Protocol Title: The Exercise And Colorectal Cancer Treatment (EXACT) Trial

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Objectives

This randomized trial will examine the biological effects of 12 weeks of moderate-intensity aerobic exercise versus wait-list control in 60 subjects who have completed standard medical therapy for stage I- III colorectal cancer. The primary objective is to determine if aerobic exercise can reduce systemic inflammation, quantified using plasma concentrations of high-sensitivity C-reactive protein and interleukin-6. The secondary objectives are to determine if exercise can reduce 1) insulin resistance quantified using an oral glucose tolerance test and 2) circulating tumor cells quantified using a microfluidic antibody-mediated capture platform. The exploratory objective is to determine if exercise can improve mitochondrial respiration rates and fatty acid oxidation from peripheral blood mononuclear cells, and tumor fraction.

Background

Colorectal Cancer Prognosis. Each year, 145,600 people are diagnosed with colorectal cancer in the United States.[1] Despite efficacious surgical and chemotherapeutic interventions, 25–30% of patients will experience recurrent and metastatic disease within three years of diagnosis, and 91% of those who recur within three years die by five years.[2] Additional therapies are critically needed to maximize the likelihood of cure for these patients. One such additional therapy may be the prescription of physical activity.[3]

Physical Activity and Colorectal Cancer. Physical activity or exercise is a modifiable lifestyle behavior that is associated with disease outcomes among colorectal cancer patients. A meta-analysis of six prospective cohort studies that included 7,523 colorectal cancer patients demonstrated that participation in physical activity after cancer diagnosis was associated with a 39% lower risk of colorectal cancer-specific mortality [Hazard Ratio (HR): 0.61, 95% CI: 0.40−0.92] and 42% lower risk of all-cause mortality [HR: 0.58, 95% CI: 0.48−0.70].[4] The relationship between physical activity and mortality is independent of various demographic, clinical, pathologic, treatment, and behavioral factors (e.g., smoking and obesity). Colorectal cancer patients who derive the largest survival benefits from physical activity are those who were sedentary before diagnosis and adopt a physically active lifestyle after diagnosis.[5] This observation substantiates the hypothesis that the prescription of physical activity after

colorectal cancer diagnosis may decrease disease-specific morbidity and mortality. However, the biological pathways through which physical activity may influence cancer recurrence and mortality are not yet completely understood.[6]

Inflammation and Colorectal Cancer. One biological pathway hypothesized to mediate the relationship between physical activity and cancer recurrence and mortality in colorectal cancer is systemic inflammation. Systemic inflammation, such as elevated concentrations of C-reactive protein (CRP),[7] interleukin-6 (IL-6),[8] and soluble tumor necrosis factor-alpha receptor 2 (sTNF-αR2),[9] is associated with a higher risk of cancer recurrence and mortality among colorectal cancer patients. Systemic inflammation induces signaling pathways, including PI3K-Akt-mTOR, JAK-STAT, and NF-κB, resulting in cell growth, migration and invasion, anti-apoptosis, and angiogenesis.[10-12] Elevated IL-6 and TNF-α induce the hepatic expression of CRP, producing a state of chronic low-grade systemic inflammation.[13] CRP is a robust overall measure of systemic inflammation given its strong correlation with IL-6 and sTNF-αR2.[14,15] The observations derived from cohort studies are supported by preclinical data that demonstrate experimentally manipulating inflammatory-related pathways, including IL-6[16,17] and TNF-α/sTNF-αR2,[18-22] regulate colorectal cancer growth and progression. Collectively, these data suggest that systemic inflammation is an important mechanism associated with a higher risk of cancer recurrence and mortality in colorectal cancer.

Insulin Resistance and Colorectal Cancer. Another biological pathway hypothesized to mediate the relationship between physical activity and cancer recurrence and mortality in colorectal cancer is insulin resistance. The hallmarks of insulin resistance are high insulin concentrations and an exaggerated insulin response to glucose.[23] Colorectal cancer patients have fasting insulin concentrations 58% higher than age- and sex-matched controls ($P<0.001$).[24] Hyperinsulinemia is often a symptom among people diagnosed with type 2 diabetes. Patients with colorectal cancer who also have type 2 diabetes have a worse prognosis than those without type 2 diabetes.[25,26] Markers of insulin secretion, such as C-peptide, predict mortality in colorectal cancer patients.[27,28] *In vitro* studies demonstrate that insulin increases cell resistance to chemotherapy agents used to treat colorectal cancer, including 5-fluorouracil[29] and oxaliplatin,[30,31] and preclinical models demonstrate that exposure to insulin promotes colorectal tumor multiplicity.[32] Collectively, these data suggest that insulin resistance is an important mechanism associated with a higher risk of cancer recurrence and mortality in colorectal cancer.

Circulating Tumor Cells in Colorectal Cancer. The development of metastases from colorectal cancer are hypothesized to result from tumor cells entering the circulation, migrating to distant organs, extravasating, multiplying, and eventually manifesting as clinically detectable lesions. This process marks the transition from localized, potentially curable to disseminated, usually incurable disease.[33] Autopsy studies suggest 66% of metastases in colorectal cancer are explained by vascular blood flow alone.[34] The early detection and characterization of circulating tumor cells (CTCs) are important to monitor and prevent the development of metastases.[35] CTCs predict disease recurrence and mortality among patients with stage I-III colorectal cancer.[36-39] For example, among 438 patients with stage I-III colorectal cancer, 31% had tumor cells in peripheral blood after completing treatment, and the presence of CTCs was associated with a 29-fold increase in the risk of cancer recurrence ($P<0.001$).[40] However, few therapeutic

interventions exist to reduce CTCs and improve clinical outcomes.[41] We hypothesize that cancer cells disseminate via the circulation during the earliest stages of recurrent metastatic growth, mirroring what occurs during the primary tumor setting. If this occurs and exercise interrupts this process, we are uniquely situated to detect this phenomenon by quantifying CTCs. Collectively, these data suggest that CTCs are a key constituent of cancer recurrence and mortality in colorectal cancer.

Circulating Cell-Free DNA (Tumor Fraction). Circulating cell-free DNA allows for detailed genetic characterization of residual tumor burden.[42] Cell-free DNA is released into the circulation through apoptosis and necrosis. Cell-free DNA are generally double-stranded fragments of 150 to 200 base pairs in length.[42] Cell-free DNA that is released from tumor cells is classified as circulating tumor DNA. Estimates of the tumor's contribution to cell-free DNA, defined as the tumor fraction, offer greater sensitivity compared to that of CTCs alone, and the combination of both CTCs and cell-free DNA provides complementary biological insight.[43] It is estimated that ≥70% of patients with stage I to III colorectal cancer have measurable cell-free DNA concentrations with cancer cell origin (i.e., a tumor fraction ≥0.1).[43,44] Tumor fraction is a pharmacodynamic biomarker that correlates with response to chemotherapy,[45] and is prognostic of disease recurrence and mortality in stage I to III colorectal cancer.[46] For example, in patients with stage II colorectal cancer, a higher tumor fraction after surgical resection is associated with an 11-fold to 18-fold higher risk of systemic recurrence.[47] The advantages of cell-free DNA include a high sensitivity to accurately quantify tumor burden in patients with curatively resected cancer (e.g., high signal-to-noise ratio), the capacity to characterize intra-tumor heterogeneity, and the ability to predict future treatment resistance.[44] However, the effect of exercise on circulating cell-free DNA in cancer patients has not been evaluated.

Preliminary Data. The pilot data to justify the current trial was a dose-response study that randomized 39 subjects with stage I-III colon cancer to 150 min/wk or 300 min/wk of aerobic exercise or a usual-care control group for 24 weeks.[48] We identified an interaction between cancer stage (a randomization stratification factor in the pilot study) and randomized group for changes in high-sensitivity [hs]-CRP ($P_{interaction}$=0.051) and IL-6 ($P_{interaction}$=0.002). Compared to baseline, 150 min/wk exercise reduced hs-CRP (−0.79±0.38 mg/L; P=0.038) and IL-6 (−0.57±0.29 pg/mL; P=0.047) in subjects with stage III but not in subjects with stage I/II disease. Compared to baseline, 300 min/wk of aerobic exercise did not reduce hs-CRP or IL-6. Additional analyses demonstrated that compared to baseline, 150 min/wk of aerobic exercise reduced fasting plasma insulin (−28.0±8.4 pmol/L; P=0.001) and glucose (−0.39±0.1 mmol/L; P=0.011),[49] and CTCs (−1.3±0.3 cells/mL; P<0.001).[50] However, due to the multiple randomized groups of this pilot trial, the sample size within each group was small (n=12−14), making between-group comparisons statistically underpowered. The results obtained from this pilot study inform the primary and secondary aims for the proposed trial, as detailed below.

Experimental Design & Methods

A. Number of Subjects

Accrual is 60 subjects (see power and sample size section below), recruited over 24 months, with a monthly accrual goal of approximately 2–3 subjects per month. In our prior trial, we successfully accrued 5.5 subjects per month.[1] Using estimates from our prior trial, we predict we will need to screen approximately 160 potential subjects to successfully randomize 60. We aim to recruit an equal ratio of males and females and ≥25% non-white minority subjects.

B. Inclusion and Exclusion Criteria

Many oncology clinical trials do not reach their minimum projected accrual goal.[51] To ensure trial completion, we implement prospectively specified stepped inclusion and exclusion criteria that enable the efficient recruitment of the most homogeneous study population that is feasible within the allowable time horizon without compromising subject safety or scientific validity.

We will begin with three months of strict eligibility criteria (Phase 1). At the end of three months, if six subjects are not accrued, eligibility criteria will be expanded for another three-month period (Phase 2). If, at the end of six months, we have still not accrued an average of two subjects per month, we will further expand eligibility criteria (Phase 3). We successfully implemented these prospectively specified stepped inclusion and exclusion criteria in the pilot study.[48]

<u>Phase 1 (most strict) inclusion criteria</u>:

1.1 Age ≥18 years
1.2 Histologically-confirmed stage III colorectal cancer
    a. Self-report during screening is acceptable if confirmed with pathology report, state tumor registry file, or physician documentation prior to randomization
1.3 Completed surgical resection within 1–24 months or completed chemotherapy (if applicable) within 1-24 months
1.4 Provide written approval by a physician or other qualified healthcare provider
1.5 No planned major surgery during the study period (including colostomy reversal; however, chemotherapy infusion port removal is permitted)
1.6 Readiness to exercise [as determined by a modified version of the Physical Activity Readiness Questionnaire (PAR-Q)]
    a. If there are any indications that home-based exercise would be unsafe based on the PAR-Q, the patient will not be enrolled until approval from the patient's provider (e.g., oncologist, cardiologist) confirms that it is safe to exercise
1.7 Allow the collection and storage of specimens and data for future use
1.8 Willing to be randomized

<u>Phase 2 (less restrictive) inclusion criteria will expand</u>:

2.1 Histologically-confirmed stage to include stage I, II, or III colorectal cancer

a. Self-report during screening is acceptable if confirmed with tumor registry, pathology report, or physician documentation prior to randomization

2.2 Time since completing surgical resection to 1–36 months or time since completing chemotherapy (if applicable) to 1-36 months

Phase 3 (least restrictive) inclusion criteria will expand:

3.1 Time since completing surgical resection ≥1 month or time since completing chemotherapy (if applicable) ≥1 month

3.2 Histologically confirmed stage I, II, or III colorectal cancer ≥1 month since completing surgical resection or ≥1 month since completing chemotherapy and/or radiotherapy

Exclusion criteria for all three accrual phases of recruitment include:

1.1 Evidence of metastatic colorectal cancer (biopsy-proven or radiologic evidence that results in the initiation of local or systemic therapy)

a. Elevated tumor markers (e.g., CEA ≥5 ng/mL), in the absence of an identified lesion(s) on imaging, that is monitored with surveillance only is not considered evidence of metastatic disease

b. Lesion(s) classified on imaging as "suspicious" that are monitored with surveillance only are not considered evidence of metastatic disease

1.2 Concurrently actively treated other cancers (except non-melanoma skin cancer or in situ cancers)

1.3 Currently enrolled in another clinical trial of weight loss, physical activity, or dietary intervention

1.4 Current body mass ≥181 kg

1.5 Unable to provide a baseline fasting blood sample

1.6 Unable or unwilling to give informed consent

1.7 Unable or unwilling to be randomized

1.8 Or any other condition that may impede the testing of the study hypothesis or make it unsafe to engage in the exercise program (as determined by the investigative team)

C. Recruitment Methods

Potentially eligible study subjects may be identified and contacted using the Louisiana Tumor Registry and the Gulf South Community Oncology Research Program.

The Louisiana Tumor Registry is part of the Surveillance, Epidemiology and End Results (SEER) Program, funded by the National Cancer Institute. The registry collects and reports high-quality and timely population-based cancer data across the state to support research activities in cancer prevention and control. The Louisiana Tumor Registry Director is Xiaochen Wu, MD, MPH, who is a Professor of Epidemiology at the School of Public Health in the Louisiana Health Sciences Center. Potentially eligible subjects will be identified in the Louisiana Tumor Registry (e.g., using tumor codes

affiliated with the diagnosis of colorectal cancer) and contacted using mail and telephone.

The Gulf South Minority Underserved Community Oncology Research Program is part of the National Cancer Institute-funded Community Oncology Research Program (NCORP). The Gulf South NCORP is a state-wide initiative that brings together an integrated clinical trials program in the state of Louisiana to promote and facilitate participation and enrollment of cancer patients into clinical trials. The Gulf South Minority Underserved NCORP Director is Augusto Ochoa, MD, who is the Director of the Stanley S. Scott Cancer Center at the Louisiana State University Health Sciences Center. The Gulf South Minority Underserved NCORP currently includes 25 oncology clinics throughout the state. Subjects will be recruited from these clinics using flyers placed in patient waiting areas, mailed postal letters, direct physician referrals, or other methods deemed appropriate.

Potential subjects will be directed to complete a web screening form on the PBRC landing page, call the PBRC Recruitment Core directly, or e-mail the Recruitment Core. Potential subjects will then undergo a phone screen to answer a series of yes or no questions to determine eligibility. Individuals meeting the criteria from the phone screen will be scheduled for a screening visit at PBRC.

Other approved recruitment materials may include a landing page, listserv, flyers, and social media developed specifically for the study. Our community outreach efforts will include but are not limited to, presentations and community events. We will also utilize media outlets, including newspapers, radio, and social media.

The Principal Investigator and study staff will also notify members of local cancer support groups, and additional cancer services organizations may be utilized.

D. Study Timelines

Subjects are expected to undergo screening, baseline, and randomization visits at Pennington Biomedical Research Center and participate in a 12-week, home-based exercise intervention or wait-list control. A final clinic visit after the completion of the 12-week intervention period will conclude study participation. The duration of an individual subject's participation will be approximately five months. The study will be conducted over a three-year period.

E. Study Procedures

Table 1. Schedule of Procedures

Procedure	Screening (SV1)	Baseline (BL)[a,b]	12 Week Intervention (W1-W12)	Month 3 (M3)[a,c]
Review Phone Screen	X			

Consent	X			
History & Physical	X			
ECG	X			
Vital Signs	X	X		X
Height	X			
Weight	X	X		X
Waist/Hip Circumference		X		X
Questionnaires		X[h]		X[h]
Concomitant Meds	X	X		X
Accelerometer	X[d]			X[e]
Lifestyle Consultation	X			
Adverse Events		X	X	X
Urine Pregnancy Test		X[f]		X[f]
DXA		X		X
IV OGTT		X		X
Lab Work		X		X
Fitness Assessment		X		X
Randomization		X[g]		
Exercise Instruction			X	
Weekly Telephone Calls			X	
Exercise Program/ Usual Care			X	

[a]Baseline and Month 3 visits can each be split into two days of measures if requested by the participant.
[b]BL is to be completed within 30 days of SV1.
[c]M3 is to be completed within 30 days W12.
[d]Accelerometer is provided to the participant at SV1 and is to be returned at the BL visit.
[e]Accelerometer is mailed to the participant at W10 and is to be returned at the M3 visit.
[f]Females of child-bearing potential only.
[g]Randomization can occur up to 7 days after BL.
[h]Questionnaires will be given to participant at BL and M3 to complete.

1. Visit Description

   Screening Visit (SV1): As part of the screening visit, each subject will review their answers provided during the phone screen and the informed consent with a member of the clinical trials unit staff. Once the consent is signed, the following will be completed:
   - Medical History and Physical;
   - Resting 12 lead ECG;
   - Height, weight, and vital signs;
   - Concomitant Meds;
   - Lifestyle consultation to assess subject readiness to initiate a physical activity program and assess barriers to participation;

- An accelerometer with instructions will be given to the participant and returned at the baseline visit.
- Clinic staff will send a fax to the subject's medical providers that describes the study, reviews the subject's answers to the PAR-Q, and requests that the medical provider approve participation in the study.

Baseline Visit (BL): At the baseline visit, the participant will report to the inpatient unit after fasting for approximately 10 hours (minimum of 8 hours). The following will be completed:

- Weight and circumferences of the hip and waist
- Vital signs
- Concomitant medications
- Collection of accelerometer
- Females of child-bearing potential will complete a urine pregnancy test
- DXA*
- Oral glucose tolerance test (OGTT)**
- Submaximal fitness assessment
- Administer and review questionnaires

*After completing the DXA, the participant will be provided a snack prior to the fitness assessment.

**OGTT will take blood samples at 0, 60, and 120 minutes.

Randomization: After the baseline visit, all collected data will be reviewed for completeness and accuracy. Once all baseline data is confirmed to be complete, accurate, and the participant is deemed eligible, he/she will be randomized into one of the two study groups. The interventional resources staff will call the subject and explain their randomly assigned group assignment and expectations. At that time, participants randomized into the intervention group will be shipped a heart rate monitor with instructions for use. A treadmill will also be shipped to the subject's home. Instructions on the exercise program will be provided in brief along with a plan for future contact. Upon confirmation of delivery and setup of the treadmill and receipt of the heart rate monitor, the interventional resources staff will re-contact the participant and review the intervention in greater detail.

Intervention. Participants will be randomized into 1 of 2 intervention groups: Exercise Intervention Group or Usual Care Control Group.

a. Exercise Intervention. The exercise intervention will consist of moderate-intensity (50−70% age-predicted maximum heart rate) treadmill walking. This exercise protocol balances participant safety with behavioral feasibility towards the goal of accumulating 150 min/wk of moderate-intensity aerobic exercise. Participants with residual lower-extremity neuropathy from chemotherapy may elect to receive an elliptical or cycle rather than a treadmill. After treadmill delivery (or other equipment) and set up, participants will speak on the phone with an exercise specialist to introduce

the exercise prescription and familiarize the participant with the use of the treadmill, the completion of exercise logs, the use of a heart rate monitor (described below), appropriate warm-up and cool-down, stretches, and proper footwear for aerobic exercise. In subsequent weeks, participants will be permitted to complete supervised exercise sessions at Pennington Biomedical's Fitness Center if they choose to do so. All other exercises will be completed at home with the study-provided treadmill (or other equipment of preferred exercise modality). All exercise sessions will begin with a five-minute warm-up of slow walking, 30-60 minutes of moderate-intensity walking, and a five-minute cool-down of slow walking. In our pilot study, the study-provided treadmill was used for 77% of exercise sessions; most of the remaining exercise included outdoor walking (19%). No serious adverse events occurred.

Monitoring Exercise Adherence. Each participant will be provided a Polar heart rate monitor (M430) for the duration of the study. The M430 heart rate monitor can record up to 12 weeks of exercise using a one-minute epoch with a failure rate of <3%. The exercise specialist will download and review the heart rate data to objectively monitor exercise adherence (frequency, duration, and intensity) approximately bi-weekly. The exercise specialist will also inquire about any adverse events or barriers to completing exercise via weekly phone calls. The compliance information gathered from the heart rate monitors will be reviewed with the participant for positive reinforcement and to develop and tailor the desired dose of exercise for the following week. Participants who demonstrate poor adherence to the study protocol will be reminded of the study objectives, discuss any barriers or obstacles to completing the exercise, and be provided with encouragement to promote exercise self-efficacy, participation, and compliance.

b. Usual Care Control Group. Participants randomized into the wait-list control group are asked to maintain their pre-study levels of physical activity and follow the recommendations provided by their physician. The study interventionist will contact control group participants each week to maintain ongoing communication about the study and inquire about any adverse events. After completing three-month measures, control group participants are provided with an in-home treadmill and individualized exercise program similar to that prescribed to the exercise group.

Month 3 Visit: The Month 3 Visit is a repeat of the Baseline Visit. At week 10 of the study, an accelerometer will be mailed to the participant with instructions for use. The participant will report to the inpatient unit after fasting for approximately 10 hours (minimum of 8 hours). The following will be completed:

- Weight and circumferences of the hip and waist
- Vital signs

- Concomitant medications
- Collection of accelerometer
- Females of child-bearing potential will complete a urine pregnancy test
- DXA*
- Oral glucose tolerance test (OGTT)**
- Submaximal fitness assessment
- Administer and review questionnaires

*After completing the DXA, the participant will be provided a snack prior to the fitness assessment.
**OGTT will take blood samples at 0, 60, and 120 minutes.

Upon completion of delivery and acceptance by the participant, the treadmill will be the participant's responsibility and will no longer be the responsibility of Pennington Biomedical Research Center or any of the researchers.

2. Procedure Descriptions

- Laboratory Collection. At baseline and three months, all participants will undergo a blood draw. Blood will be performed after fasting for approximately 10 hours (minimum of 8 hours), abstinence from alcohol consumption, and any structured exercise for 24 hours. 70 mL of blood will be collected, centrifuged, and aliquoted into 1.8 mL cryovials at 0.5 mL/vial. Whole blood, plasma, serum, and buffy coat will be collected. Blood samples will be stored at −80°C. Females of child-bearing potential will complete a urine pregnancy test.

Plasma Inflammatory Marker Concentrations.

- The concentration of high-sensitivity CRP will be determined using an immunoturbidimetric assay (Roche Diagnostics, Indianapolis, IN). This high-sensitivity assay has a limit of detection of 0.03 mg/L. The day-to-day variability of the assay at concentrations of 0.91, 1.60, and 18.40 mg/L are 3.8, 3.3 and 1.9%, respectively.
- IL-6 is measured by an ultra-sensitive quantitative sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN). The assay has a sensitivity of 0.094 pg/mL, and the day-to-day variability of the assay at concentrations of 0.49, 2.78, and 5.65 pg/mL are 9.6, 7.2, and 6.5%, respectively.
- sTNFα-R2 is measured by a quantitative sandwich enzyme immunoassay assay (R&D Systems). The assay has a sensitivity of 0.6 pg/mL. The day-to-day variability of the assay at concentrations of 89.9, 197, and 444 pg/mL are 5.1, 3.5, and 3.6%, respectively. Masked quality-control samples are interspersed among cases, and all lab personnel are blinded to patient outcomes.

Insulin Resistance. An assessment of insulin resistance will be performed using a 75-gram oral glucose tolerance test. Plasma glucose and insulin concentrations will be determined at pre-specified intervals to calculate the incremental area under the curve during the oral glucose tolerance test.

Circulating Tumor Cells (CTCs). CTCs from participant venous blood will be isolated using a geometrically enhanced differential immunocapture (GEDI) platform. GEDI is a microfluidic platform that utilizes antibody-coated obstacles to capture rare cells within the blood. This allows for anticoagulated whole blood to be applied to the "chip" the size of a microscope slide in the laboratory using standard syringe pumps. To capture CTCs, obstacles will be coated with an antibody specific to epithelial cell-adhesion molecule (EpCAM), an epithelial cell-specific marker. After washes, captured cells will be stained with the nuclear marker DAPI and fluorescently labeled antibodies to the leukocyte marker CD45 and the epithelial cell marker Pdx-1. Using fluorescence microscopy, Pdx1+/DAPI+/CD45- EpCAM captured cells with intact cellular morphology will be counted as CTCs by a blinded technician. Cells will be stored at -80°C for additional analyses in subsequent grant submissions.

Circulating Cell-Free DNA (Tumor Fraction). The Broad Institute of MIT and Harvard has developed a comprehensive, versatile, and scalable platform to measure circulating cell-free DNA.[52] Venous blood samples will be drawn into 10 mL cell-free DNA blood collection tubes (Streck, Inc., La Vista, NE). Blood will be processed into component plasma, buffy coat, and erythrocytes within four hours of collection through standard density gradient centrifugation. Plasma samples will be subjected to additional high-speed centrifugation and frozen at –80°C until processing.

Mitochondrial Function and Fatty Acid Oxidation. The Buffy coat will be isolated within 24 hours of sample collection and stored at room temperature. The Buffy coat will be diluted with an equal amount of PBS, layered over a density gradient medium, and centrifuged according to the manufacturer's guide. Mononuclear cells will be washed with PBS and centrifuged, and any remaining red blood cells will be lysed and separated via centrifugation, followed by a final PBS wash and centrifugation. The mononuclear cell pellet will be prepared for oxidative phosphorylation by suspending it in a mitochondria respiration medium (MiR05), and the number of live mononuclear cells will be quantified. The integrated mitochondrial function will be assessed using a high-resolution respirometer, Oxygraph-2 K using DatLab 4 software (Oroboros Instruments, Innsbruck, Austria) to record the oxygen consumption rate for the transport of substrates into the mitochondria, the generation of reducing equivalents by specific dehydrogenases, entry into ETC (electron transport chain), and coupling to ATP synthesis.[53] Using two established protocols, the respiration rates of complex I, II, III, and IV as well as fatty acid oxidation will be quantified.[54]

Fitness Assessment. An assessment of sub-maximal fitness will be performed at baseline and three months with a treadmill using the modified Bruce Protocol.[9] The

speed and elevation will slowly increase until 80% of the age-predicted maximal heart rate (or RPE of 18 for patients on beta-blocker therapy), adhering to guidelines by the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription.

Body Composition. Body mass, height, weight, waist, and hip circumferences will be assessed using a digital scale, scale-mounted stadiometer, and Gulick tape measure, respectively. DXA (Hologic) will be used to quantify changes in bone mineral density and body composition.

Accelerometry. Participants will be asked to wear an Actigraph GT3X+ triaxial accelerometer for one week before and after the study to objectively assess activity levels.

Clinical Characteristics. Clinical characteristics, including tumor pathology, surgical history, and chemotherapy treatment plan, will be obtained from the clinical and cancer registry data files prior to randomization.

Patient Outcomes. Participants will complete questionnaires relating to demographics, alcohol and smoking habits, current medicine, and supplement usage. Participants will complete a patient-reported version of the Common Terminology Criteria for Adverse Events.

Data and Specimen Banking

The participant will be asked to allow blood specimens and data to be stored and used for research at a later time. Participants who refuse to have this information kept for future research will be excluded from the study. The specimens and data will be stored indefinitely. Future research may take place at Pennington Biomedical and may involve Pennington Biomedical researchers in this study. The future research may not take place at Pennington Biomedical Research Center and may not be reviewed by Pennington Biomedical Research Center's Institutional Review Board.

For privacy and confidentiality, all specimens and scans will be labeled with a unique series of letters and numbers. Pennington Biomedical will store the information with this unique identifier. The research done with data from these specimens and scans may help to develop new products in the future or may be used to establish a diagnostic test that could be patented or licensed. The participant will not receive any financial compensation for any patents, inventions, or licenses developed from this research.

The de-identified data will be stored indefinitely on the network shares at PBRC. Hard copy records for each subject will be kept in the participant's chart located in the Medical Records Department. These data will be used in publications but not shared outside of the PI's.

Long-term storage of specimens will be at -80° C. The samples will be stored in a designated freezer housed at Pennington Biomedical Research Center. The Principal Investigator, unless otherwise noted, will be the person who has access to the samples. Each sample will be labeled with the subject's ID and date.

Power analysis

After completion of baseline measurements, participants will be stratified by sex (male *vs* female) and then randomized in a 1:1 ratio to either the exercise or control group using a computer-generated algorithm. The co-primary outcomes for this study are hs-CRP and IL-6. For the CRP outcome, assuming a baseline concentration of 1.87 mg/L with a standard deviation of 1.32 mg/L, correlation of 0.8 between time points, a reduction of −0.71 mg/L in the exercise group over 12 weeks, and a type-I error rate of 5%, 46 participants total will provide 80% statistical power to detect between-group differences. For the IL-6 outcome, assuming a baseline concentration of 1.7 pg/mL with a standard deviation of 0.7 pg/mL, a correlation of 0.58 between time points, a reduction of −0.57 pg/mL in the exercise group over 12 weeks, and a type-I error rate of 5%, 48 participants will provide 80% statistical power to detect between-group differences. sTNF-αR2 is a secondary inflammatory measure. In the pilot study, we observed a 3% dropout rate (defined as participants not providing endpoint data), an 8% crossover rate (defined as control group participants who disclose initiating a structured, progressive exercise program during the trial), and 8% recurrence rate (defined as the clinical discovery of locoregional or distant metastatic disease).[70] We will inflate these estimates to accommodate a higher-than-anticipated dropout, experience a recurrence, and/or crossover. Enrolling 60 study participants provides a robust estimate of the largest necessary sample size needed for this trial. This sample size provides adequate statistical power to detect moderate between-group effect size differences (e.g., Cohen's *d* of ≥0.3) for measures of insulin resistance (Aim 2) and CTCs (Aim 3).

Statistical Analysis

The primary hypothesis in this trial is that exercise will significantly lower plasma inflammation concentrations over 12 weeks compared to control. All inferential analyses will be conducted on an intention-to-treat basis. Plasma inflammatory concentrations will be log-transformed in the inferential analysis to improve normality. Changes in outcomes will be evaluated from baseline to follow-up in the two groups using a repeated-measures mixed-effects regression model that accounts for the correlation between repeated measures and is robust to missing data. The baseline value of the dependent variable and sex (randomization stratification factor) will be included as a covariate in the regression models.[55] Group-by-time interaction terms will be included as fixed effects in the regression model. Model fit will be assessed using standard methods. A closed testing procedure for the two study outcomes (hs-CRP and IL-6) will be used, which is hierarchically ordered to test CRP and then IL-6 to maintain the overall experiment-wise error rate at 5%.[56] Other inflammatory outcomes and measures of insulin resistance (Aim 2) and CTCs (Aim 3) will be analyzed using the same statistical techniques and reported at the nominal type I error rate of 5%.

Data and Specimen Management

Study participants will be assigned unique subject identification (ID) numbers. Study subject ID numbers will be used on all data collection instruments, including questionnaires, data collection forms, biological specimen tubes, and computer records. A master list linking the participants' names and ID numbers will be kept in a password-protected computer file with access restricted to the PI and co-PIs. Biological samples that are moved off-site for analysis will not contain any personally identifiable information and will be labeled with only the unique subject ID numbers. Staff at these sites will not have access to the master list at any time.

Data collection forms will be kept under lock and key or password-protected if computerized and under the control of the PI, co-PIs, and medical investigator. Only personnel assigned to the research study by the PI will have access to the data. Hard-copy data records will be stored for a minimum of three years after study completion.

The PBRC has a fully integrated, campus-wide, automated data management system, REDCap. REDCap, a web-based application used to build and manage surveys and databases, will be used for questionnaire collection in this study. REDCap (Research Electronic Data Capture) is a secure, web-based application that is flexible enough to be used for a variety of types of research. REDCap provides an intuitive user interface that streamlines project development and improves data entry through real-time validation rules (with automated data type and range checks). REDCap also provides easy data manipulation (with audit trails for reporting, monitoring, and querying patient records) and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

All data are entered into a central database using existing methodology that has been fully validated and undergoes continuous quality assurance by the PBRC Research Computing Core. All data are backed up daily, and the Research Computing Core at the PBRC oversees all data management. The research team has extensive experience using the procedures and methods required to conduct this study. Standard operating procedures in place throughout the units at Pennington Biomedical will be utilized for repeatable, valid data collection and quality.

In accordance with the standards of practice followed by the Clinical Chemistry core, blood samples will be stored frozen at PBRC until analysis can be completed. Specific blood samples will be shipped to Boston Children's Hospital and Cornell University for further analysis of plasma inflammatory markers and circulating tumor cells, respectively. Packaging and shipping of biological samples will be overseen by the PI, and they will be shipped by the laboratory and completed in accordance with International Air Transport Association regulations to ensure that viable biological samples reach their intended destination.

Provisions to Monitor the Data to Ensure the Safety of Subjects

The study has a minimal level of risk to study participants and does not warrant the establishment of an independent Data and Safety Monitoring Board. This plan describes the safety monitoring procedures for the proposed study, including a description of how often and

to whom serious and unexpected adverse events will be reported. The plan will help ensure the safety of all participants. The PI will communicate via electronic submission to the IRB all unanticipated problems as defined by the IRB and all serious adverse events (SAEs) to the medical investigator within 72 hours.

The investigators will monitor the study's conduct. Study staff will report adverse events or other problems directly to the PI as they occur. The PI will schedule monthly meetings with study staff to review data on adverse events and recruitment or adherence to regimen problems. Any significant health problems coming to our attention during the study will be referred to the participant's usual source of medical care with his/her permission. We will cooperate fully with his/her physician by providing relevant medical records. The following criteria, if detected during any part of the study regimen, will lead to referral to the participant's usual source of medical care:

- Clinical symptoms or signs of CVD include chest pain suggestive of angina pectoris, unusual dyspnea on exertion, severe ankle edema, and symptoms suggestive of transient ischemic attacks or intermittent claudication.
- Musculoskeletal injuries or problems causing severe pain during exercise or interference with daily activities.

A. Adverse Events

Adverse events that occur will be classified as a Serious Adverse Event (SAE) or an Adverse Event (AE). Serious adverse events are defined to include:

- Death
- A life-threatening event
- Severe illness, including worsening of a pre-existing condition, injury, or accidents
- An inpatient hospitalization, surgical procedure, or treatment to prevent an SAE
- A permanent disability or incapacity
- A clinically significant abnormal laboratory or diagnostic test result
- Any other event that, in the opinion of the principal investigator or study physician, might have resulted in a serious adverse event if medical intervention had not been initiated

Serious adverse events will be reported to the study PI, Project Manager, and MI throughout the trial. Serious adverse events will be collected from randomization until the final visit. SAE data will be analyzed quarterly, but serious or life-threatening adverse events require immediate reporting and follow-up in the event that an adverse event occurs on campus and results in a serious or life-threatening situation, the investigator or other project staff present will begin emergency measures, as appropriate, and call 911.

- For minor physical injury, the individual will be encouraged to see a health care practitioner of his or her choice.
- If the study participant experiences psychological or emotional distress, the project staff will cease research activities and attempt to calm and reassure the participant. The participant will be directed to an appropriate healthcare practitioner for further assessment and treatment as needed.
- The investigator and/or project staff will record detailed narrative notes describing the adverse event they witnessed or that was reported by the participant. The medical investigator will complete the Notification form for a Serious Adverse Event.

Serious adverse event reporting will follow the requirements of the IRB of the Pennington Biomedical Research Center. Serious adverse events that are unanticipated problems will be reported within 48 hours.

For this trial's purposes, an **adverse event** is defined as any health-related unfavorable or unintended medical occurrence that happens after randomization. Examples of Adverse Events include but are not limited to the following:

- An event that requires a visit to a physician because it alters the participant's ability to do physical activity
- An event that occurs as a result of a study procedure which is not listed in the risks section of the consent

Adverse events will be reported to the study PI and the MI throughout the trial as necessary. Adverse event data will be collected from randomization until the final visit. Adverse events classified as serious will be reported from the date of consent through the final closeout visit. Adverse event data will be analyzed quarterly, but serious or life-threatening adverse events require immediate reporting and follow-up. It is anticipated that most adverse events will be mild, and the participant will be able to resume intervention activities within a week of reporting the event.

B. Stopping Rules

There is minimal risk for participating in this trial. The most likely scenario that would indicate a cessation of the study would be a failure to recruit participants or implement the intervention as planned. Nevertheless, in addition to monitoring recruitment and compliance with the intervention, the staff will monitor the participant rates of injury. The medical investigator, in conjunction with the study investigators, will alert the IRB and funder if a larger than reasonably expected injury rate occurs in the treatment groups.

Other issues that are related to the stopping rules include:

- New information – It is unlikely that new information will become available during this study, which would result in the discontinuation of the trial.
- Limits of assumption – It is possible that the value of data analysis will be limited by differences between the intervention groups because of study dropouts or missing data.
- Limit of rules – We acknowledge that circumstances other than what is listed may justify stopping the study.

Withdrawal of Subjects

There is no risk associated with withdrawal from the study. Participants may voluntarily withdraw or be withdrawn from the study if either the PI or MI feels that their continued participation would compromise the participant's safety or the results of the study.

Risks to Subjects

The following is a list of reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related to the procedures in this study:

OGTT: There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line. Trained personnel minimize this risk. The drink may cause nausea, vomiting, abdominal bloating, or a headache.

DXA: The amount of radiation used for this procedure is very small. The radiation dose for this scan is equivalent to the radiation you are naturally exposed to in the environment in less than one day. Scans will not be performed on any subject who is pregnant. A pregnancy test will be performed within 72 hours before the scan on females of child-bearing potential.

Electrocardiogram (EKG or ECG): There are minimal risks associated with this test. There is a small possibility there may be some redness or irritation while cleaning the skin prior to applying the electrodes or if you happen to be allergic to the adhesive on the electrodes.

Blood Draws: There is the possibility of infection and/or pain and bruising at the vein on your arm where the needle is inserted. Aseptic (sterile) technique and trained personnel minimize these risks.

Submaximal Fitness Assessment: All exercise testing core operating procedures are in accordance with the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, as well as the American Heart Association. There is minimal risk of injury or a cardiovascular event during exercise testing. We believe the risk of an event during exercise testing is minimized with a pretest review of the medical history, physical examination, use of highly trained staff, and well-defined emergency procedures. Participants may experience temporary discomfort during blood pressure recordings due to the pressure of the blood pressure cuff on the arm. All tests are conducted in the presence of Exercise Testing Core personnel with extensive experience in conducting maximal exercise testing. All laboratory staff

are trained in BLS (basic life support – CPR) and/or ACLS (advanced cardiac life support). In the event of a life-threatening emergency, the subject would be treated with BLS/ACLS by trained individuals and subsequently be transported to the nearest acute care medical-surgical facility via Emergency Medical Services, which is a parish-wide paramedic response unit.

Accelerometry & other activity monitoring devices: There is no known risk associated with measuring activity with accelerometers and other activity monitoring devices. Accelerometers fit comfortably on your waist or arm and can easily be adjusted should they become uncomfortable. In rare cases, the device may irritate the skin. If this should happen, the device can easily be repositioned to be more comfortable.

Blood Pressure Testing: Participants may experience temporary discomfort during blood pressure recordings due to the pressure of the cuff on their arm.

Height measurement: There is no risk to participants who record their height.

Body weight: There is no risk to participants who record their body weight. Measurements will be taken in a secluded area to protect the participant's privacy.

Self-report Questionnaires: There are no anticipated risks from completing self-report questionnaires. If signs of minor stress or fatigue are apparent, participants will be given time to take a break from completing the questionnaires. It is estimated that the questionnaires will take approximately 30 minutes to complete. The questions contained in some of the questionnaires may make people feel uncomfortable. Responses to the questions will be coded to protect confidentiality, and participants may choose not to answer questions.

Exercise interventions: The proposed exercise interventions are unlikely to cause major harm. We have conducted numerous exercise training studies and have never had a serious adverse event. There is the possibility of adverse events ranging from minor musculoskeletal problems to, in very rare cases, cardiovascular events. Exercise bouts will include aerobic exercise in the form of treadmill walking at moderate intensity. The exercise prescription will be at the discretion of the trainer to ensure compliance with the intervention and minimize injury. Occasionally, study participants experience minor orthopedic problems, but most self-correct with rest and standard first aid. These orthopedic injuries will be minimized by gradually progressing participants to their abilities as necessary. Exercise staff are trained in first aid and basic CPR. Each staff member is trained in either advanced or basic life support, and an automated external defibrillator and a fully stocked crash cart are kept on site. Although some study participants will be at moderately elevated risk for CVD, they will receive a thorough health screen, including a physical examination and an EKG. According to the available data on adverse events resulting from exercise, risk should be low. Fatal events during exercise are extremely rare.

Potential Benefits to Subjects

There are no direct benefits to the participants; however, the knowledge to be gained is critical for the advancement of science.

Vulnerable Populations

No vulnerable populations will be included in the study.

Multi-Site Research

This is a single-site research study.

Sharing of Results with Subjects

Subjects will be provided reports of medical information obtained from the body composition and exercise stress test following the completion of the study if requested.

Resources Available

The outpatient and inpatient research units are well-equipped and staffed to carry out the requirements of this study, and appropriate standards of practice are in place to ensure appropriate research procedures are followed.

Setting

This study will be conducted at Pennington Biomedical Research Center.

Compensation

Enrolled participants will be compensated up to $300 for the completion of the study. Participants will receive $100 after the completion of baseline and randomization if eligible. Participants will receive up to $100 for completion of all intervention-related activities (e.g., returning telephone calls, tracking, and adhering to the exercise protocol). The remaining $100 will be reimbursed upon the completion of the study (defined as providing complete endpoint data measures). Reimbursements may be prorated according to the degree of adherence and willingness of participants to accommodate staff requests. Reimbursement will be requested from the LSU payroll department, and it usually takes about 3-4 weeks for it to arrive at Pennington Biomedical Research Center.

As part of the study, all exercise intervention participants will receive a treadmill for home use. After completing the three-month measures, control group participants will be provided with an in-home treadmill and individualized exercise program similar to that prescribed to the exercise group. As additional compensation for participation in the study, participants will keep the treadmill provided.

Confidentiality

All participants are assured of their confidentiality, both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research participants. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, the director of the clinical facilities, and the PIs. Participants' medical records are filed according to ID numbers. All forms on the chart display the ID number. Electronic data storage is similarly restricted, with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e., those containing names OR other identifying information.

Data, including body weight, body composition, exercise testing, etc., will be collected from participants. Data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

Provisions to Protect the Privacy Interests of Subjects

All attempts will be made to maintain a subject's privacy. Safeguards such as password-protected computers and networks have been put in place to limit access to subject data. Subjects will be given ample time to read over the consent, ask questions, and agree to participate in the research study. Subjects may decline to answer questions they are not comfortable with. Each procedure will be explained to the subject before it is performed. All discussions will be held with study subjects in examination or private rooms to protect their privacy.

Compensation for Research-Related Injury

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from the Pennington Biomedical Research Center. In the event of injury or medical illness resulting from the research procedures in which a subject participates, they will be referred to a treatment facility. Medical treatment may be provided at the subject's expense or at the expense of their health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.), which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should the study subject require ongoing medical treatments, they must be provided by community physicians and hospitals.

Economic Burden to Subjects

All study-related tests and procedures will be at no cost to the subject. The subject will incur transportation costs to and from PBRC.

Consent Process

The clinic staff will obtain informed consent during the first screening visit as per established policies and procedures. All clinic staff have completed the required GCP training and have trained on the consenting policies and procedures. An ample opportunity will be given for the subject to review the consent form and ask any questions prior to signing it. If subjects wish, they can take the form home and return it at a different time of the visit. A copy of the consent will be provided to the subject.

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