask us to destroy it. However, once we have analyzed your private information and biospecimens, we may not be able to take it out of our future research.

We may share your private information and biospecimens, so that others can use it in their research. Their research may be similar to this study or may be completely different. Once we have shared your private information and biospecimens with other researchers, we will not be able to get it back.

Future research use of your private information and biospecimens will be conducted in compliance with applicable regulatory requirements.

You will not find out the results of future research on your private information and biospecimens. Allowing us to do future research on your private information and biospecimens will not benefit you directly.

The private information and biospecimens used for future research may be used for commercial profit. There are no plans to provide financial compensation to you should this occur.

Initial your choice below:

_____I agree to allow my private information and biospecimens to be kept and used for future research.

_____I do not agree to allow my private information and biospecimens to be kept and used for future research.

Initial your choice below:

_____I agree for my genetic and other relevant study data, such as health information, to be shared broadly in a coded form for future research or analysis.

_____I do not agree for my genetic and other relevant study data, such as health information, to be shared broadly in a coded form for future research or analysis.

Questions

If you have any questions, concerns, or complaints about the research or a research-related injury including available treatments, please contact the study doctor. You may contact Dr. Laura Rogers at 205-934-9735.

If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the UAB Office of the IRB (OIRB) at (205) 934-3789 or toll free at 1-855-860-3789. Regular hours for the OIRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday.

Legal Rights

You are not waiving any of your legal rights by signing this consent form.

Signatures

Your signature below indicates that you have read (or been read) the information provided above and agree to participate in this study. You will receive a copy of this signed consent form.

_____

Signature of Participant Date

_____

Signature of Person Obtaining Consent Date

Supplemental Material 3

Equations for calculating daily calorie needs for energy balanced controlled feeding used in the ROME study (R01CA235598)

Step 1: Calculate a base equation (used for all participants with Step 2 adjusting it for participants randomized to the aerobic exercise condition)

To calculate total energy expenditure (TEE), insert resting energy expenditure (REE) measured by indirect calorimeter from the most recent study assessment into the following equations developed per race based on prior datasets generated in the laboratory of Dr. Gary Hunter.[105,106]

European Americans: TEE = 1124 + (.725 * REE)
African Americans: TEE = 1074 + (.725 * REE)

Note: The ROME study uses the European American equation for individuals of Asian descent.

Step 2: Refine base equation for participants randomized to the aerobic exercise condition

Exercise energy expenditure for each workout based on the a priori exercise progression protocol ($\dot{V}O_{2peak}$ in ml/kg/min and BDW [body weight] in kg) are entered into the equation with the weekly total averaged over 7 days (to get a daily average needed for the daily controlled feeding menu). This daily average is added to the base equation calculated under Step 1 to determine the daily calorie needs for participants randomized to the aerobic exercise condition.

	Continuous training		Interval training (added in later weeks per protocol)
WK1:	3 * 0.05 * $\dot{V}O_{2peak}$ * BDW/7		
WK2:	3 * 0.0597 * $\dot{V}O_{2peak}$ * BDW/7		
WK3:	3 * 0.08 * $\dot{V}O_{2peak}$ * BDW/7		
WK4:	3 * 0.103 * $\dot{V}O_{2peak}$ * BDW/7		
WK5:	2 * 0.13 * $\dot{V}O_{2peak}$ * BDW/7	+	1 * 0.0675 * $\dot{V}O_{2peak}$ * BDW/7
WK6:	2 * 0.13 * $\dot{V}O_{2peak}$ * BDW/7	+	1 * 0.0675 * $\dot{V}O_{2peak}$ * BDW/7
WK7:	2 * 0.13 * $\dot{V}O_{2peak}$ * BDW/7	+	1 * 0.0743 * $\dot{V}O_{2peak}$ * BDW/7
WK8:	1 * 0.13 * $\dot{V}O_{2peak}$ * BDW/7	+	2 * 0.078 * $\dot{V}O_{2peak}$ * BDW/7
WK9:	1 * 0.13 * $\dot{V}O_{2peak}$ * BDW/7	+	2 * 0.0844 * $\dot{V}O_{2peak}$ * BDW/7
WK10:	1 * 0.13 * $\dot{V}O_{2peak}$ * BDW/7	+	2 * 0.0911 * $\dot{V}O_{2peak}$ * BDW/7

Note: Exercise-related energy expenditure is greater during first 4 weeks (vs. later weeks) because interval training decreases volume and thus, decreases energy expenditure required.

Note: Rationale for coefficients used to estimate energy expenditure during exercise as follows:

- The week 1 coefficient of 0.05 is based on:
  - Subjects train at 50% $\dot{V}O_{2peak}$ (60% max heart rate is about 50% $\dot{V}O_{2peak}$) or the proportion 0.5.
  - The $\dot{V}O_{2peak}$ is in ml/kg/min and must be converted to l/kg/min, therefore we must divide by 1000.

- There are 5 kcal burned for each liter of oxygen used and the subjects train for 20 minutes during the first week.

Therefore, the equation is 0.5 * 5 * 20/1000 = 0.05 for week 1. The same methods are used for subsequent weeks as the intensity (proportion $\dot{V}O_{2peak}$) and duration increase.

SPIRIT-Outcomes 2022 Checklist (for combined completion of SPIRIT 2013 and SPIRIT-Outcomes 2022 items)[a]

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Administrative information				
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	-	
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	-	
	2b	All items from the World Health Organization Trial Registration Data Set	-	
Protocol version	3	Date and version identifier	-	
Funding	4	Sources and types of financial, material, and other support	-	
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	-	
	5b	Name and contact information for the trial sponsor	-	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	-	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	-	
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	-	
	6b	Explanation for choice of comparators	-	
Objectives	7	Specific objectives or hypotheses	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	-	
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	-	
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	-	
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (for specific guidance see TIDieR checklist and guide)	-	
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	-	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	-	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-	
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
	12.1		Provide a rationale for the selection of the domain for the trial's primary outcome	
	12.2		If the analysis metric for the primary outcome represents within-participant change, define and justify the minimal important change in individuals	
	12.3		If the outcome data collected are continuous but will be analyzed as categorical (method of aggregation), specify the cutoff values to be used	
	12.4		If outcome assessments will be performed at several time points after randomization, state the time points that will be used for analysis	
	12.5		If a composite outcome is used, define all individual components of the composite outcome	
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	-	
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	-	
	14.1		Define and justify the target difference between treatment groups (eg, the minimal important difference)	
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-	
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	-	
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	-	
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	-	
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	-	
Methods: Data collection, management, and analysis				
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	-	
	18a.1		Describe what is known about the responsiveness of the study instruments in a population similar to the study sample	
	18a.2		Describe who will assess the outcome (eg, nurse, parent)	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	-	
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	-	
	20a.1		Describe any planned methods to account for multiplicity in the analysis or interpretation of the primary and secondary outcomes (eg, coprimary outcomes, same outcome assessed at multiple time points, or subgroup analyses of an outcome)	
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-	
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-	
Methods: Monitoring				
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	-	
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-	
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	-	
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	-	
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	-	
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	-	
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	-	
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-	
	31b	Authorship eligibility guidelines and any intended use of professional writers	-	

Section	Item No.	SPIRIT 2013 Item	SPIRIT-Outcomes 2022 item	Location Reported[b]
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-	
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-	

[a]It is strongly recommended that this checklist be read in conjunction with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) Statement paper for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license and is reproduced with permission.
[b]Indicates page numbers and/or manuscript location: to be completed by authors.