CLINICAL PROTOCOL

PROTOCOL NUMBER: 1B-12-1

TITLE: Combined Exercise Program for Early Breast Cancer Survivors

STUDY PHASE: N/A

STUDY ARMS: Control vs Exercise Intervention

IND OR IDE #: N/A

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Amendment #5
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1.0 BACKGROUND AND HYPOTHESES

Physical Activity and Breast Cancer Survivorship.
Recent estimates show more than 2.4 million breast cancer survivors with a 5-year survival rate of 88.6% 1. In California, 21,740 women will be diagnosed with breast cancer this year and 4,030 women will die as a result of their breast cancers 2. These survivors are at an increased risk of cancer recurrence, comorbidities such as diabetes, osteoporosis and cardiovascular disease, and premature death 3,4. They have special needs as a consequence of the adverse effects associated with common treatments, such as surgery, chemotherapy, and radiation therapy. One important consequence of these adverse effects is a profound decline in physical activity 5. This is of particular concern as physical activity is thought to lower the risk of cancer recurrence and mortality 6. Studies of survivors 6-12 months following treatment describe individuals with decreased cardiorespiratory function, muscle strength, bone mineral density, and physical well-being 7,8. In addition, these survivors experience fatigue, depression, anxiety, and weight gain 9,10. These effects of decreased physical activity appear to have more profound effects on cardiovascular and psychosocial health in those undergoing radiation and some forms of chemotherapy 4,12. While the relationship between decreased physical activity and factors of overall well-being has been established in moderate and long-term survivors 13-16, less information is available with regards to early (0-3 month post-treatment) survivors.

In 2006, the American Cancer Society released guidelines for cancer patients and survivors promoting physical activity to improve cancer outcomes 17, emphasizing the beneficial effects of exercise for the health of breast cancer survivors. Not only has physical activity been associated with decreased breast cancer risk 18-20, it has also been shown to be beneficial for breast cancer survivors. For example, aerobic exercise has been found to improve cardiorespiratory function in survivors 6 months-5 years post-treatment 13,14,21,22, which is particularly important for survivors who have had cardiac damage from chemotherapy. Resistance exercise has been found to increase muscle strength 8,15,23,24, which subsequently reduces injuries and improves balance. Additionally, combined (aerobic and resistance) exercise programs result in improvements in cardiorespiratory function and muscle strength in breast cancer survivors 25-27. Combined exercise involves both resistance and aerobic exercises in a single session and therefore, is effective in improving cardiovascular, musculoskeletal, and psychological factors.

Metabolic Syndrome and Breast Cancer Survivorship.
Breast cancer survivors are at an increased risk of cancer recurrence, comorbidities such as diabetes, osteoporosis and cardiovascular disease, and premature death 3,4. Current evidence suggests that breast cancer treatments such as chemotherapy lead to excessive weight gain, fatigue, physical inactivity, and negative alterations in components of metabolic syndrome (MetS) 5,11,28. Metabolic syndrome (MetS), which is associated with increased risk of cardiovascular diseases and type 2 diabetes (6), is a cluster of risk factors including visceral adiposity, insulin resistance, hyperglycemia, hyperinsulinemia, low serum high-density lipoprotein cholesterol, and hypertension 29. MetS is highly prevalent and present in at least 25% of American and European adults 30. Therefore, despite high breast cancer survival rates, many breast cancer survivors are at risk of and may experience mortality from diabetes and cardiovascular disease, which can be modified by lifestyle interventions 31,32. Obese postmenopausal breast cancer survivors receiving adjuvant hormone therapy present with MetS and elevated levels of C-reactive protein, placing them at a higher risk for cardiovascular and metabolic diseases 28,33,34. In addition, premenopausal breast cancer patients experience detrimental effects such as increased body mass index (BMI) and central obesity from adjuvant chemotherapy potentially contributing to MetS 35. Chemotherapy in premenopausal breast cancer patients frequently induces premature menopause, which is associated with increases in body fat, cholesterol, and triglycerides 35,36. These changes may contribute to earlier development of cardiovascular disease or type 2 diabetes among women already at risk or to increased risk among those not already predisposed to these diseases 29.

Treatment and management of MetS mainly consists of symptomatic drug treatments of the syndrome’s individual components 37. Candidate drugs that reduce hyperglycemia may have additional metabolic benefits.
Examples are metformin, more recently known for its potential actions in treating breast cancer, PPAR\(\gamma\) agonists, GLP-1 agonists, and DPP-4 inhibitors. However, since lifestyle factors such as physical activity, dietary intake, and smoking habits affect the risk of developing MetS, it is important and possibly preferable to target lifestyle factors to prevent the onset of metabolic-related diseases in breast cancer survivors. The few studies that have examined the effects of exercise training on particular components of MetS in postmenopausal breast cancer survivors have shown reduced insulin levels and waist circumference, but no change in insulin resistance, fasting glucose, or body weight. Some of these results are promising and emphasize the need for additional studies in this area. Studies of premenopausal survivors will be particularly important. Given that chemotherapy induces many of the components of MetS, an effort to offset potential treatment side effects can greatly benefit breast cancer patients. The proposed study aims to determine whether a 16-week exercise intervention induces changes in components of MetS (body weight, waist circumference, blood pressure, serum levels of glucose, insulin, lipids, C-reactive protein and HbA1c) among premenopausal and postmenopausal breast cancer survivors if initiated within 3 months of completion of chemotherapy.

Elevated CV risk among patients with obesity, hypertension, and MetS has been associated with increased arterial stiffness, endothelial dysfunction, and negative vascular alterations measured by carotid intima media thickening (cIMT) and impaired flow-mediated dilation (FMD) using B-mode ultrasonography. Despite the greater risk of CV events in breast cancer patients, no studies have been conducted to examine the vascular abnormalities in breast cancer patients with obesity, hypertension, and MetS. Only clinical blood markers such as glucose, cholesterol, and triglyceride have been used to predict CV health in a recent breast cancer study. Further, there are limited data from clinical trials evaluating the effect of exercise on vascular health in breast cancer patients with MetS. Thus, there is a critical need to identify CV alterations to reduce the MetS-associated CV risk in breast cancer patients.

The proposed research project and a future program in exercise interventions has translational potential if successful and can be applied to the growing number of breast cancer survivorship clinics including the newly form breast cancer survivorship clinic at the City of Hope. Such an effort would lead to a rehabilitation program for breast cancer patients that would mimic programs currently in place for cardiac rehabilitation. If successful, this may present an opportunity to drastically improve the health of breast cancer survivors by presenting them with lifestyle intervention options allowing them to take control of one aspect of their survivorship experience.

**Exercise Needs and Preferences of Breast Cancer Survivors.**

Physical activity after breast cancer diagnosis may reduce mortality and thus, is an important aspect of survivorship. Although a limited scope of research is available to describe specific exercise needs of these survivors, a recent report addresses this topic in rural breast cancer survivors. This indicated that survivors prefer to receive face-to-face exercise counseling from an exercise specialist in the community anytime before or after treatment. Accommodating this need is achieved in our proposal as we will provide face-to-face exercise instruction soon after treatment is completed in an effort to enhance sustainability of the exercise program by demonstrating that expensive equipment and extraneous costs do not have to be associated with an exercise. Significant value is placed on exercise by survivors who may or may not participate in exercise as means of health promotion and support, which reiterates the importance of exercise and the need for a feasible exercise program to encourage participation in exercise. Barriers to exercise reported by survivors include lack of motivation and appropriate facilities, treatment side effects and fatigue.

Timing of an intervention is a key aspect in order to capture a time point following treatment where survivors change their lifestyle behaviors, often referred to as the “teachable moment.” Currently, the optimal time to participate in an exercise intervention following breast cancer diagnosis is unknown. Our proposal will investigate whether an early exercise intervention is feasible and applicable to this community. Due to the harsh side effects associated with treatment and AIs, it is important to encourage a healthy lifestyle earlier in the recovery process in order to prevent additional negative consequences from occurring and as a potential means to improve AI compliance which can be vital for survivorship. Capitalizing on the “teachable moment” soon after treatments by enrolling in a community-based group exercise program will foster a behavioral change that...
will be sustainable for longer periods of time due to the positive and encouraging environment elicited through group exercise. Breast cancer survivors are more likely than other cancer survivors to harbor psychological stress of a diagnosis for longer periods of time and may have higher levels of interest in interventions\textsuperscript{50}, stressing the importance of an early intervention. To deliver an effective intervention, the needs and preferences of survivors must be considered.

**Safety and Efficacy of Exercise Interventions in Breast Cancer Survivors.**

Based on the large number of exercise intervention studies that have reported few to no adverse effects, participation in exercise is a safe way to improve cardiovascular, musculoskeletal, and psychological health, QOL, and functional capacity. To ensure a safe environment, exercise is often performed under supervision following clearance to participate from a physician\textsuperscript{13,14,21,40,51}. Exercise interventions in breast cancer survivors reported few occurrences of musculoskeletal injuries including shin splints, muscle strains, tendonitis and back pain, with no adverse events\textsuperscript{16,52,53}. To address safety concerns, our study team includes a physical therapist with specialized oncology training as well as an exercise physiologist trained in exercise for cancer populations. In addition to proving to be a safe intervention method, physical activity is highly effective for improving survivorship. A recent evaluation of the efficacy of physical activity interventions reported the percentage of studies that found significant improvements which included: aerobic fitness (72%), muscle strength (66%), body composition (44%), QOL (66%), physical function (66%), fatigue (54%), and psychosocial function (63%)\textsuperscript{54}. Based on this report, physical activity interventions appear to be effective in improving various outcome measures, yet an important aspect in the efficacy of interventions is targeting early breast cancer survivors. Since most interventions involve longer-term survivors, it is important to investigate the safety and feasibility of a group exercise program for early breast cancer survivors included in this study.

**Adipose Tissue Macrophages and Breast Cancer.**

Globally, obesity and breast cancer represent two common diseases, both with increasing prevalence\textsuperscript{55}, and each independently having a profound impact on public health. A well-established relation between obesity and breast cancer exists, with most large epidemiological studies demonstrating an increased risk of developing postmenopausal breast cancer in overweight or obese women\textsuperscript{56-59}. Obesity is associated with poorer overall and breast cancer-specific survival\textsuperscript{60} suggesting that adipose tissue may play a role in breast cancer prognosis and recurrence. Obese patients have approximately double the death rate from breast cancer compared to non-obese patients\textsuperscript{61} thus making body weight an important target for therapeutic interventions. Maintaining a healthy body weight, is a serious concern for breast cancer survivors; there are currently over 2.5 million breast cancer survivors with a 5-year survival rate of 88.6%\textsuperscript{1}, an estimated 64% of whom are overweight (BMI 25-30 kg/m\textsuperscript{2}) or obese (BMI >30 kg/m\textsuperscript{2})\textsuperscript{62}.

In obese individuals, adipose tissue macrophages (ATMs) with the M1 phenotype secrete pro-inflammatory cytokines including TNF-a and IL-6\textsuperscript{63}. These "classically" activated ATMs (M1) are stimulated with T helper 1-type cytokines and promote insulin resistance while "alternatively activated" ATMs (M2) are stimulated with T helper 2-type cytokines and protect against insulin resistance by attenuating inflammation\textsuperscript{64}. During obesity, expanded adipose tissue could contribute to metabolic dysfunctions and cancer recurrence by deregulated secretion of pro-inflammatory cytokines, which activate the NF-kB pathway to contribute to tumor growth and metastasis via TNF-a and activation of STAT3 (activator of transcription) pathways to increase cell proliferation via IL-6\textsuperscript{65}. Increasing obesity induces a phenotypic switch in macrophage activation from M2 to M1 macrophages leading to increased insulin resistance\textsuperscript{66}. Examining ATM characterization for phenotypic switching from M1 to M2 predominance after exercise will provide insight into adipose tissue dysregulation and may serve as targets of future clinical trials, including different strategies to decrease inflammation in breast cancer survivors.

Exercise has a positive impact on adiposity by reducing body mass, particularly fat mass and distribution, and changing adipocyte size and number in obese adults\textsuperscript{67}. For example, a 15-week combined diet and exercise intervention reduced adipose tissue inflammation determined by a decreased gene expression of macrophage activation specific markers (CD14, CD68), IL-6, IL-8, and TNF-a in subcutaneous abdominal adipose tissue of obese (BMI > 30 kg/m\textsuperscript{2}) men and women\textsuperscript{68}. Other than serum inflammatory markers such as C-reactive protein...
subcutaneous abdominal adipose tissue inflammation has not been studied in breast cancer survivors, including those participating in an exercise intervention. We will utilize validated histochemical and molecular biology techniques utilized in Dr. Mittelman's laboratory to examine ATM phenotypes and ATM-secreted cytokine gene expression in adipose tissue of obese breast cancer survivors in an effort to collect pilot data for larger randomized trials in cancer survivorship.

**Hypotheses:**

Based on supportive existing literature, we propose the following hypotheses:

1.1 A 16-week exercise intervention will improve components of metabolic syndrome in breast cancer survivors soon after completion of cancer-related treatments (i.e. surgery, chemotherapy, radiation) when compared to the Control group.

1.2 A 16-week exercise intervention will improve physical fitness in breast cancer survivors soon after completion of cancer-related treatments (i.e. surgery, chemotherapy, radiation) when compared to the Control group.

1.3 Early breast cancer survivors on the Exercise arm will attend 90% of all exercise sessions included in the exercise program.

1.4 A 16-week exercise intervention will result in the phenotypic switching of ATMs from M1 to M2 in obese breast cancer survivors.

1.5 A 16-week exercise intervention will result in reductions in ATM cytokine expression by decreasing pro-inflammatory cytokines, IL-6, IL-8, TNF-α, and MCP-1.

1.6 A 16-week exercise intervention will exhibit a decrease in cIMT and improved FMD when compared to the Control group.

2.0 **OBJECTIVES AND PURPOSE**

The purpose of this proposal is to investigate components of metabolic syndrome (MetS) and adipose tissue inflammation in breast cancer survivors following an exercise intervention. The objective is to improve survivorship utilizing an exercise intervention to diminish elevated components of MetS and adipose tissue inflammation among breast cancer survivors. It is our hypothesis that a 16-week exercise intervention performed soon after the completion of treatment will improve 1) components of metabolic syndrome (body composition, waist circumference, blood pressure, and serum levels of insulin, glucose, lipids, C-reactive protein, and HbA1c) 2) physical fitness, 3) ATM phenotype, and 4) ATM cytokine expression among breast cancer survivors. This is a randomized, controlled 16-week exercise intervention in premenopausal and postmenopausal breast cancer survivors. The control group will refrain from increasing physical activity levels from exceeding 120 minutes per week, and their physical activity patterns will be recorded and monitored. The exercise group will participate in three weekly 60-minute combined (resistance and aerobic) exercise sessions. All participants will undergo the following outcome measure tests at baseline and upon study completion: blood draw for analysis of lipids, insulin, glucose, C-reactive protein, and HbA1c, body composition, blood pressure, resting energy expenditure, cardiopulmonary fitness walk test, muscle strength, physical activity and dietary assessments. All participants will be given the option to undergo deep subcutaneous abdominal adipose tissue biopsies (performed by Dr. Sattler) at baseline and study completion in order to examine ATM phenotype and ATM cytokine expression.

**Specific Aims/Objectives:**

2.1 To determine whether a 16-week exercise intervention will improve components of MetS in breast cancer survivors soon after completion of cancer-related treatments (i.e. surgery, chemotherapy, radiation) by measuring changes in body composition, waist circumference, blood pressure, and serum levels of insulin, glucose, lipids, C-reactive protein, and HbA1c.

2.2 To determine whether a 16-week exercise intervention will improve physical fitness in breast cancer survivors soon after completion of cancer-related treatments (i.e. surgery, chemotherapy, radiation) by measuring cardiorespiratory fitness and muscle strength.

2.3 To assess the feasibility of a supervised exercise intervention in early breast cancer survivors.

2.4 To determine whether a 16-week exercise intervention will result in a reduction in adipose tissue inflammation in obese breast cancer survivors soon after completion of cancer-related treatments.
treatments (i.e. surgery, chemotherapy, radiation) by measuring ATM phenotype and ATM cytokine expression.

2.5 To determine whether a 16-week exercise intervention will alter endothelial function, arterial stiffness, and vascular atherosclerosis.

It is important to investigate the degree to which the exercise participants retain a physically active lifestyle following the intervention: therefore an additional aim is proposed:

2.6 To determine whether breast cancer survivors can maintain positive benefits of an exercise intervention following a 12-week follow-up period by measuring changes in body composition, waist circumference, blood pressure, and serum levels of insulin, glucose, lipids, C-reactive protein, and HbA1c, cardiorespiratory fitness and muscle strength.

3.0 STUDY DESIGN

3.1 Subject Identification and Recruitment
To identify potential subjects, recruitment efforts will include posting flyers at USC Health Science Campus, and Huntington Memorial Hospital, and notifying local oncologists at those institutions and in the San Gabriel Valley who may invite their patients to participate based on our eligibility criteria. Specifically, we will inform breast cancer patients prior to and during chemotherapy and/or radiation therapy such that the patients are aware of the study and will remain eligible. In addition, we would like to post an announcement on the Clinical Trials webpage for USC. The approval from the subject’s primary physician will be obtained prior to recruitment of the subject. Specifically at USC, Dr. Dieli-Conwright will recruit participants from the Lee Breast Clinic at Norris Comprehensive Cancer Center and at LAC+USC County Medical Center, under the guidance of Dr. Tripathy. Dr. Dieli-Conwright will attend regular Breast Tumor Board meetings to inform all members of the Women’s Cancers Program of study recruitment and patient eligibility. Dr. Dieli-Conwright will contact potential participants from the registry by telephone once receiving physician permission to contact the patient.

3.2 Research Plan Outline
One hundred women will be recruited from USC breast cancer clinics and local oncology practices once they receive clearance from their physicians to participate in a regular exercise program. Once deemed eligible, participants will visit the Integrative Center for Oncology Research in Exercise (ICORE) at USC within one week prior to the start of the intervention where they will undergo a series of tests (see Outcome Measures below) and will be randomly assigned to either the Exercise (receive intervention) or Control (no intervention) groups. Participants will return to the ICORE within one week following the completion of the study intervention for post-testing. All participants will be provided the option to undergo dSAT biopsies at pre-intervention visit 1 and post-testing, which will take place at the CTU during additional visits from the initial outcome measures (as described below in the Methods). For the participants not willing to undergo the biopsy- pre-intervention visits 1 and 2 will occur on the same day as originally executed. The adipose tissue aim is a pilot to collect preliminary data for a larger intervention therefore we propose to recruit 30 within the 100 participants to undergo the biopsy procedure.

The Exercise group will participate in three ~60 minute weekly supervised exercise sessions led by the PI, an American College of Sports Medicine (ACSM) Cancer Exercise Trainer (CET), held at the ICORE, which houses aerobic and resistance exercise equipment regularly utilized to conduct clinical exercise interventions. The Control Group will be asked not to change their exercise level, which cannot exceed 60 minutes of total exercise per week, as specified in the inclusion criteria, during study participation and will be offered 4 months of supervised exercise sessions at the ICORE led by the CET specialist upon completing the 16 week study. The
Control Group, similarly to the Exercise Group, will complete weekly physical activity logs to ensure adherence and prevent occurrence of ‘drop ins.’

3.3 Methods
Several measures will be performed at pre-intervention visits 1 and 2 and following the 16-week study period for participants in the exercise and control groups and will be conducted by Dr. Dieli-Conwright. Additionally, following completion of the 16-week exercise intervention, participants in the exercise group will be asked to return to the ICORE 12 weeks later to repeat the outcome measure testing. During the 12-week period, participants will be encouraged to exercise on their own without study team supervision. This can be independent or group activity and participants may seek assistance from fitness professionals as they see fit. They will be asked to maintain weekly physical activity logs and wear an accelerometer on a daily basis. The purpose of this follow-up period is to determine whether the participants remain active and can maintain the benefits gained from the exercise intervention.

Measures of MetS: Pre-intervention Visit (Week 0) and post-testing (Week 17; 29 for Exercise Group)

a. Blood draw. Fasting blood will be drawn from the antecubital vein (~30 cc) by a trained phlebotomist at the ICORE. Participants will be asked to fast for 12 hours prior to the blood draw. Refreshments will be provided following the blood draw and prior to proceeding with further testing (Location: ICORE).

b. Serum Assays. The Clinical Pathology Laboratory at the USC will perform appropriate standard assays to measure serum lipids (cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), insulin, glucose, C-reactive protein (CRP), and glycosylated hemoglobin (HbA1c). Fasting glucose, insulin, and HbA1c serve as metabolic biomarkers while CRP as an inflammatory biomarker.28 (Location: Keck Hospital).

c. Body composition. BMI in kg/m^2 will be calculated from height and weight measurements using a medical scale (Detecto® 437, Webb City, MO). Body composition (total lean mass and percent body fat) will be measured from a whole body scan using Dual-Energy X-ray Absorptiometry (DEXA, Lunar DPX-IQ). A tape measure will be used to obtain waist circumference defined as the distance around the waist using the navel as the reference point. (Location: ICORE)

d. Blood pressure. The participant will be asked to sit quietly for 5 minutes while resting her arm on a table so the brachial artery is level with the heart. An automated sphygmomanometer will be used to measure blood pressure. (Location: ICORE)

The primary outcome was MetS z-score calculated from modified z-scores of the following variables:70-73 WC; systolic blood pressure (SBP); diastolic blood pressure (DBP); HDL-cholesterol (HDL-C); triglycerides (TG); and glucose using individual participant data, US National Cholesterol Education Program Adult Treatment panel III (ATP III) criteria, and SDs (denominator of each factor in the formulas) using baseline data of the entire study cohort (n=100): [(50-HDL)/5.5]+[(TG-15)/25.5]+[(Glucose-100)/15.9] +[(WC-88)/8.8]+[(SBP-130)/11.4]+[(DBP-85)/10.8]70,73

Metabolic Syndrome Criteria. Based on the ATP III definition,74 MetS constituted ≥3 of the following risk factors: WC≥88 cm, SBP≥130 mmHg or ≥85 mmHg DBP or taking blood pressure medication, fasting levels of HDL-C<50 mg/dL, TG≥150 mg/dL, and glucose≥100 mg/dL or taking diabetes medication. Each participant’s ATP III score was calculated by summing ATP III criteria met at each timepoint.

e. Carotid Intima Media Thickness (cIMT). The participant will be asked to lie in the supine position on a plinth and cIMT of the right common carotid artery will be measured using B-mode ultrasound for 1 minute by a trained ultrasound technician. The ultrasound scan of cIMT provides lumen diameter, intima-media thickness, and presence and extent of plaques. To
measure cIMT, carotid bifurcation should be detected as a reference. Digitally stored images will be manually analyzed from 10mm proximal to the bifurcation point for the start of the measurement using ultrasonic calipers. The calculation of cross-sectional area of right carotid artery is \[
\left\{\frac{\text{maximal lumen diameter}}{2}\right\}^2 \times \pi - \left\{\frac{\text{maximal lumen diameter}}{2} - \text{cIMT}\right\}^2 \times \pi
\]

However, mild discomfort may result if the transducer presses too hard. Skin irritation can be caused induced if allergic to ultrasound lubricant gel. This procedure takes approximately: 2 minutes (Location: ICORE)

f. **Flow-Mediated Dilation (FMD).** The participant will be asked to lie in the supine position on a plinth to assess endothelial function non-invasively measured using B-mode ultrasound which captures the brachial artery on the arm. A rapid inflation and deflation pneumatic cuff will be positioned on the imaged arm immediately distal to the antecubital fossa to provide a stimulus to forearm ischemia. Using the mechanisms of shear stress measured by FMD, lumen diameter will be obtained to determine endothelial dysfunction. Either right or left unaffected arm will be occluded, depending on the side of surgery (i.e. mastectomy or lumpectomy). Each recording lasts a total of 11 minutes including 1 minute of baseline measurement, 5 minutes of occlusion with 250mmHg of blood pressure, and 5 minutes post-occlusion measurement. All participants will be asked to keep their arm as still as possible after deflation of blood pressure cuff. Since ultrasound is a non-invasive, no serious side effect has been found. However, the discomforts involved with FMD may include redness of the skin, numbness, pain, tingling of the finger and discomfort while the cuff is inflated, as well as skin irritation caused by ultrasound lubricant gel. This procedure takes approximately 11 minutes. (Location: ICORE)

g. **Arterial Stiffness.** Arterial stiffness (central/pheripheral stiffness) will be non-invasively measured from pulse wave velocity, which is the speed of the pressure wave traveling through the arteries using ultrasound. Increased arterial stiffness leads to a faster return of the backward pressure (reflected) wave to the heart, increasing central pressure. The magnitude of the reflected wave (augmentation index) and the central aortic pressures will be quantified using the ultrasound device. ECG-gated carotid and femoral artery waveforms will be sequentially recorded. The time delay (t, seconds) between the onset of carotid and femoral waveforms (foot-to-foot) will be determined from the onset of the waveform and the R wave recorded on the ECG during the cardiac cycles. Pulse wave velocity will be calculated as the ratio of distance to time (PWV = L/t (m/s)). The length will be measured as the difference in distances from the carotid sampling site to the suprasternal notch and from the suprasternal notch to the radial artery. Measurements of central, as opposed to peripheral, pressures is more physiologically relevant to CV function. This procedure takes approximately 5 minutes. (Location: ICORE)

**Additional Lifestyle Measures: Pre-intervention Visit (Week 0) and post-testing (Week 17; 29 for Exercise Group)**

a. **Resting energy expenditure (REE).** Participants will be asked to lie still in the supine position on a plinth (padded 7 foot medical table). CardioCoachCO2® portable metabolic device will be used to measure REE over the course of 8 minutes. (Location: ICORE)

b. **Cardiopulmonary fitness.** Participants will be instructed to walk comfortably (so they are able to talk while walking) on a treadmill for 4 minutes and heart rate will be measured at the end of the test to estimate maximal oxygen uptake. (Location: ICORE)

c. **Muscle strength.** Maximal voluntary strength will be evaluated by the 10-repetition maximum (10-RM) method for the following exercises: chest press, latissimus pulldown, knee extension, knee flexion, and leg press (Tuff Stuff, Pomona, CA) which will be used to calculate 1-RM (maximum strength) values for the exercise intervention. (Location: ICORE)

d. **Physical activity assessments.** Physical activity history will be assessed at baseline using an interviewer-administered physical activity questionnaire. Throughout the duration of the study period, weekly 7-day physical activity logs will be completed by all participants and returned to the PI by mail for the Control group.
   a. Approximate time to complete baseline questionnaire: 20 minutes
b. Approximate time to complete weekly physical activity logs: 20 minutes/week

e. **Dietary assessments.** Dietary history will be measured at baseline using the NIH-DHQ 83. Three-day dietary records will be completed at baseline and at the completion of the study period to assess recent dietary patterns.
   a. Approximate time to complete baseline questionnaire: 70 minutes
   b. Approximate time to complete weekly records: 35 minutes/week

f. **Quality of life assessments.** Quality of life (QOL) will be assessed using the SF-36 and FACT-B. The SF-36 is a multi-purpose, short-form health survey with 36 items used to assess physical and mental health 84. The FACT-B (Functional Assessment of Cancer Therapy-Breast) is a breast cancer-specific questionnaire comprised of 44 items to specifically assess quality of life in breast cancer patients 85.
   a. Approximate time to complete SF-36: 15 minutes
   b. Approximate time to complete FACT-B: 15 minutes

g. **Psychosocial assessments.** Depressive symptoms will be assessed using the 20-item CES-D (Center for Epidemiologic Studies Depression) scale, which was designed to measure one’s current level of depressive symptomatology 86. Self-efficacy will be assessed using the SHAPE (Shoulder and Arm Post-breast cancer Efficacy) which is a newly designed 25-item questionnaire related to arm and shoulder care after breast cancer treatment 87.
   a. Approximate time to complete CES-D: 10 minutes
   b. Approximate time to complete SHAPE: 15 minutes

h. **Musculoskeletal disorders assessment.** Upper limb musculoskeletal disorders will be assessed using the DASH (Disabilities of the Arm, Shoulder, and Hand) and Penn Shoulder Scale (PSS). DASH is a 30-item questionnaire designed to measure physical function and symptoms of possible musculoskeletal disorders of the upper limb 88. The PSS is a 100-point shoulder-specific self-report questionnaire consisting of 3 subscales of pain, satisfaction, and function 89.
   a. Approximate time to complete DASH: 15 minutes
   b. Approximate time to complete PSS: 15 minutes

**Shoulder Health and Lymphedema Measures: Pre-intervention Visit (Week 0) and post-testing (Week 17; 29 for Exercise Group; Location: ICORE)**

We will assess shoulder health and lymphedema to determine the following: 1) can a combined exercise program improve shoulder strength, and range of motion, and 2) does an exercise program have deleterious effects with respect to lymphedema?

a. **Shoulder strength.** Maximal muscle force produced by the primary agonist during scapular plane elevation (SE) and external rotation (ER of the upper extremity will be measured using a hand held dynamometer (Hoggan Health Industries). The participants will sit in an armless chair with her back flush to the back of the chair, feet flat on the floor approximately shoulder width apart, and sitting with neutral posture. To obtain a neutral posture, the participant will be asked sit with her back straight, shoulders rolled back, and ears aligned over shoulders and hips.

The participant will be told, “This (indicating HHD) is used to measure muscle force and the angle at which you are holding your arm. When I tell you to, I want you to hold your arm like this (testing position will be demonstrated). I will place the apparatus that is connected to the dynamometer on your arm like this (demonstrate accordingly). When I ask you to, push against the HHD until I say stop, which will be about 4 seconds. Keep trying to push as hard as you can for the 4 seconds. We will be doing two trials in each position (to get an average) with 30 seconds in between each trial and position. If you need more than 30 seconds please tell me.” The HHD apparatus will be positioned appropriately. Once the apparatus is aligned correctly on the participant’s arm and secured to the doorframe the participant will be instructed to begin pushing. For each test (SE and ER), the dynamometer on the apparatus will be aligned so that the resistance is in exactly the opposite direction of the direction of motion being resisted. Two trials will be performed for each muscle test, taken sequentially. The subject will be allowed to
rest for 30 seconds between the two trials. The average of two trials will be used for data analysis.

**Scapular Elevation (SE)-** With the participant in position as described above, she will hold the affected arm at 90 degrees of humeral elevation and in the scapular plane (40 degrees of horizontal adduction), and with the thumb up which is essentially the “full-can” position. The scapular plane will be verified by aligning the arm with a piece of tape on the floor that has been premeasured. The 90 degrees of scapular elevation position will be verified by centering the HHD upside down and longitudinally over the radial styloid process and adjusting humeral elevation until 0 is in the right screen. Once the participant is positioned correctly, the HHD apparatus will be clamped onto the doorframe with the HHD centered on the radial styloid process for force measurement. Humeral elevation will be resisted via the HHD while the examiner’s other hand is placed on the subject’s shoulder for stabilization. Examiner (PI: Dieli-Conwright) will instruct the subject to begin to apply pressure to the HHD. Pressure will initiate the timer on the left screen of the HHD. The participant will be instructed to stop pushing after approximately 4 seconds.

For our purposes a bad/unacceptable trial is one that includes one or more of the following:
- Trial lasts less than 4 or exceeds 6 seconds
- Improperly placed HHD
- Prematurely initiated HHD timer
- HHD settings are not as described above
- Participant states they did not give best effort during the trial
- Participant does not maintain proper positioning
- Administrator fails to properly position participant
- Participant does not follow instructions
- Randomization of trial sequence is compromised

A good/acceptable trial is defined as anything not included above.

**External Rotation (ER)-** With the participant in position as described above, they will hold the affected arm at their side at 0 degrees of elevation, elbow bent to 90 degrees, and humerus held in neutral. The participant will be told, “during this test, I want you to keep your elbow at your side and push with your forearm so that it works like a door on a hinge.” This motion (ER) will be demonstrated. The administrator will use one hand to stabilize the participant’s arm on the lateral side of the elbow. The examiner will instruct the participant to begin to apply pressure to the HHD in the direction of ER. Pressure will initiate the timer on the left screen of the HHD. The participant will be instructed to stop pushing after approximately 4 seconds.

For our purposes a bad/unacceptable trial is one that includes one or more of the following:
- Trial lasts less than 4 or exceeds 6 seconds
- Improperly placed HHD
- Prematurely initiated HHD timer
- HHD settings are not as described above
- Participant states they did not give best effort during the trial
- Participant does not maintain proper positioning
- Administrator fails to properly position participant
- Participant does not follow instructions
- Randomization of trial sequence is compromised
- The HHD apparatus is loosened or compromised for any reason

A good/acceptable trial is defined as anything not included above

a. Approximate time to complete shoulder strength measures: 8 minutes
b. **Shoulder Active Range of Motion (AROM).** AROM will be measured for shoulder forward flexion and shoulder ER at 90 degrees abduction.

**ER at 90 degrees of abduction.** The participant will be placed in supine, trunk stabilized on the table, knees bent so that the feet are placed flat on the table. The participant’s arm will be placed in 90 degrees of shoulder abduction (towel may be placed under the arm to align the humerus), the elbow flexed to 90 degrees, and the wrist in neutral position. The position of 90 degrees of abduction will be confirmed with a universal goniometer with the axis at the glenohumeral joint, one arm parallel to the sternum, and the other aligned with the shaft of the humerus. The participant’s arm will be passively moved into external rotation within their pain free range of motion 3-5 times to precondition the tissue. Then, the participant will be asked to ACTIVELY perform ER. Active ER will be measured with the Acumar™ digital inclinometer aligned between the olecranon and ulnar styloid process to measure external rotation of the glenohumeral joint. Two trials will be performed with approximately 10 seconds between each trial.

**Forward Flexion.** The subject will be placed in a standardized seated position, with their back directly against the back of a straight back chair. Their feet will be placed on the floor, with their hips and knees at approximately 90 degrees. Participants will be asked to actively elevate their arm as far as they can into flexion. The Acumar™ digital inclinometer will be aligned along the mid-humeral shaft with the elbow in extension and shoulder in neutral rotation by asking the subject to point their thumb towards the ceiling. Two trials will be performed with approximately 10 seconds between each trial.

  a. Approximate time to complete shoulder AROM: 3 minutes

c. **Lymphedema Assessment.** Geometric arm volume calculations will be performed to assess lymphedema. We will calculate arm volume using circumferential measurements taken at anatomic landmarks as described by Taylor et al.\(^9\) This method was determined to be a reliable and valid method of limb volume measurement. The participant will be seated at a table with their shoulder positioned at approximately 90° of scapular plane flexion with their straight arm resting on a table. Circumferential measurements will be taken with a thin plastic tape measure with a spring that standardizes how tightly the tape is pulled. The anatomic landmarks include the wrist to middle forearm, middle forearm to elbow, elbow to middle upper arm, and middle upper arm to a 65% mark. The 65% mark of the upper arm is 65% of the distance from the elbow (olecranon) to the shoulder tip (acromion). This mark was used as the upper boundary level because the women in the comparison study by Taylor et al were able to submerge their arm for the water displacement to this point. Calculations for limb geometric volume will be calculated using the frustum (truncated cone) volume as described by Taylor et al.\(^9\). The formula is:

\[
V = \frac{h(C_1^2 + C_1C_2 + C_2^2)}{12\pi}
\]

where \(V\) is the volume of the segment, \(C_1\) and \(C_2\) are the circumferences at the ends of the segment, and \(h\) is the length of the segment. The arm volumes calculated by anatomic landmarks had the smallest error associated with a single measure (SEM). The interrater reliability for this method has been found to be excellent (>0.98)\(^2\). A percentage difference between lymphedema limb and the uninvolved limb will be calculated to determine the amount of lymphedema. Lymphedema has been defined in recent literature as a greater than 10% difference in volume calculation for the arm compared to the uninvolved upper extremity\(^9\).

  a. Approximate time to complete lymphedema assessment: 5 minutes
Adipose Tissue Inflammation Measures: Pre-intervention Visit (Week 0) and post-testing (Week 17); Location: CTU

a. Fat Biopsy Collection: Following a 12-hour fast, participants will undergo an deep abdominal subcutaneous fat biopsy by punch biopsy under local anesthesia using 6 mm biopsy attachment with a 1-cm scalpel incision (Bergstrom biopsy needle). One portion will be immediately fixed in formalin and another portion will be frozen in liquid nitrogen and stored at -80°C until RNA isolation. The primary risk of an abdominal subcutaneous fat biopsy is pain and discomfort at the biopsy site. The methodology used for this biopsy will minimize the risk for more serious adverse events, such as bleeding and infection. There is a small risk of dimpling at the site at which the fat tissue is extracted. There is a moderate risk of visible hypertrophic scarring with a likely change in coloration. There is a 10% risk of significant scarring such as keloid formation. In an effort to determine whether we are sampling from superficial or subcutaneous adipose tissue, some of the biopsies may be performed under ultrasonic guidance.

b. Adipose Tissue mRNA Profiles: mRNA levels will be determined from SAT biopsy samples. To determine mRNA expression, microarray gene chip technology will be utilized in collaboration with the Mitelman Laboratory (Directed by Steven Mittelman, MD, PhD). Total RNA will be isolated with RNeasy RNA isolation kits (Qiagen, Inc, Chatsworth, CA). RNA will be quantitated using the Ribogreen RNA Quantitation assay (Invitrogen, Carlsbad, CA), and quality of RNA assessed on a Bioanalyzer 2100 (Agilent, Santa Clara, CA). Global gene expression will be tested using an Illumina Inc. (San Diego, CA) HumanRef-8 Expression Bead Chip (contains ~30 different 50-nucleotide transcripts for each of the 23,000 known genes) and an Illumina BeadLab 1000 gene expression system at the Southern California Gene Expression Consortium. The hybridization reactions will be processed and scanned according to the standard Illumina protocols. Raw data will be converted to expression values using GenomeStudio. After any necessary pre-processing, variance-stabilizing transformation will be implemented.

c. Immunoblotting: Immunoblotting will be performed on genes related to inflammation, and confirmed to change by real-time PCR as well as other proteins.

d. Adipocyte Histology / Flow Cytometry: Adipose tissue will be dissected and fixed in 5% neutral buffered formalin overnight, and then rinsed and stored in phosphate buffer solution. The fixed tissue will be shipped to Dr. Greenberg’s lab at Tufts University, packed in boxes containing cold packs. No data will accompany the tissue samples. After embedding in paraffin, adipose tissue sections will be stained with hematoxylin and eosin. Images will be taken on an Olympus BX-51 microscope with an Olympus DP70 color digital camera. Adobe Photoshop will be used to enhance the contrast of the images before analysis. MetaMorph software (version 6.1; Molecular Devices, Downingtown, PA) will be used to calculate cellular area and the number of adipocytes per field with integrated morphometry analysis. Analysis of macrophages in adipose tissue will be performed on 5 µm-thick paraffin sections of adipose tissue fixed in Bouin's solution. Sections will be microwave-pretreated in 10 mM citric acid, pH 6.0, for 10 min to enhance antigenicity. Endogenous peroxidase activity will be blocked by 3% H2O2 in methanol for 10 minutes, followed by a 30 minute block with normal horse serum; macrophages will be identified by KP1 mouse monoclonal antibodies (Abcam). Sections will be developed with coumarin-based tyramide signal amplification fluorescence system (PerkinElmer Life Sciences). Five random sections will be photographed and the number of KP1+ cell/field and KP1+ cell/nuclei will be determined. Part of the sample will placed in PBS and will be examined at a histology core for macrophage infiltration. Flow cytometry will be used to separate lymphocytes, Tregs, T effectors, IFNγ-secreting helper T-cells, macrophage subtypes, and adipocytes in the fat biopsies.

Exercise Intervention (Weeks 1-16):
This project is a prospective, randomized controlled trial. After enrollment, participants will be randomly assigned to an Exercise group or Control group. The Exercise group will receive a 16-
week exercise intervention. The Control group will be asked not to initiate a structured exercise program during the 16-week study period (>60 minutes of total exercise per week). To reduce attrition, the control group will be offered the same intervention at the end of their 16-week trial period. The exercise intervention consists of a 16-week supervised aerobic and strength training protocol. All exercise sessions will be supervised by Dr. Dieli-Conwright, an American College of Sports Medicine (ACSM) certified Cancer Exercise Trainer, and will take place at USC-ICORE.

Participants in the Exercise group will be required to visit ICORE three times per week for 16 weeks to complete the exercise protocol. The exercise protocol will consist of 50 minute aerobic exercise sessions three times per week for 16 weeks. Twice a week the aerobic exercise sessions will be followed by resistance exercise sessions (see Table 1 below). Participants will be required to wear a Polar heart monitor during each aerobic exercise session. Heart rate (HR) will be monitored throughout the aerobic exercise sessions to maintain an exercise HR at 65-80% of maximum HR. This protocol is designed to include moderate to vigorous forms of activity, which are more beneficial in decreasing risk of mortality from breast cancer, cardiovascular disease and diabetes. \(^{31,32,91}\) Aerobic exercise types will include treadmill walking or stationary cycling. Each resistance exercise session will include the following exercises: leg press, leg flexion, leg extension, chest press, seated row, biceps curls, and triceps pulldown. Initial resistance will be set at 80% of the 1-repetition maximum (1-RM) for lower body exercises and 60% 1-RM for upper body exercises which will be determined during baseline testing. When the participant is able to complete 3 sets of 10 repetitions at the set weight in 2 consecutive sessions then the weight will be increased by 10%. Each daily session will begin with a 5 minute warm up on the treadmill or cycle and 10 minutes of static stretching. Collectively, the participants will visit ICORE a total of 3 times per week which includes 2 visits where aerobic and resistance exercise will be performed sequentially. The described exercise protocol has been deemed safe, and effective in reducing insulin measures in breast cancer survivors. \(^{41}\) Additionally, the exercise sessions are based on current recommendations for breast cancer survivors following treatment which includes 20-60 minutes of aerobic exercise performed at least 3 times per week and 6-10 resistance exercises performed at least 1-3 days per week. \(^{92-94}\) The exercise classes are based on current recommendations for breast cancer survivors following treatment which includes 20-60 minutes of aerobic exercise performed at least three times per week and 6-10 resistance exercises performed at least 1-3 days per week. \(^{92,93}\) Over the course of the 16-week intervention, exercises will be altered and resistance will be increased based on the guidance of Dr. Dieli-Conwright as participants gain muscular strength and endurance. To prevent any financial burden on the participants, we will provide financial compensation for travel to and parking at USC. Compliance with the exercise sessions will be measured by the percentage of scheduled sessions attended and self-reported aerobic exercise.

### Table 1. Weekly Exercise Intervention Protocol.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Exercise: 50 minutes at 65-80% HR maximum; and Resistance Exercise: 3 sets of 10 repetitions, 45 second rest between sets</td>
<td>Rest</td>
<td>Aerobic Exercise: 50 minutes at 65-80% HR maximum; and Resistance Exercise: 3 sets of 10 repetitions, 45 second rest between sets</td>
<td>Rest</td>
<td>Aerobic Exercise: 50 minutes at 65-80% HR maximum; No Resistance Exercise</td>
<td>Rest</td>
<td>Rest</td>
</tr>
</tbody>
</table>
Follow-up Period (Weeks 17-29): After the trial has ended, participants in the Exercise group will be given an appointment to return to the ICORE 12 weeks later to repeat the outcome measure testing. During the 12-week period, participants will be encouraged to exercise on their own without study team supervision. This can be independent or group activity and participants may seek assistance from fitness professionals as they see fit. They will be asked to maintain weekly physical activity logs and wear a pedometer on a daily basis. The purpose of this follow-up period is to determine whether the participants remain active and can maintain the benefits gained from the exercise intervention. Participants will be given up to week 33 to return to the ICORE to complete the follow-up testing. This additional 4-week window of time will provide participants flexibility in scheduling to return to the lab in light of potential scheduling conflicts.

4.0 DRUG/DEVICE INFORMATION

N/A

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria:

- Women ≥ 18 years of age newly diagnosed (0-III) with a first primary invasive breast cancer
- Have undergone a lumpectomy or mastectomy
- Have completed surgery, neoadjuvant/adjuvant cytotoxic chemotherapy and/or radiation therapy and able to initiate Exercise program (if randomized to that arm) within 24 weeks of therapy (i.e. surgery, chemotherapy, radiation) completion
- BMI > 25 kg/m² or body fat > 30% or waist circumference >88 cm (determined by Dr. Dieli-Conwright at baseline visit)
- Currently participate in less than 60 minutes of physical activity per week
- May use adjuvant trastuzumab or endocrine therapy if use will be continued for duration of study period
- Nonsmokers (i.e., not smoking during previous 12 months)
- Willing to travel to the exercise facility and USC
- Able to provide physician clearance to participate in exercise program
- Women of all racial and ethnic backgrounds will be included in the study enrollment process

5.2 Exclusion Criteria:

- History of chronic disease including uncontrolled diabetes, uncontrolled hypertension or uncontrolled thyroid disease
- Weight reduction ≥ 10% within past 6 months
- Metastatic disease
- Planned reconstructive surgery with flap repair during trial and follow-up period
- Cardiovascular, respiratory or musculoskeletal disease or joint problems that preclude moderate physical activity.

5.3 Withdrawal Criteria

- Participants may choose to withdrawal from the study at any time.
- Participants in the Exercise Group will be asked to withdraw from the study if they fail to complete more than 3 consecutive exercise sessions.
6.0 STRATIFICATION/DESCRIPTIVE FACTORS/RANDOMIZATION SCHEME

6.1 Stratification factors.
The study population will be further stratified by menopausal status (premenopausal and postmenopausal) resulting in a total of 50 premenopausal and 50 postmenopausal participants.

6.2 Descriptive factors.
Participant age, menopausal status, stage of breast cancer, body weight, and cancer treatment regimen will be reported at time of data analysis.

6.3 Randomization.
A randomized controlled design will be implemented to determine the control and exercise groups. Participants will be randomly assigned to either the Control or Exercise groups. A modern blocked design will be implemented to execute randomization, which will be performed by the Clinical Investigations Support Office (CISO) at the time of registration.

7.0 STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN

7.1 See “Exercise Intervention”, section 3.3.

7.2 Drug studies: N/A

7.3 Criteria for removal from treatment.

7.31 The intervention will be discontinued if participants experience serious exercise-related injuries requiring medical attention.

7.32 Patients will discontinue the intervention if breast disease progression occurs at any time.

7.33 A participant may always be removed from the intervention whenever she wishes.

7.4 Ancillary treatments. N/A

8.0 ASSESSMENT OF EFFICACY AND SAFETY

8.1 Side effects/Toxicities to be monitored.

8.11 List all side effects/toxicities that the patient is to be asked about at each evaluation while on treatment and those to be measured. Toxicities will be assessed weekly during the exercise intervention and documented using the toxicity worksheet. Completed worksheets will be placed in the participant binder.
**Exercise Bouts:** There is a chance that the subject may experience muscle fatigue immediately following the exercise bouts and possibly muscle soreness the next day. The subjects will be provided proper warm-up and instruction by an exercise specialist to minimize these discomforts. During the exercise intervention, all subjects will be monitored to prevent adverse events. Heart rate monitors will be worn at all times during the exercise sessions and will be asked to provide feedback on rates of perceived exertion. Any signs of over-exertion from the exercise bouts such as nausea, vomiting, severe muscle soreness will result in withdrawal from the study and referral for treatment will be advised. Appropriate follow-up following an adverse event will be made and the subject will be contacted to ensure appropriate treatment was received.

**DEXA:** Radiation exposure for the scans (whole body) is very low. The amount of radiation to which subjects will be exposed during DEXA scan is so small that the precise risk is unknown.

**Blood draws:** There is a chance the subject may experience slight discomfort or nausea during the blood draw, bruising after the draw, and there is a very small risk of bleeding or infection. The subjects will be given proper care under the guidance of the trained phlebotomist and PI. Refreshments will be provided to each subject following each blood draw.

**dSAT biopsies:** There is a chance the subject may experience slight discomfort or nausea during the biopsy, bruising after the biopsy, and there is a very small risk of bleeding or infection. The subjects will be given proper care under the guidance of the Dr. Sattler and the PI. Refreshments will be provided to each subject following each biopsy.

**Ultrasound:** The subjects may experience redness of the skin, numbness, pain, tingling of the finger and discomfort while the cuff is inflated, as well as skin irritation caused by ultrasound lubricant gel.

8.12 Long-term toxicities to be monitored after completion of therapy. N/A

8.2 Dosage change based on toxicity. N/A

8.3 Adverse Event Reporting.
Any adverse events will be initially reported to Dr. Dieli-Conwright at (323) 865-3000 or to the listed research personnel, who will inform the IRB within 7 days. The Principal Investigator will comply with all safety reporting regulations as set forth in the Code of Federal Regulations. Toxicities and adverse events including laboratory adverse events will be graded and summarized according to the National Cancer Institute CTCAE, v4.3 (available on the NCI website at: http://ctep.cancer.gov/forms/CTCAEv4.pdf).

Serious Adverse Events

**Definition of a Serious Adverse Event**
Any adverse drug experience occurring at any dose that results in any of the following outcomes:
• Death
• A life threatening adverse drug experience
• Inpatient hospitalization or prolongation of existing hospitalization
• A persistent or significant disability/incapacity or a congenital anomaly/birth defect
All serious adverse events will be reported to the IRB and DSMC within 3 days. Also, the Principal Investigator will report SAEs via the MedWatch Form 3500A to the FDA, which can be accessed at: http://www.accessdata.fda.gov/scripts/MedWatch/
MedWatch forms will be sent to the FDA online at the above internet address or to the following:
MEDWATCH
5600 Fishers Lane
Rockville, MD  20852-9787
FAX:  1-800-FDA-0178 (1-800-332-0178)

8.31 Serious exercise-related injuries will be reported as adverse events and appropriate documentation will take place.

8.32 Reports will be submitted to: IRB and NCI/NIH.

8.4 Data Monitoring Plan.
If an injury or adverse event does occur, the study physician (Dr. Tripathy) will be contacted immediately and the IRB will be notified within 24 hours.
## 9.0 CLINICAL AND LABORATORY EVALUATIONS AND STUDY CALENDAR

Study Calendar:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-intervention Visit 1 - CTU (Week 0a)</th>
<th>Pre-intervention Visit 2 - ICORE (Week 0b)</th>
<th>Weeks 1-16</th>
<th>Post-testing (Week 17)</th>
<th>Follow-up (Week 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical examination^2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Height, weight, BMI*</td>
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<tr>
<td>Pregnancy test</td>
<td>X</td>
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</tr>
<tr>
<td>Blood Draw (Fasting Insulin, glucose, lipids, CRP, HbA1c)^3</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Blood pressure*</td>
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<tr>
<td>dSAT biopsy^3</td>
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<tr>
<td>DEXA scan (body composition)</td>
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<td>Physical activity history questionnaire</td>
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<tr>
<td>Physical activity logs^4</td>
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<td>NIH-DHQ</td>
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<td>3-day diet logs</td>
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<tr>
<td>QOL assessments (SF-36, FACT-B)</td>
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<td>X</td>
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</tr>
<tr>
<td>Psychosocial assessments (CES-D, SHAPE)</td>
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<td>X</td>
<td></td>
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<td>X</td>
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<tr>
<td>DASH, PSS</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder strength, AROM, Lymphedema^*</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

1. Participants will attend pre-intervention visits 1 and 2 if they elect to undergo a biopsy; otherwise if they choose not to undergo the biopsy, all other outcome measures will occur in 1 baseline visit as originally executed.

2. All participants willing to undergo a dSAT biopsy will have a physical examination completed by Dr. Sattler prior to the biopsy procedure.

3. Biopsies will take place at CTU; all other parameters will take place at the ICORE.

4. The follow-up testing applies only to the participants in the Exercise Group; Physical activity logs will be completed during the follow-up period.

* Measurements are captured by automated device or by hand and written directly into the data collection form.

## 10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

All subjects completing the 16-week study period (and 12-week follow-up period for the Exercise Group) will be evaluated for analysis.

## 11.0 SPECIAL INSTRUCTIONS:

N/A
12.0 DATA COLLECTION AND MONITORING

All data collected will be documented in individual participant de-identified data folders. Each participant will receive a patient Identification Number. Data folders will be stored in a locked file cabinet in CHP-149A. Data will be stored on a password-protected computer.

The Data and Safety Monitoring Committee (DSMC) at the USC Norris Comprehensive Cancer Center (NCCC) is an independent body and is responsible for the safety of study subjects through the review of all new institutional protocols and those studies deemed by the CIC to require DSMC oversight to ensure an adequate adverse event reporting plan and through the real-time and periodic monitoring of severe adverse events (SAEs) or those that require expedited reporting. The DSMC also performs real-time, quarterly and annual study progress and safety review, as well as efficacy/futility review as outlined in the NCCC Data and Safety Monitoring Plan (available through CISO website). After each review, the DSMC reports the results of each review and make recommendations to the study PI, IRB, and CIC.

13.0 STATISTICAL CONSIDERATIONS

13.1 Statistical Analyses:

13.1.1 Sample Size Determination

Although a pilot study, this project is designed to detect a treatment effect based on insulin results with 80% statistical power at a 5% level of statistical significance. Ligibel et al. assessed insulin levels before and after a 16-week exercise intervention in breast cancer survivors 41. Using their results, a sample size of 80 participants would detect a difference in mean insulin levels of 2.6 μU/ml assuming that the common standard deviation is 4.0 μU/ml using a two group t-test with two-sided 5% level of statistical significance. Therefore, to allow for a 20% maximum withdrawal rate, 100 participants will be recruited, of whom 50 will be premenopausal and 50 postmenopausal.

Adipose Tissue Inflammation- As this is a pilot study our goal will be to get an estimate of the change in ATM characterization and ATM gene expression to develop a fully powered clinical trial. In a similar study, Aron-Wisnewsky et al. assessed ATM changes before and after a weight loss program in obese women and found a difference in adjusted mean ATM CD40 quantification of 2.0 macrophages/100 adipocytes; large effect- Cohen’s d = 1.795. Using the standard deviation from the Aron-Wisnewsky study, we used G*Power 3.1 software to determine the detectible group difference for our proposed total sample size of 30 participants (n=15 Exercise group; n=15 Control group) with $\alpha = 0.05$ and power $(1-\beta) = 0.80$. Within the expected attrition range of up to 25%, there should be sufficient power to detect an effect (d) between 1.1 and 1.3, lower than that reported previously.

13.1.2 Data Analysis

Specific Aim 1: To determine whether a 16-week exercise intervention will improve components of MetS in breast cancer survivors soon after completion of cancer-related treatments by measuring changes in body composition, waist circumference, blood pressure, and serum levels of insulin, glucose, lipids, C-reactive protein, and HbA1c.

A 2 (group) x 2 (time) repeated measures analysis of variance (ANOVA) will be used to examine changes in a body composition, waist circumference, blood pressure, fasting serum levels of insulin, glucose, lipids, C-reactive protein,
HbA1c. When a significant group by time interaction is found, Bonferroni post hoc tests will be performed to examine the changes in outcomes from pre- and post-exercise training between and within groups with adjustment for multiple comparisons. Stratification by type of therapy (i.e. surgery, chemotherapy, radiation) may result based on therapy regimens completed by enrolled subjects.

**Specific Aim 2:** To determine whether a 16-week exercise intervention will improve physical fitness in breast cancer survivors soon after completion of cancer-related treatments by measuring cardiorespiratory fitness and muscle strength.

A 2 (group) x 2 (time) repeated measures analysis of variance (ANOVA) will be used to examine changes in REE and 1-RM values. When a significant group by time interaction is found, Bonferroni post hoc tests will be performed to examine the changes in outcomes from pre- and post-exercise training between and within groups with adjustment for multiple comparisons. Stratification by type of therapy (i.e. surgery, chemotherapy, radiation) may result based on therapy regimens completed by enrolled subjects.

**Specific Aim 3:** To assess the feasibility of a supervised exercise intervention in early breast cancer survivors.

To determine if the exercise intervention is feasible, we will record attendance to the exercise sessions for each participant. The exercise intervention will be deemed feasible if the participants attend 90% of the exercise sessions.

**Specific Aim 4:** To determine whether breast cancer survivors can maintain positive benefits of an exercise intervention following a 12-week follow-up period by measuring changes in body composition, waist circumference, blood pressure, and serum levels of insulin, glucose, lipids, C-reactive protein, and HbA1c, cardiorespiratory fitness and muscle strength.

A 2 (group) x 2 (time) repeated measures analysis of variance (ANOVA) will be used to examine changes in a body composition, waist circumference, blood pressure, fasting serum levels of insulin, glucose, lipids, C-reactive protein, HbA1c. When a significant group by time interaction is found, Bonferroni post hoc tests will be performed to examine the changes in outcomes from pre- and post-exercise training between and within groups with adjustment for multiple comparisons.

**Specific Aim 5:** To determine whether a 16-week exercise intervention will result in a reduction in adipose tissue inflammation in obese breast cancer survivors soon after completion of cancer-related treatments (i.e. surgery, chemotherapy, radiation) by measuring ATM phenotype and ATM cytokine expression.

Repeated measures ANCOVA will be used to determine whether macrophage phenotype (outcome) is significantly altered following an exercise intervention (Exercise vs Control groups). A priori covariates include baseline body weight, and we will use interaction terms to determine if the exercise intervention is significantly modified by age. **Aim 2:** Changes in gene expression and CLS pre- and post-intervention will be determined using 2-way ANCOVA (Exercise vs Control, baseline vs follow-up) with possible covariates such as age, BMI, lean mass or fat mass. The effect size of the exercise intervention will be evaluated based on the adjusted mean change estimates from the regression analysis on ATM characterization and gene expression levels.
Specific Aim 6: To determine whether a 16-week exercise intervention will alter endothelial function, arterial stiffness, and vascular atherosclerosis. A 2 (group) x 2 (time) repeated measures analysis of variance (ANOVA) will be used to examine changes in FMD, arterial stiffness, and cIMT. When a significant group by time interaction is found, Bonferroni post hoc tests will be performed to examine the changes in outcomes from pre- and post-exercise training between and within groups with adjustment for multiple comparisons.

Note: Additional statistical tests, including correlations and t-tests, will be performed as deemed appropriate once data collection has occurred.

14.0 REGISTRATION GUIDELINE
The Study Coordinator must complete the protocol eligibility form to ensure that the patient is eligible. The Study Coordinator or data manager will then register the patient into the Cancer Center database, café, by accessing the Registration forms. Likewise, after the patient has completed the study, the Off Study forms in café will need to be completed, for Off Treatment and Off Study.

14.1 RECORDS AND DATA SUBMISSION
A. Confidentiality of Records
The original data collection forms will be kept in secure file cabinets in CHP-149A

B. Patient Consent Form
At the time of registration, signed and dated copies of the patient Informed Consent with the Human Rights and the HIPAA authorization must be given to the patient and sent to True at HRA. The original will be kept by the Data Manager.

C. Registration Eligibility Worksheet
At the time of registration, the completed Eligibility Worksheet will be submitted to the QA Monitor at CISO for review of eligibility compliance.

D. Data Collection Forms and Submission Schedule
- Within two weeks of registration, the data manager will complete the initial set of On Study forms and baseline Toxicities
- Within two weeks of completion of each course of treatment, the data manager must complete the Course Assessment, Toxicities, and if appropriate Response data.
- After Off Treatment, within two weeks of each follow up, complete the Follow Up forms.

15.0 BIOHAZARD COMTAINMENT
Radiation Safety approved the usage of the DEXA within the Clinical Exercise Research Center and Human Performance Laboratory in CHP-155.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS
All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.
17.0 REFERENCES


33. Healy LA, Ryan AM, Carroll P, et al. Metabolic Syndrome, Central Obesity and Insulin Resistance are Associated with Adverse Pathological Features in Postmenopausal Breast Cancer. *Clinical oncology (Royal College of Radiologists (Great Britain)).*


49. Stull VB, Snyder DC, Demark-Wahnefried W. Lifestyle interventions in cancer survivors: designing programs that meet the needs of this vulnerable and growing population. *J Nutr.* 2007;137(1 Suppl):243S-248S.


