MITIGATING CARDIOTOXICITY: THE EFFECTS OF PHYSICAL ACTIVITY ON ECHOCARDIOGRAPHIC PARAMETERS AND THE METABOLOME IN BREAST CANCER PATIENTS RECEIVING ANTHRACYCLINE TREATMENT

by

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Dalhousie University is located in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We are all Treaty people.

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Abstract

Background: Metabolomic profiling is a novel technique to identify anthracycline-induced cardiotoxicity (CTX). Higher physical activity levels may mitigate this damage. Therefore, the primary objectives of this thesis were to determine the effects of anthracyclines on the metabolome, investigate the relationship between echocardiographic parameters and the metabolome, and assess the impact of physical activity levels on echocardiographic and metabolic changes. **Methods:** 17 adult females with stage I to III breast cancer scheduled to receive anthracyclines completed baseline and 24-week follow-up assessments. Assessments included an echocardiogram to measure left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS), a questionnaire to assess physical activity levels, and a blood draw for the targeted metabolomic analysis. **Results:** No significant changes in LVEF% (t_{16} = 1.90, P = .08) or GLS% (t_{16} = 1.97, P = .07) were identified. Lipids were the most frequently affected metabolite class. Overall, the altered metabolites were unrelated to changes in LVEF% ($F_{2,14}$ = 1.89, P = .40, R^2 = 0.93) or GLS% ($F_{2,14}$ = 2.37, P = .34, R^2 = 0.94). Baseline levels of physical activity were associated with changes in GLS% (ß = 1.05, t_{16} = 2.92, P = .01) and phosphatidylcholine 16:0/16:0 (ß = 1.04, t_{16} = 2.68, P = .02). Baseline physical activity levels were also associated with changes in 71% of assessed metabolites compared to follow-up physical activity levels. Follow-up reported physical activity levels impacted changes in urea relative intensity (ß = -1.10, t_{16} = 2.71, P = .02). **Conclusions:** Anthracyclines altered metabolite levels, particularly in lipid-based metabolites. Physical activity levels, notably baseline, were associated with changes in metabolic parameters and GLS%. Changes in the metabolome were not predictive of echocardiographic parameters. Future longitudinal research should be conducted to identify metabolic biomarkers for CTX, and investigate the effect of physical activity, particularly before anthracycline treatment, on CTX development.

List of Abbreviations Used

2DE	Two-dimensional Echocardiography
a’	Late Diastolic Velocities
AEX	Aerobic Exercise
ATP	Adenosine Triphosphate
BIOMEX	Biological Interpretation Of Multi-omics EXperiments
BNP	Brain-type Natriuretic Peptide
BMI	Body Mass Index
BMS	Biological Mass Spectrometry
CRP	C-reactive Protein
CTX	Cardiotoxicity
CVD	Cardiovascular Disease
e’	Early Diastolic Velocities
ER +	Estrogen Receptor-positive
EXACT	Exercise to Prevent Anthracycline-Based Cardio-Toxicity
GLS	Global Longitudinal Strain
HER2 +	Human Epidermal Growth Factor two-positive
HILIC	Hydrophilic Interaction Liquid Chromatography
hiSPC	Human-induced pluripotent stem cell-derived cardiomyocytes
hs-cTn	Highly Sensitive Cardiac Troponin
IPAQ	International Physical Activity Questionnaire
LC-MS/MS	Liquid Chromatography LC Tandem Mass Spectrometry
LVEF	Left Ventricular Ejection Fraction
LVID	Left Ventricular Internal Dimension
LVIDed	Left Ventricular Internal Diameter at the end of Systole
LVIDes	Left Ventricular Internal Dimension at the end of Diastole
MeOH-ILIS	Methanol-Isotopically Labelled Internal Standard
MET	Metabolic Equivalents of Task
MRM	Multiple Reaction Monitoring
OPLSDA	Orthogonal Partial Least Squares Discriminant Analysis
p53	Protein 53
PC	Phosphatidylcholine
PCA	Principal Component Analysis
PGC-1	Peroxisome Proliferator-activated Receptor-γ Co-Activator 1
PR +	Progesterone Receptor-positive
REDCap	Research Electronic Data Capture
S’	Systolic Velocities
SOC	Standard of Care
SM	Sphingomyelin

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Chapter 1: Introduction

Cancer encompasses a variety of diseases characterized by uncontrolled cell growth.[1] Cancer remains the leading cause of death in Canada.[2] Estimates indicate that 43% of Canadians will develop cancer at some point in their lifetime, and 25% will die from the disease.[3] For Canadian women, breast cancer is the most frequently diagnosed cancer, accounting for 25% of all new cases.[3] Each day, 78 women, on average, will receive a breast cancer diagnosis, with 15 dying from the disease.[3] While the incidence remains strikingly high, breast cancer survivorship has consistently improved over the past century. Breast cancer mortality rates are 46% lower than 35 years ago;[2] successes attributed to increased screening rates,[4] and improved therapies.[5]

With improved survivorship, the long-term effects of breast cancer therapies are becoming more apparent. Therapies are increasingly effective at managing malignancies, but often cause adverse and long-term side effects. Among breast cancer treatment's most common and severe side effects is anthracycline-induced cardiotoxicity (CTX).[6–12] Doxorubicin and epirubicin are specific anthracyclines frequently prescribed to treat breast cancer and are associated with CTX, leading to a wide range of heart problems,[7] including valvular disease, arrhythmias, left ventricular dysfunction, and, most notably, heart failure.[13]

Anthracycline-induced CTX is a severe and common condition such that Canadian breast cancer patients receiving any dosage of anthracyclines have an 11% chance of developing significant acute, early-onset or long-term cardiac damage.[14] The incidence and severity of such cardiac complications increase proportionately to the

anthracycline dosage a breast cancer patient receives and may manifest many years after initial anthracycline treatment.[15]

Currently, there are various ways to prevent and manage CTX. CTX can be mitigated by treating breast cancer patients with concurrent dexrazoxane upon detection of cardiovascular damage via cardiac imaging,[16] or by modifying the anthracycline pharmacokinetics.[17,18] CTX can be managed using conventional cardiovascular disease (CVD) medications.[19] However, such prevention and treatment modalities are not entirely effective. For example, dexrazoxane is often not administered promptly, routine cardiac imaging can fail to detect early cardiovascular damage,[16] altering anthracycline pharmacokinetics is not possible for all cancer types,[20] and conventional CVD medications are often not prescribed until the patient is symptomatic, indicating irreversible cardiac dysfunction has occurred.[19]

High-throughput molecular profiling techniques may detect CTX better than current diagnostic modalities.[21] Specifically, metabolomic profiling to analyze blood samples from breast cancer patients treated with anthracyclines could identify CTX.[22] Metabolomic profiling is used to assess the metabolome, which includes the full complement of low molecular weight metabolites, small molecules involved in metabolism. The metabolome is highly variable, and its composition and quantitative levels vary in response to lifestyle, disease status and other environmental disturbances. Thus, changes in metabolite levels could predict the earliest signs of CTX, allowing for proactive interventions before irreversible structural changes.[23] Metabolomic profiling can predict CTX *in vivo* and *in vitro*, but has not been extensively investigated in clinical populations.[24,25]

Current research indicates that maintaining high physical activity levels during anthracycline treatment may be an effective alternative intervention to manage CTX. Physical activity reduces CTX risk by minimizing CVD risk factors and improving cardiovascular fitness.[26] The beneficial effects of physical activity to prevent and treat CTX are well established in animal models, and, more recently, in some clinical research.[27–34] However, research investigating the effects of exercise on CTX in breast cancer patients has resulted in inconsistent findings regarding cardiac health without a consensus on the cardioprotective effects of physical activity.[26,35–37] Nonetheless, reports highlight the feasibility and safety of physical activity prescription for preventing CTX in patients before, during, and after anthracycline breast cancer treatment.[26,36] The recent Exercise to Prevent Anthracycline-Based Cardio-Toxicity (EXACT) in Women with Breast Cancer pilot study corroborates these findings.[37]

Thus, the ongoing EXACT 2.0 study aims to investigate if a home-based aerobic-exercise program can mitigate the cardiotoxic effects of anthracyclines in breast cancer patients currently receiving treatment. In the EXACT pilot study, researchers reported difficulties recruiting breast cancer patients, and many participants stated that the time to travel to in-person exercise programs was a barrier.[37] Hence, EXACT 2.0 investigates the impact of a 24-week home-based aerobic-exercise program on various indices of heart function. Participants complete a baseline assessment, are allocated to either an aerobic-exercise (AEX) intervention group or a standard-of-care (SOC) control group and return 24 weeks later for a follow-up assessment. Preliminary findings from the EXACT 2.0 study suggest that the exercise intervention did not increase participant activity levels.[38–40] Thus, the present thesis explored the impact of reported physical activity levels on

markers of CTX instead of conducting a between-group analysis. The specific objectives of this thesis were to investigate (1) the impact of anthracyclines on echocardiographic parameters and the metabolome; (2) the correlation between changes in echocardiographic parameters and the metabolome; and (3) the impact of physical activity levels on changes in metabolic and echocardiographic parameters.

Chapter 2: Literature Review

The following literature review employed a search strategy described in Appendix A.

2.1 Anthracycline-induced Cardiotoxicity

Anthracycline drugs are a class of highly effective chemotherapy commonly used alone or in combination with other chemotherapies (e.g., taxanes) to treat cancer.[41,42] Doxorubicin and epirubicin are anthracyclines often used as adjuvant or neoadjuvant treatments for breast cancer.[43] Anthracyclines exhibit high efficacy for treating breast cancer independent of tumour size or differentiation, and estrogen or nodal status.[41] The Early Breast Cancer Trialists' Collaborative Group reported lower mortality rates in breast cancer patients treated with anthracyclines.[41] Specifically, the group reported that any dosage of anthracyclines decreased breast cancer patient mortality rates by 20% and up to 30% in breast cancer patients accumulating higher dosages (doxorubicin ≥ 240 mg/m^2 or epirubicin ≥ 360 mg/m^2).[41] Because of their efficacy, anthracyclines have been a mainstay in treating cancer since their isolation from bacteria in 1964.[44]

CVD is a leading cause of death for breast cancer patients treated with anthracyclines.[45] Patnaik *et al.*[45] retrospectively studied 63 566 female breast cancer patients > 65 years of age and found that of the women who died, a similar proportion of women died of CVD (31%) compared to those who died of breast cancer (29%). However, of the women who died of CVD, only 25.5% presented with it at the time of their breast cancer diagnosis, suggesting the treatment induced cardiotoxic effects.[45] It is also important to note that anthracyclines are often used alongside taxanes, such as trastuzumab, which further increase a breast cancer patient's risk for developing CVD.[42]

2.1.2 Clinical Manifestation and Prevalence of Cardiotoxicity

While there is no standard definition, CTX refers to cardiac dysfunction caused by chemotherapy.[12] The literature often defines CTX as a decrease in left ventricular ejection fraction (LVEF) > 10% and up to 55%, as determined by 2-dimensional echocardiography (2DE).[6,12,13,15,19,46] CTX is challenging to define as it encompasses a wide range of cardiac abnormalities.[47] CTX can present as myocardial dysfunction, heart failure, coronary artery disease, valvular disease, arrhythmias, arterial hypertension, thromboembolic disease, peripheral vascular disease, stroke, pulmonary hypertension, and pericardial complications.[13]

Given the variety of cardiovascular abnormalities, the prevalence of CTX is challenging to characterize. In an early report of 4018 cancer patients receiving doxorubicin, the prevalence of coronary heart failure was 2.2%.[48] Furthermore, the report highlighted the dosage dependence of CTX. Of patients who developed coronary heart failure, 3%, 7%, and 18% developed coronary heart failure when receiving 400 mg/m^2, 550 mg/m^2, and 700 mg/m^2 of doxorubicin, respectively.[48] These findings are corroborated in more recent studies. Swain and Whaley[49] found that cardiac events (defined as a decline in LVEF% ≥ 20% from baseline) occurred dose dependently. Specifically, cardiac events occurred in 7% of patients receiving 250 mg/m^2, 9% of patients receiving 350 mg/m^2, 18% of patients receiving 450 mg/m^2, 38% of patients receiving 550 mg/m^{2}and 65% of cancer patients receiving 650 mg/m^2 of doxorubicin.[49] Additionally, of patients who develop CTX, 2% to 4% exhibited congestive heart failure, 9% to 11% exhibited subclinical structural changes, > 12% exhibited arrhythmias, and 30% to 35% exhibited cardiac biomarker elevations.[12] While cancer patients receiving

anthracyclines can present with various CVDs, all patients receiving anthracyclines are at a clinically relevant risk for developing CTX.

CTX can also be categorized temporally based on the time between initial treatment and onset of cardiovascular damage. Acute CTX occurs within 2 weeks of beginning treatment, presents in 1% of cancer patients, and is usually reversible.[6,15] Early-onset CTX occurs within the first year of treatment, accounts for 98% of all cases, and is often asymptomatic.[6,13,15] Late-onset CTX occurs years (a median of 7 years) after a patient receives anthracyclines, and is frequently irreversible.[6,13] Late-onset CTX is often the result of undiagnosed early-onset CTX.[6,13]

When cancer patients develop cardiovascular abnormalities detectable via imaging, the damage is often irreversible.[13,15] Unfortunately, breast cancer patients often do not undergo routine cardiac surveillance unless previously identified as high risk for developing CVD.[13,15] Thus, CTX is usually not detected in breast cancer patients until it is severe and irreversible, making treatment challenging.[13,15] Diagnosing CTX through identifying substructural level changes at the cellular level may allow for earlier detection and, thus, earlier interventions to reverse or prevent anthracycline-induced CTX potentially.

2.1.3 Pathophysiological Mechanisms of Cardiotoxicity

The mechanism by which anthracyclines exhibit antineoplastic effects and CTX is hypothesized to occur through the same cellular pathway.[20,50] Anthracyclines primarily inhibit topoisomerase II, an essential enzyme in DNA replication.[51] Specifically, topoisomerase II is responsible for disentangling supercoiled DNA.[51] After systemic intravenous administration, anthracyclines passively diffuse into cells and intercalate with

nuclear DNA, forming a ternary complex.[26] This complex prevents topoisomerase II from disentangling DNA, causing DNA double-stranded breakages. Thus, the cells cannot resynthesize their DNA, are forced to enter growth arrest, and apoptotic pathways are triggered.[50,52] There are two distinct isoforms of topoisomerase II – topoisomerase IIα found in highly proliferative cells like neoplasms, and topoisomerase IIß in quiescent cells like cardiomyocytes.[50] Anthracyclines bind to both isoforms. Thus, cell death occurs. While it is advantageous when anthracyclines inhibit topoisomerase IIα and kill cancer cells, it is disadvantageous when anthracyclines inhibit topoisomerase IIß and kill cardiomyocytes. Such effects are the underlying mechanisms of CTX.

Anthracyclines also cause CTX by impairing mitochondrial biogenesis.[50] Impaired mitochondrial biogenesis leads to the activation of apoptotic and oxidative stress pathways.[50] Research in mouse models found that doxorubicin treatment increased the expression of genes involved in protein 53- (p53) induced apoptosis. Increased p53 synthesis suppressed peroxisome proliferator-activated receptor-γ co-activator 1-α and 1-ß (PGC-1α and PGC-1ß).[53] PGC-1α and PGC-1ß are critical transcriptional regulators of genes involved in mitochondrial biogenesis.[50] Suppression of such pathways reduces mitochondrial production by downregulating genes responsible for producing transcripts involved in the electron transport chain, tricarboxylic acid cycle, and ß-oxidation of fatty acids.[53] Thus, numerous metabolic pathways are negatively impacted by anthracyclines. Zhang *et al.*53 also showed that cardiomyocytes lacking topoisomerase IIß did not show decreases in LVEF% or significant mitochondrial dysfunction when exposed to anthracyclines. Therefore, CTX is highly dependent on the presence of topoisomerase IIß.[53]

In summary, anthracyclines are hypothesized to cause CTX through association with topoisomerase IIß. Anthracyclines cause DNA double-stranded breakages, suppression of PGC-1α and PGC1-ß, and activation of p53.[53] Such effects cause impaired mitochondrial biogenesis, increased production of reactive oxygen species, decreased adenosine triphosphate (ATP) production and activation of apoptotic pathways leading to cardiomyocyte cell death (Figure 1).[50]

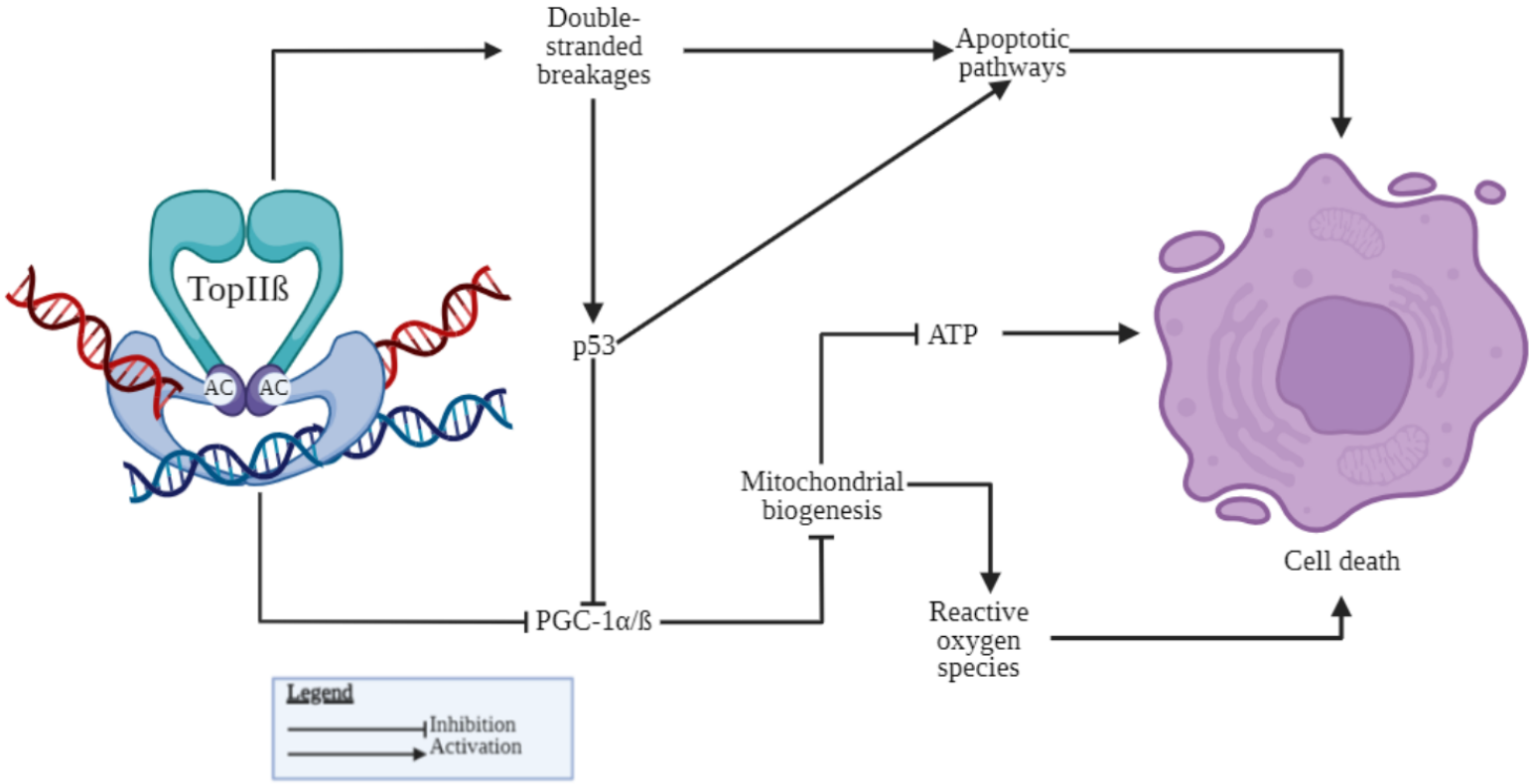

Figure 1: Topoisomerase IIß Mechanism of Cardiotoxicity

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Adapted from Vejpongsa and Yeh, 2014.[50] When anthracyclines (AC) bind with topoisomerase IIß, DNA double-stranded breakages occur, which trigger apoptotic pathways. Additionally, genes encoding for protein 53 (p53) are upregulated, leading to suppression of peroxisome proliferator-activated receptor-γ co-activator 1-α and 1-ß (PGC-1α and PGC-1ß), causing impaired mitochondrial biogenesis. Decreased mitochondrial production results in reduced adenosine triphosphate (ATP) production and increased reactive oxygen species production. Increased reactive oxygen species production and decreased ATP production combined with the triggering of apoptotic pathways causes cell death.

2.1.4 Assessing Risk for Cardiotoxicity

While the mechanisms of anthracycline-induced CTX occur at the cellular level, various external factors may reduce or increase breast cancer patient CTX risk. Such factors to consider are previous CVD, demographic and cardiovascular risk profiles (e.g., age, hypertension, diabetes mellitus, chronic kidney disease), previous cardiotoxic cancer treatment, and lifestyle risk factors (e.g., smoking, obesity).[54]

Indeed, patient risk can be stratified by completing cardiovascular health screening. Various modifiable, nonmodifiable, and cancer-related CVD risk factors can predict breast cancer patient CTX risk. Risk factors should be considered before, during and after the patient receives anthracyclines.[54] In patients with many CVD risk factors, regular cardiac surveillance is crucial as prompt surveillance can detect 98% of CTX within 12 months of follow-up.[15] Lyon *et al.*[54] recommended that cancer patients undergo a comprehensive cardiovascular health assessment before receiving potentially cardiotoxic treatment, and the preassessment should be used to inform cardiac surveillance and cancer treatment regimens. In brief, low-risk patients can receive anthracyclines with minimal cardiovascular surveillance. Medium-risk patients should be monitored closely and referred to a cardiooncologist. High and very-high-risk patients must receive a personalized cancer treatment plan, ideally using fewer cardiotoxic treatments in consultation with a cardiooncologist.[54] Unfortunately, in Canada, such comprehensive cardiovascular health screenings are not part of the standard of care for breast cancer patients.

2.2 Detecting Cardiotoxicity

Various tools can be used to screen and monitor cardiac health, including cardiac imaging (e.g., echocardiography), and cardiac biomarker analysis.[19] Each screening tool has certain advantages and disadvantages.

2.2.1 Cardiac Imaging

Echocardiography is a form of cardiac imaging to assess cardiac structure, function, and hemodynamics. A benefit of echocardiography is that it is non-invasive, widely available, reproducible and cost-effective compared to other cardiovascular health assessment tools.[55] Current recommendations suggest that all cancer patients undergo repeat echocardiograms to assess left ventricular function, especially in patients at risk for CVD and those scheduled to receive cardiotoxic treatments.[19]

Traditionally, 2DE is used to assess a patient's baseline cardiac health or degree of cardiac damage following cardiotoxic treatment, such as anthracyclines.[55] 2DE is used to measure LVEF% from linear or volumetric measurements. Specifically, motion-mode tracing is used to determine the left ventricle's internal linear measurements from the heart's parasternal long-axis view. The modified biplane Simpson's technique determines volumetric measurements[55] at the apical 4- and 2-chamber views.[56] 2DE linear and volumetric assessments of LVEF% create reproducible images with high resolution. However, linear measurements can only be collected in one dimension, and the images produced from volumetric measurements appear to shorten the apex of the heart. Linear measurements cannot detect changes in ventricle shape outside of the apical 2-and 4-chamber planes.[56] Additionally, LVEF% as a clinical determinant of heart health is only reliable when there is a > 10% change in LVEF% compared to baseline measurements.[19]

Thus, 2DE is often insensitive to asymptomatic and minor changes in cardiac function, so other imaging modalities should be considered.[56]

Measurements of global longitudinal strain percentage (GLS%) resolve many limitations of standard 2DE. Specifically, GLS% is determined using speckle-tracking echocardiography to assess the change in length of the left ventricle myocardium between end-diastole and end-systole.[56] Díaz-Antón *et al.*[57] reported that an increase in GLS% is an earlier detector of CTX than LVEF% at one month following initial anthracycline treatment. Ali *et al.*[58] reported that patients with a baseline GLS% > -17.5% have more than a 6-fold risk of heart failure or cardiac death following anthracycline treatment. Clinically, patients with > 15% increase in GLS% have a significant risk of developing CTX.[59] While GLS% is a more sensitive measure than LVEF% for assessing the cardiac health of breast cancer patients, its availability is limited, requires an experienced clinician, exhibits variability across equipment, and is restricted by the image quality.[56,57]

2.2.2 Cardiac Biomarker Analysis

As 2DE and GLS% have some limitations, the most recent (2016) recommendations from the Canadian Cardiovascular Society suggest conducting cardiac biomarker analyses numerous times throughout a patient's treatment with anthracyclines in addition to imaging.[19] Despite not being used in clinical practice, cardiac biomarkers are helpful alongside imaging to identify and monitor CTX.[15] Blood biomarkers non-invasively indicate ultrastructural damage that imaging is not sensitive enough to identify.[60] CTX biomarkers include brain-type natriuretic peptide (BNP), highly sensitive cardiac troponin (hs-cTn), N-terminal pro-BNP, and C-reactive protein (CRP). BNP and hs-cTn are proteins unique to the heart, where elevated levels can indicate a myocardial

infarction.[60] A recent meta-analysis that assessed data from 5691 cancer patients found that patients receiving anthracyclines had increased hs-cTn, and those with elevated hs-cTn were more likely to have left ventricular dysfunction.[61] Additionally, BNP and N-terminal pro-BNP were significantly elevated.[61] In another review, CRP was highlighted as a possible biomarker predictive of CTX.[62] CRP levels likely increase in individuals treated with anthracyclines because of the increased presence of reactive oxygen species leading to systemic inflammation.[62] Although biomarker level increases rarely justify cessation or change in cancer therapy, the Heart Failure Association of the European Society of Cardiology advised oncologists to consider increased cardiac monitoring and the possible initiation of cardioprotective treatments in patients with elevated cardiac biomarkers.[60] Analyzing biomarkers, such as BNP, N-terminal pro-BNP, CRP, and hs-cTn, can assist in diagnosing and identifying pre-symptomatic CTX.

The previously described cardiac biomarker analyses can indicate ultrastructural changes; however, new advances in high-throughput molecular profiling techniques may allow for the earlier identification of CTX. Metabolomics is one such technique which allows for the identification of small chemical intermediaries produced during cellular metabolism, called metabolites.[63] Metabolite levels are dependent on the genome, transcriptome, proteome and environment — as such, analyzing the metabolome may provide insight into intracellular changes indicative of pathophysiological changes due to environmental disturbances, including disease status and lifestyle modification, prior to structural changes.[21,63,64] The literature highlights the promising ability of metabolomic profiling to detect conditions earlier and more accurately than conventional diagnostic tools, allowing for precision[65] and individualized medicine.[21] Further, metabolomic

profiling can be conducted on serum and plasma, which is relatively easy to obtain and implementing such profiling in clinical practice would not pose an excessive testing burden to breast cancer patients.

The current literature highlights the potential importance of metabolomic profiling in detecting anthracycline-induced CTX. Numerous models – *in vitro*,[66,67] and *vivo*[22,64,68–76] – present evidence indicating that anthracycline-induced CTX can be identified using metabolomic profiling (Table 1). For example, a recent review by Choksey and Timm[77] recommended metabolomic profiling for patients receiving anthracyclines because myocardial metabolism dysfunction is thought to play a critical role in the pathophysiology of CTX. The review suggested that metabolomic analyses can identify early changes in cardiac health because of the high energy demands of the heart and the disruption of mitochondrial biogenesis during anthracycline treatment.[63,77] Notably, a recent study by Thonusin *et al.*,[22] investigated the diagnostic applicability of metabolomic profiling for CTX identification. They conducted comprehensive metabolomic profiling and assessed the cardiac health of rats treated with doxorubicin, trastuzumab or saline.[22] The group found 59 metabolites involved in amino acid, fatty acid, energy, nucleotide, and phospholipid metabolism to be altered in the plasma of doxorubicin-treated rats. Further, the group found strong correlations between changes in heart health as indicated by echocardiography and changes to the metabolome as indicated by metabolomic profiling, including amino acids, free fatty acids, acylcarnitines, and phospholipids.[22] Overall, anthracycline treatment is most commonly reported to alter metabolites classified as, or related to amino acids and lipids (Table 1 and Figure 2). However, minimal research has been conducted to explore the

metabolomic profiles of clinical populations treated with anthracyclines and which metabolites should be monitored to optimize CTX screening (Table 1).[24,25] Current clinical research reported in the literature is limited to an abstract by Cocco *et al.*[25] and a case-control study conducted by Asnani *et al.*[24] (Table 1).

Table 1: Changes in Metabolite Levels Indicative of Anthracycline-induced Cardiotoxicity

Study	Species	Species and Sample	Altered Metabolites
Andreadu *et al.*, 2009[68]	Wistar rats	Aqueous myocardial extracts	Leucine, isoleucine, valine, acetate, succinate
Tan *et al.*, 2011[69]	Institute of Cancer Research mice	Myocardial tissue	Glycerol-3-phosphate, stearic acid, cholesterol, linoleic acid, arachidonic acid, alanine, glycine, proline, glutamine, phenylalanine, valine, isoleucine, threonine, succinate, citrate, malate, lactate, 3-hydroxybutyric acid, dihydroxyacetone phosphate, fructose, glucose, myoinositol, phosphate
Cong *et al.*, 2012[70]	Sprague-Dawley rats	Urine	N-acetyl glutamine, N-acetyl tryptophan, citrate, lactate, D-gluconate-1-phosphate
Li *et al.*, 2015[71]	Wistar Rats	Plasma	19-hydroxydeoxycorticosterone, LPC (14:0), LPC (20:2), carnitine
Schnackenberg *et al.*, 2016[72]	B6C3F1 mice	Heart tissue	5 acylcarnitines, 18 different amino acids, acetylornithine, kynurenine, putrescine, serotonin
		Plasma	16 acylcarnitines, 16 amino acids, acetylornithine, hydroxyproline, formate, acetate
Chaudhari *et al.*, 2017[66]	Human stem cell-derived cardiomyocytes	Culture medium	Formate, acetate
QuanJun *et al.*, 2017[73]	BALB/c mice	Serum	5-hydroxylysine, 2-hydroxybutyrate, 3-hydroxybutyrate, 2-oxoglutarate glutamate, cysteine, methionine, aspartate, isoleucine, glycylproline, glucose, acetone,
Asnani *et al.*, 2020[24]	Breast cancer patients treated with anthracyclines and trastuzumab	Plasma	Citrate, aconitic acid
Cocco *et al.*, 2020[25]	Breast cancer patients	Plasma	Fatty acids, tricarboxylic acid cycle intermediates
Geng *et al.*, 2020[74]	Sprague-Dawley rats	Serum, hear, liver,	Cholesterol, glycerol, palmitic acid, phenol, stearic acid, glycine, alanine, valine, propanoic acid,

Study	Species	Species and Sample	Altered Metabolites
		kidney, and brain tissue	lactate, 3-methyl-1-pentanol, glucose, phenol
Palmer *et al.*, 2020[67]	Human stem cell-derived cardiomyocytes	Culture medium	Arachidonic acid, 2'-deoxycytidine, thymidine, lactate
Timm *et al.*, 2020[75]	Wistar rats	Myocardial tissue and plasma	Glutamate, malate, carnitine, NAD, AMP, ADP, ATP
Lin *et al.*, 2021[76]	Sprague-Dawley rats	Serum	Arachidonic acid, linoleic acid, tryptophan
Thonusin *et al.*, 2022[22]	Wistar rats	Aqueous myocardial extracts and plasma	59 metabolites involved in amino acid, fatty acid, energy, nucleotide, and phospholipid metabolism.

Abbreviations: Adenosine Diphosphate, ADP, Adenosine Monophosphate, AMP, Adenosine Triphosphate, ATP; Lysophosphatidylcholine, LPC; Nicotinamide Adenine Dinucleotide, NAD, PC, Phosphatidylcholine.

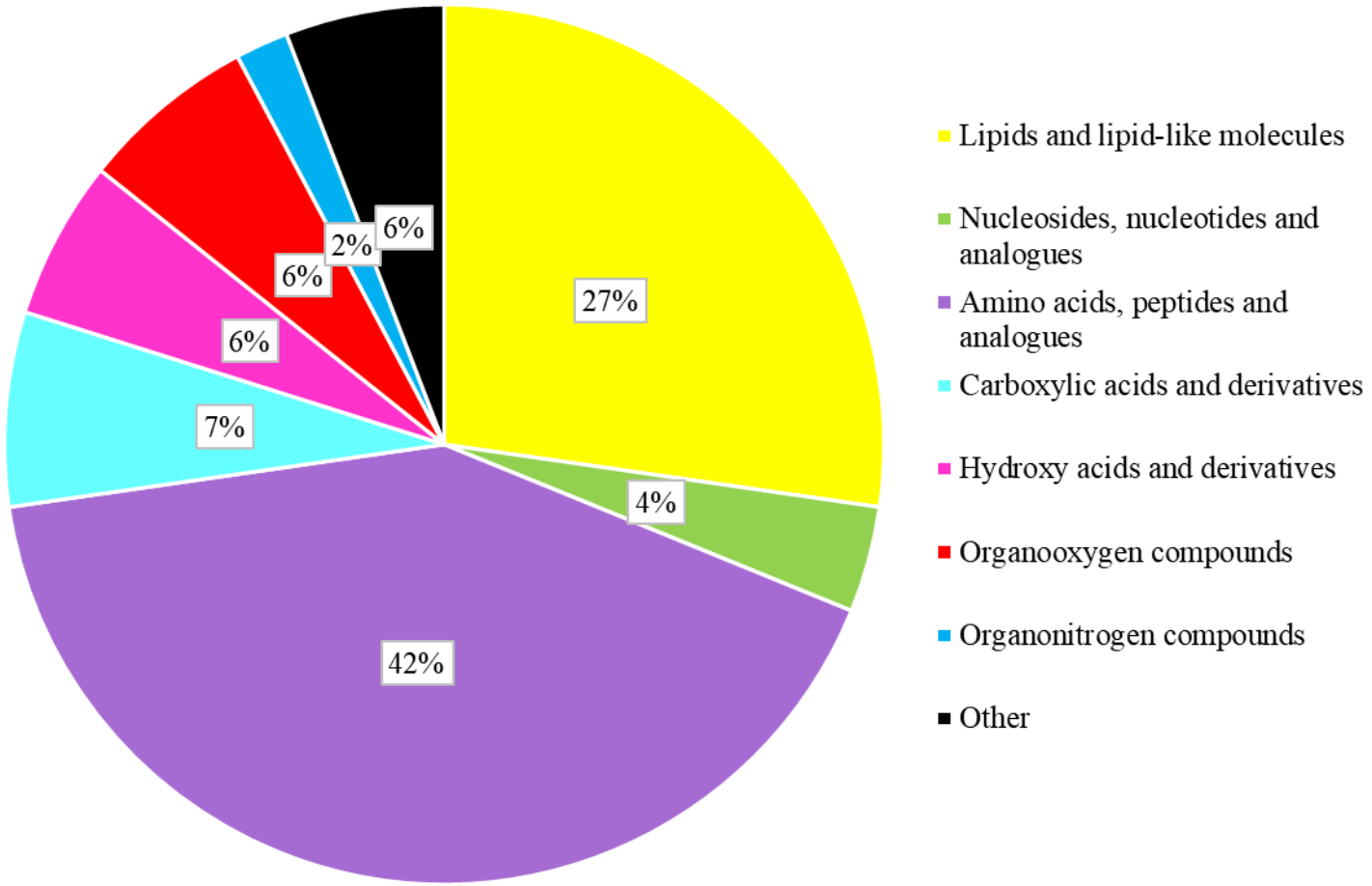

Figure 2: Reporting Frequency of Metabolites Significantly Altered Following Anthracycline Treatment by Molecule Classification

Lipids and lipid-like molecules,[22,68,70,72,73,75,77] nucleosides, nucleotides and analogues,[22,68] amino acids, peptides and analogues,[22,69–71,73–76] carboxylic acids and derivatives,[22,24,25,67,69–71,75] hydroxyacids and derivatives,[22,68,70,71,74–76] organooxygen compounds,[22,70,73–75] organonitrogen compounds,[72,73,76] and other compounds[22,70,71,73–75,77] were reported.

In summary, cardiac imaging and occasionally traditional cardiac biomarker analysis are used to detect CTX.[13,19] Once CTX is detected, anthracycline treatment is either stopped or reduced; thus, anthracycline efficacy is impacted. Unfortunately, CTX screening is not frequently utilized in Canada because imaging availability is limited and expensive. [78] Metabolomic profiling of breast cancer patient serum or plasma at baseline and intermittently throughout anthracycline treatment may allow for earlier identification of CTX without being overly invasive or burdensome on the patient. Prompt detection of CTX would allow for the implementation of preventative interventions, and cancer treatment regimens would not necessarily need to be changed.

2.3 Managing Cardiotoxicity

Following CTX screening, preventative measures and, when applicable, CTX treatment should be promptly implemented. CTX prevention must be prioritized as it is often irreversible.[6] Preventative interventions include minimizing cumulative anthracycline dosage, modifying treatment regimens, and initiating pharmaceutic and non-pharmaceutic interventions.[13,20]

2.3.1 Minimizing Cumulative Dosage

The most effective way to prevent anthracycline-induced CTX is by limiting anthracycline treatment. The European Clinical Guidelines on Cardiovascular Toxicities highlight the effectiveness of minimizing cumulative anthracycline dosage as the primary strategy to prevent CTX.[13] The guidelines indicate that the relative prevalence of heart failure is < 5% in patients who receive ≤ 150 mg/m^2, 400 mg/m^2, 800 mg/m^2, 900 mg/m^2 of idarubicin, doxorubicin, daunorubicin, or epirubicin, respectively.[13] Additionally, any anthracycline dose warrants cardiovascular surveillance, and CTX varies by patient and

anthracycline type.[13] Minimizing cumulative anthracycline dosage in patients with aggressive malignancies may increase the risk of metastases and malignant-related death. Therefore, detection of CTX must be prompt to allow for early intervention and minimal changes to the breast cancer patient's anthracycline treatment regimen to ensure the best cancer- and cardiac-related outcomes for the individual.

2.3.2 Modifying Treatment Regimens

While anthracyclines remain a standard of care for their highly effective antineoplastic effects, modifications to anthracycline treatment regimens decrease the drug's cardiotoxic profile. Preclincal[79] and clinical[17] studies indicate that continuous anthracycline administration is preferred over bolus injections, which does not decrease the antineoplastic effects of anthracyclines. Legha *et al.*[17] reported that 46.7% of lung cancer patients receiving standard bolus dosages of doxorubicin developed severe changes in their endomyocardial biopsies, compared to only 9.5% of patients receiving the drug via continuous infusion. Further, a pharmacodynamic study in animal models indicated that anthracycline concentrations in tumours were the same in mice treated continuously compared to bolus anthracycline injections.[79] Thus, continuous anthracycline administration does not reduce anthracyclines' efficacy in treating cancer, and decreases CTX risk compared to bolus injections.

Furthermore, CTX can be prevented by modifying the pharmacokinetics of anthracyclines. Specifically, anthracyclines delivered via liposomal encapsulation increase the drug's delivery to neoplastic tissue, but reduce the drug's delivery to the heart. Van Dalen *et al.*[18] reported that liposomal encapsulation is associated with a risk reduction in clinical and subclinical heart failure of 0.38. Unfortunately, liposomal

encapsulation usage is limited as it is expensive and only currently approved for ovarian cancer, AIDS-related Kaposi sarcoma and multiple myeloma.[20]

2.3.3 Pharmaceutic Interventions

Dexrazoxane is the only pharmaceutic intervention widely used for preventing CTX. Dexrazoxane is an iron-chelator that prevents anthracyclines from binding to topoisomerase IIß via competitive inhibition.[80,81] Thus, the cascades of intracellular reactions leading to CTX do not occur.[20] As discussed earlier, topoisomerase IIß is the primary isozyme in cardiac tissue, and thus dexrazoxane primarily acts upon cardiomyocytes to prevent CTX. However, dexrazoxane is only effective as a prophylactic treatment for preventing CTX. Dexrazoxane must be administered concurrently with anthracycline treatment, and only patients with CVD risk factors or those who develop cardiac complications receive dexrazoxane treatment.[16] As the evidence highlights the importance of early interventions to prevent CTX, challenges with detecting CTX and the delayed onset of CTX, dexrazoxane usage and effectiveness are limited.

Other conventional heart medications, like beta-blockers, statins, and renin-angiotensin-aldosterone system blockades, can be administered concurrently with anthracyclines upon detecting asymptomatic left ventricular dysfunction.[6] However, managing anthracycline-induced CTX using conventional heart medications has limitations. Conventional heart medications can slow the progression of CTX, manage CVD-related symptoms, and reduce the risk of cardiac events, but do not prevent or treat patients with cardiovascular-related declines caused by anthracycline therapy.[12,82] Additionally, conventional heart medications are often not administered before the

development of irreversible cardiac dysfunction because of challenges with detecting CTX.

Thus, pharmaceutic treatment options do not fully manage CTX, so alternative treatment and complementary treatment strategies must be researched.

2.3.4 Nonpharmaceutical Interventions

Exercise is a non-pharmaceutic intervention that decreases a breast cancer patient's risk for developing CTX pre-, during and post-anthracycline treatment. Aerobic and resistance training may be viable tools to mitigate and/or prevent CTX (Figure 3).[26] Numerous preclinical studies report that physical activity attenuates oxidative stress and apoptosis, intracellular processes hypothesized to cause CTX.[26] Studies in mouse models treated with anthracyclines indicate that exercised mice receiving anthracyclines have higher levels of antioxidant enzymes (e.g., superoxide dismutase),[30,34,83] a lower prevalence of CTX,[28,29,32,84–86] and improved mitochondrial health[29,30,32,33,83–86] than sedentary mice receiving anthracyclines. Additionally, physical activity is reported to counteract the mechanisms of cardiac dysfunction. Physical activity reduces the prevalence of DNA double-stranded breakages,[87] and PGC-1α/ß[88] and p53[89] deactivation. These findings, however, are based on general cardiac dysfunction, not CTX specifically, and thus, must be further researched.

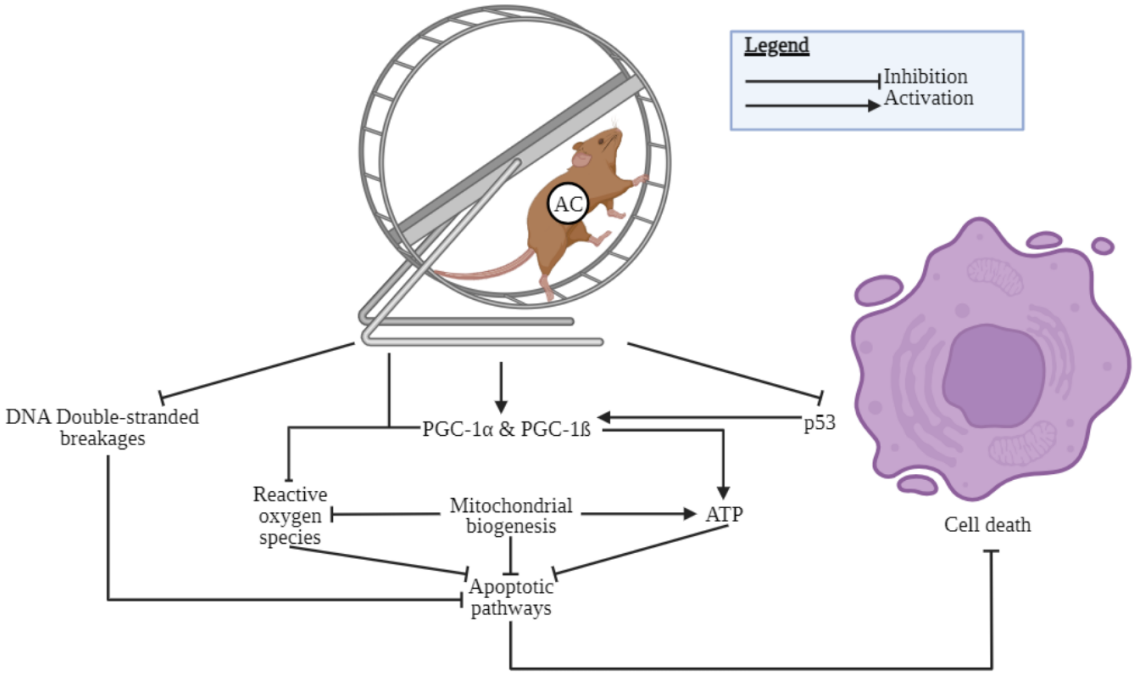

Figure 3: Cardioprotective Effects of Physical Activity Following Anthracycline Treatment.

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Exercise inhibits DNA double-stranded breakages (DSB), reactive oxygen species production and protein 53 (p53) while activating peroxisome proliferator-activated receptor-γ co-activator 1-α and 1-ß (PGC 1 α & ß). PGC 1 α & ß activation causes inhibition of reactive oxygen production and increased ATP production. All processes inhibit apoptotic pathways and, thus, cell death.

Numerous clinical studies indicate that physical activity improves multiple indices of cardiac health, including echocardiographic measurements,[90–93] cardiac biomarker levels,[35,90,94] physical fitness,[91,93,95–98] and vascular health[90,99,100] in breast cancer patients (Table 2). Kirkham *et al.*[101] evaluated the effects of a multimodal exercise intervention on heart health in early-stage breast cancer patients receiving adjuvant chemotherapy. The exercise program mitigated CTX by attenuating increases in resting heart rate, hypotension, tachycardia, and impaired heart rate recovery.[101] Additionally, Kirkham *et al.* found that a single exercise bout performed 24 hours before anthracycline treatment improved hemodynamic (e.g., systemic vascular resistance) measurements in breast cancer patients,[100] and attenuated increases in NT-proBNP.[90] However, exercise did not attenuate CTX over the entire treatment regimen as participant LVEF% decreased by 10%.[100] To date, much of the current literature has investigated the effects of low- to moderate-intensity aerobic exercise in breast cancer patients over a relatively short time.[37,90,100] Thus, the cardioprotective benefits of physical activity may not be detected by current screening modalities (e.g., LVEF% as determined by 2DE), and a more extended exercise program could elicit clinically significant changes.

Further, exercise programs are safe and feasible[37,90,98,99] when used to manage CTX in breast cancer patients (Table 2). Notably, the EXACT 2.0 pilot study investigated the feasibility, safety and effectiveness of an exercise program for breast cancer patients and found no adverse events, high exercise program adherence (defined as the ratio of the number of attended exercise sessions compared to the number of recommended sessions) and a positive effect of aerobic exercise on markers (e.g., inflammatory markers) of CTX and aerobic capacity.[37] However, researchers noted that participant travel was a barrier to

recruitment because participants were required to attend in-person exercise programming.[37] Thus, the EXACT 2.0 study was developed. EXACT 2.0 is an ongoing randomized clinical trial (NCT03748550) utilizing a home-based aerobic-exercise prescription approach to manage CTX in breast cancer patients. Recruitment began in April 2019, with an expected completion date of December 2023. EXACT 2.0 participants are randomized to a SOC control or AEX intervention group. While no published reports on the EXACT 2.0 data are available, two theses[38,39] and one conference proceeding[40] indicate that the trial is safe and feasible for breast cancer patients. However, the EXACT 2.0 exercise program does not appear to have a sufficient exercise stimulus (i.e., intensity and/or volume) to significantly alter participant physical activity levels ($F_{1,18} = 0.10$, $P = .76$) (Figure 4, Panel A), and elucidate cardiac adaptations.[38–40] Even within the leisure time physical activity domain, which encompasses exercise and sport, the intervention did not appear to alter activity levels ($F_{1,18} = 0.007$, $P = .93$) (Figure 4, Panel B). Rather than comparing AEX and SOC EXACT 2.0 participants, analyzing the physical activity levels of all participants and using more sensitive detection methods (i.e., metabolomic profiling) could better illuminate clinically significant changes.

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Table 2: The Effects of Physical Activity on Cardiac Health in Breast Cancer Patients Treated with Anthracyclines

Study	Exercise Intervention	Key findings
Chung *et al.*, 2022[93]	32 breast cancer patients completed 3 months of multimodal exercise training or usual care.	Physical activity prevented declines in systolic and diastolic function. Physical activity also increased $\dot{V}O_2$max.
Kerrigan *et al.*, 2022[96]	28 breast or leiomyosarcoma cancer patients completed 10 weeks of aerobic-exercise training or usual care.	Physical activity improved $\dot{V}O_2$max. GLS% and troponin levels did not change.
Ansund *et al.*, 2021[35]	88 breast cancer patients completed 16 weeks of multimodal, aerobic-exercise, or usual care training.	Physical activity prevented increases in NT-proBNP 1-year postbaseline, but did not attenuate increases in plasma troponin.
Heinze-Milne *et al.*, 2021[37]	10 breast or hematological cancer patients completed 12 weeks of aerobic-exercise training.	Exercise is feasible, but did not change $\dot{V}O_2$max and inflammatory cytokine levels.
Foulkes *et al.*, 2020[94]	56 breast cancer patients completed 4 months of multimodal exercise training or usual care.	Physical activity attenuated troponin increases, but did not prevent reductions in LVEF%, GLS% or diastolic function.
Howden *et al.*, 2020[97]	28 breast cancer patients completed 8 to 12 weeks of multimodal exercise training or usual care.	Physical activity attenuated declines in $\dot{V}O_2$max, but did not prevent reductions in LVEF% or cardiac reserve and did not prevent increases in troponin levels. GLS% and brain natriuretic peptide levels did not change.
Lee *et al.*, 2020[102]	22 breast cancer patients with a BMI $\geq$ 25.0 kg/m^2 completed an 8-week aerobic-exercise training program or usual care.	Exercise is feasible. $\dot{V}O_2$max did not change.
Lee *et al.*, 2019[98,99]	30 breast cancer patients completed an 8-week aerobic-exercise training program or usual care.	Physical activity prevented declines in $\dot{V}O_2$max,[98] improved brachial artery FMD[99] and was feasible.[98] Also, carotid IMT significantly increased in the usual care group, but not in the exercisers.[99]
Kirkham *et al.*, 2018[100]	24 breast cancer patients completed an aerobic-exercise session or usual care 24 hrs before each of the 4 anthracycline treatments.	Physical activity prevented increases in resting heart rate and systemic vascular resistance and prevented reductions in cardiac output. GLS% and troponin levels did not change.
Ma *et al.*, 2018[92]	64 breast cancer patients completed a 16-week aerobic-	Physical activity prevented reductions in LVEF% and the E/A ratio and prevented increases in the DT interval.

Study	Exercise Intervention	Key findings
	exercise training program or usual care.	
Fraser *et al.*, 2017[91]	20 breast cancer patients completed a 3-month multimodal exercise training program or usual care.	Physical activity prevented reductions in $\dot{V}O_2$max and cardiac function.
Kirkham *et al.*, 2017[90]	24 breast cancer patients completed 1 aerobic-exercise session prior to receiving anthracyclines.	Physical activity decreased systemic vascular resistance and increased ejection fraction and systolic strain rate. Also, exercisers had lower NT-proBNP than the usual-care group.
Hornsby *et al.*, 2014[95]	20 breast cancer patients completed a 12-week aerobic-exercise training program or usual care.	Exercise is feasible and increases $\dot{V}O_2$max.

Abbreviations: CTX, cardiotoxicity; E/A, early and late atrial filling velocity ratio, FMD, flow-mediated dilation; GLS, global longitudinal strain; IMT, intima-media thickness; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; $\dot{V}O_2$max, maximum oxygen consumption.

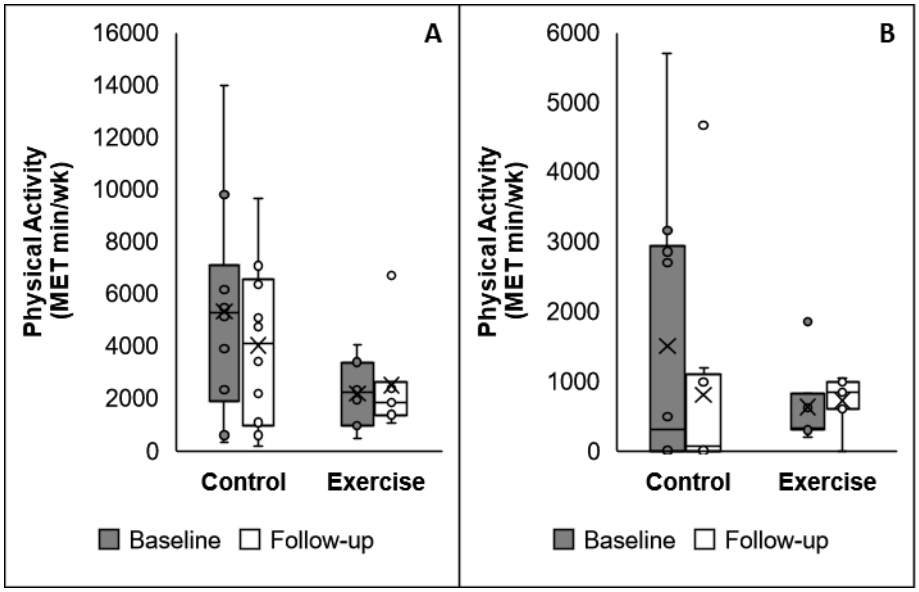


Figure 4: Physical Activity Levels in the Exercise Group Compared to the Control Group in the EXACT 2.0 Study.

Taken from Kendall *et al.*, 2022.[40] Panel A shows no significant difference in total physical activity levels between participants in the exercise and control group from baseline to follow-up. Panel B shows no significant difference in leisure-time physical activity levels between exercise and control group participants from baseline to follow-up. Data are expressed in metabolic equivalent of task multiplied by minutes per week (MET min/wk). Outliers were defined as data exceeding 1.5 interquartile ranges above the third quartile and indicated by points outside the whisker plot. No significant differences existed between the physical activity levels reported by the AEX and SOC groups.

2.4 Physical Activity, Cardiotoxicity and Metabolomic Profiling

Using alternate measures of cardiac health, such as metabolomic profiling, may provide evidence to support the use of physical activity to prevent CTX in breast cancer patients. As previously stated, metabolomic profiling is more sensitive to ultrastructural changes in cardiac tissue.[24,77] The literature supports the hypothesis that physical activity and exercise impact the metabolome.[103] Physical activity commonly alters fatty acid metabolism, the tricarboxylic acid cycle, glycolysis, amino acid metabolism, carnitine metabolism, purine metabolism, cholesterol metabolism and insulin sensitivity, as rKelly *et al.*'s[103] review reports. Furthermore, as physical activity improves mitochondrial function[30,32,83] and metabolite levels are highly variable in response to environmental disturbances,[21] the metabolome is likely to be impacted by physical activity in breast cancer patients with or at risk for developing CTX (e.g., those receiving anthracyclines). Thus, examining the impact of physical activity on the metabolomic profiles of patients receiving anthracyclines would be a valuable addition to the literature.

Some previous research has investigated the effects of physical activity on the metabolomic profiles of preclinical models with nononcologic-related cardiac damage. For instance, Shi *et al.*[104] investigated the metabolomic profiles of rats with heart failure across different physical activity intensity groups. Most metabolic changes in rat cardiac tissue following the exercise program occurred in creatine, aspartate, and glucose metabolism. Higher-intensity physical activity was also associated with more significant changes in glucose, phosphocreatine, creatine, taurine and glycerophosphocholine metabolism.[104] Thus, physical activity impacts the metabolome of heart tissue, and more intense or prolonged exercise programs may exert a more significant effect. Indeed, CTX manifests similarly to some CVDs, but more research is needed.

Furthermore, minimal preclinical research has been conducted to investigate the impact of physical activity on the metabolome of models at risk for developing CTX. De Lima *et al.*[31] investigated the effects of physical activity and metformin on skeletal muscle metabolism in the gastrocnemius of mice receiving doxorubicin treatment. The authors developed a control, exercise-only, metformin-only, and exercise-plus metformin group. Mice in the exercise plus metformin group had significantly lower levels of skeletal muscle cell PGC-1α than controls.[31] As previously stated, PGC-1α levels are associated with the activation of cell death pathways in cardiomyocytes, and thus, lower levels of PGC-1α suggest lower levels of cellular apoptosis. Additionally, mice in the exercise-only and exercise + metformin groups had higher expression of adenosine monophosphate-activated protein kinase, a regulator of cell metabolism, indicating that physical activity may ameliorate the harmful effects of anthracyclines on bioenergetics.[31] While cardiac and skeletal muscle are related tissues, future research on anthracycline-induced damage in cardiac muscle cells is necessary.

2.5 Rationale, Purpose, Hypotheses, and Research Objectives

CTX is a severe side-effect of anthracyclines that many breast cancer patients experience.[11] The development of CTX often results in the need to stop or reduce anthracycline treatment as patients can no longer tolerate their treatment, placing them at a higher risk of cancer-related mortality. CTX cases are expensive for healthcare systems and difficult to diagnose as conventional cardiac imaging such as LVEF%, as determined by 2DE, is only sensitive to changes > 10%.[19] Novel imaging techniques in cardiooncology, including GLS%, are more sensitive to structural changes; however, such imaging is not frequently conducted in clinical practice.[59] The assessment of serum

or plasma cardiac biomarkers, particularly metabolites, may aid in the early diagnosis of CTX, is non-invasive, and is highly repeatable.[60,77]

Notably, the maintenance of high physical activity levels may prevent anthracycline-induced CTX. The literature indicates that any amount of anthracyclines can be cardiotoxic; thus, preventative measures should be taken for all individuals.[13] While dexrazoxane and conventional heart medications are often administered to prevent CTX, exercise is an additional intervention with added benefits and should be implemented.[105] Preclinical research highlights the cardioprotective benefits of physical activity in preventing CTX at the cellular pathway level, as indicated by numerous measures, including metabolomic profiling.[31,86,104] Importantly, observational and clinical studies indicate that breast cancer patients with higher physical activity levels are significantly less likely to develop CTX.[105,106] However, no studies have investigated the relationship between physical activity levels and the metabolome of breast cancer patients receiving anthracyclines.

Participants completing the exercise intervention in the ongoing EXACT 2.0 clinical trial completed a similar amount of activity to those in the non-exercise group.[38–40] The controls reported high physical activity levels comparable to those in the exercise intervention group.[38–40] Thus, this study aimed to use the EXACT 2.0 baseline and 24-week follow assessment data to explore anthracycline treatment's effects on metabolism, identify if altered metabolites predict changes in echocardiographic parameters, and if physical activity levels impact this relationship.

2.5.2 Research Objectives

The primary objectives of this thesis were to determine the 1) changes in cardiac health from the baseline to 24-week follow-up assessments using echocardiography and metabolomic profiling; 2) relationships between echocardiographic outcomes (i.e., LVEF% and GLS%) and altered metabolites; and 3) impact of physical activity levels on the relationship between echocardiographic parameters (LVEF%, GLS%) and metabolites.

The secondary objective was to describe the EXACT 2.0 study participants and determine participant adherence to the EXACT 2.0 study exercise program.

2.5.1 Hypotheses

1. H_0: A. Echocardiographic parameters (i.e., LVEF% and GLS%) were the same at baseline and follow-up.

   H_0: B. Metabolite levels were the same at baseline and follow-up.

2. H_0: A. Altered metabolite levels and GLS% do not predict follow-up LVEF% while controlling for baseline LVEF%.

   H_0: B. Altered metabolite levels and LVEF% do not predict follow-up GLS% while controlling for baseline GLS%.

3. H_0: A. Baseline physical activity levels do not impact the relationships between metabolite relative intensities, LVEF%, or GLS% from baseline to follow-up.

   H_0: B. Follow-up physical activity levels do not impact the relationships between metabolite relative intensities, LVEF%, or GLS% from baseline to follow-up.

Chapter 3: Methods

An overview of the experimental design is provided in Figure 5.

3.1. Study Design

This thesis presents an interim analysis of the EXACT 2.0 study data, including data collected between April 2019 and November 2022. EXACT 2.0 is an ongoing randomized controlled trial approved by the Nova Scotia Health Research Ethics Board (ROMEO file #: 1024489) and is a registered trial at ClinicalTrials.gov (NCT03748550).

3.2 Study Sample and Participant Recruitment

EXACT 2.0 is an ongoing collaboration between researchers and clinicians in Halifax, Nova Scotia, at the Queen Elizabeth II (QEII) Health Sciences Centre, and St. Boniface Hospital, Winnipeg, Manitoba.

Eligible breast cancer patients can self-refer to the study or be referred by their healthcare provider (e.g., oncology nurse, clinician) to begin the study enrollment process. The study is advertised to patients using an in-clinic brochure (Appendix B), and to clinicians through information sessions and email communications. For clinician-referred patients, the research team contacts the individual. For self-referred patients, the patient directly contacts the study team. Eligible patients 1) are female and at least 18 years of age; 2) have a stage I to III breast cancer diagnosis; 3) have not received anthracycline therapy and are scheduled to receive a minimum cumulative dosage of 100 mg/m^2 of doxorubicin, 120 mg/m^2 of daunorubicin or 150 mg/m^2 of epirubicin; and 4) can participate in a 24-week home-based aerobic-exercise program. Patients are excluded from the study if they present with 1) significant cognitive limitations; 2) a previous history of cancer, myocardial infarction, cerebrovascular disease, peripheral

vascular disease, congestive heart failure or cardiomyopathy; or 3) known bone metastases.

An EXACT 2.0 study team member determines patient eligibility, conducts informed consent conversations, and obtains the consent of those wishing to participate (Appendix C), either in-person or through Research Electronic Data Capture (REDCap) software hosted at Nova Scotia Health.[107] "REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources."[107]

Once consented, the patient completes a cardiac exercise stress test and receives medical clearance from a cardiologist before enrolling in the study. The stress test aims to determine if the individual can safely complete a home-based aerobic-exercise program and identify their peak heart rate. A stress technician delivers, and a cardiologist supervises the stress test. The test is terminated when the patient reaches volitional fatigue or experiences the onset of adverse physiological changes.[108,109] Patients are connected to a 12-lead electrocardiograph (General Electric Case System, Boston, Massachusetts) to determine cardiac electrical activity. Patients who did not present with abnormalities were enrolled in the study, and their peak heart rate was used to prescribe individualized exercise. A patient presenting with an abnormality is withdrawn from the study, given a copy of their stress test results, and advised to consult a healthcare provider. Of note, due to equipment limitations in Winnipeg, not all participants

underwent a stress test, with some completing an indoor walk under a cardiologist's supervision.

3.3 Exercise Intervention

For EXACT 2.0, all consented breast cancer patients who safely complete the exercise stress test are randomized to either the AEX or SOC groups. A random generator was used to assign groups (http://methodologymedia.psu.edu/most/rannumgenerator).

3.3.2 Exercise Group

Participants in the AEX group receive a 24-week home-based aerobic-exercise program and their standard care. Each participant performs 2 aerobic-exercise sessions (e.g., walking, cycling, swimming, etc.) on non-consecutive days each week.

Exercise session intensity and duration vary throughout the program. Sessions range from low, moderate, moderate-high, and high intensities corresponding to 35-45%, 46-55%, 56-70%, and 71-85% of the participant's maximal heart rate reserve, respectively. Peak heart rate reserve is determined using the participant's resting heart rate and the peak heart rate they achieve during the exercise stress test per the following formula: heart rate reserve = (resting heart rate – maximal heart rate) / maximal heart rate. For individuals completing the indoor walk, the formula 220-age was used to predict their maximal heart rate. The exercise sessions range from 20 to 45 minutes, with lower-intensity sessions lasting longer than more vigorous-intensity sessions. Throughout the 24-week exercise program, the sessions gradually increase in load with some rest days incorporated. Generally, longer sessions occur at a lower intensity, and shorter sessions occur at a higher intensity (Figure 5). Exercise sessions begin with a 5-minute warm-up and conclude with a 10-minute cooldown.

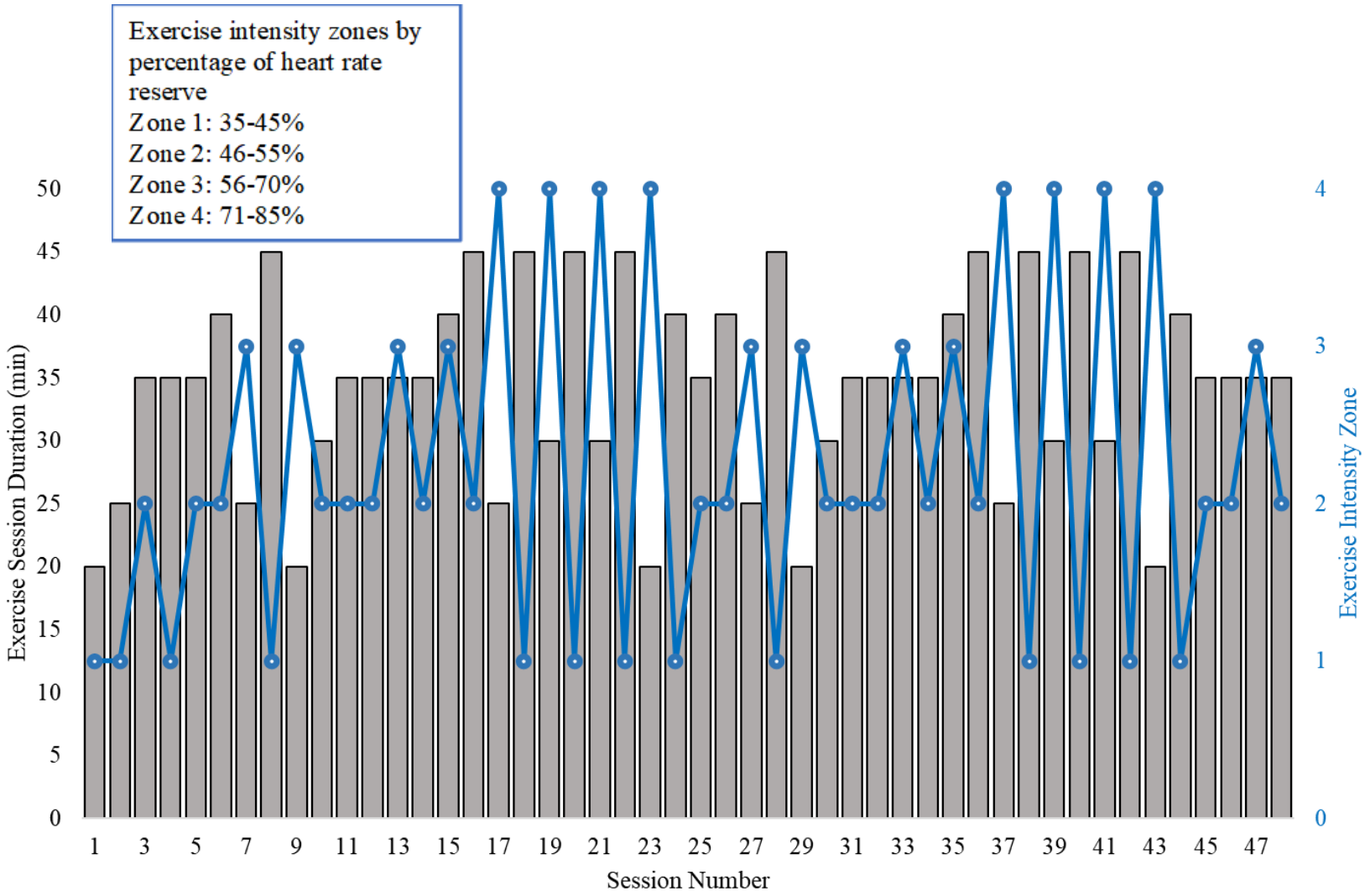


Figure 5: Exercise Intervention Intensity Zones by Heart Rate Reserve

Exercise session durations (min) are depicted by the bars in grey, and exercise intensity zones are depicted by the lines in blue.

Participant program attendance is monitored throughout the program. Participants monitor their adherence to the prescribed durations and intensities using a wrist-worn Polar A370 Activity/ Heart Rate monitor (Polar Electro, Kempele, Finland). Study staff contact participants weekly and monitor participant activity data remotely using a secure web server (www.flow.polar.com). Participants accumulating > 4 incomplete consecutive exercise sessions are excluded from the analysis. The study team is available to the AEX group participants for watch technical support.

3.3.3 Control Group

Participants in the SOC group do not receive an exercise program to follow. SOC group participants are advised to maintain their current level of physical activity and use the Polar A370 monitor to track their activity levels during activity bouts ≥ 10 min. The study team is available to the SOC group participants for watch technical support.

3.4 Outcome Measures

For the current thesis, measures were collected at baseline and follow-up.

3.4.1 Participant Demographics

Nova Scotia Cancer Care's personal health information database, OneContent, and Manitoba's CancerCare medical record database, ARIA, were used to extract information to describe the participant population. From these databases, age, height, weight, cancer diagnosis and treatment details were recorded. Participants completed a health habits questionnaire to assess their socioeconomic status, lifestyle, and other demographic information (Appendix D). Participants were asked to disclose CVD risk factors (presence of hypertension, hyperlipidemia, family history of coronary artery disease, and diabetes).

3.4.2 Physical Activity Behaviour

The International Physical Activity Questionnaire (IPAQ)[110] was administered to assess the physical activity behaviour of all participants (Appendix E). The IPAQ was employed to determine total physical activity behaviour (i.e., leisure time, domestic, work, and transportation).[110] Based on reported values, participant metabolic equivalents of task multiplied by minutes per week (MET min/wk) were calculated. MET min/wk estimates how much energy an individual expends beyond their basal metabolic rate.

3.4.3 Echocardiographic Parameters

A Vivid 7 platform (General Electric) was used to collect the echocardiographic data. All cardiac scans were conducted at the QEII or the St. Boniface Hospital. A cardiac sonographer conducted the echocardiograms using a probe placed on the participant's chest and followed an imaging protocol (Appendix F). A cardiologist blinded to the

participant's group assignment in Winnipeg analyzed and interpreted the results. The presented echocardiographic figures are samples from various participants.

The sonographer used a standard multi-frequency transducer to collect 2DE serial transthoracic echocardiographic images from the parasternal and apical views of each participant's heart. Left ventricular internal diameter at the end of diastole (LVIDed) and the end of systole (LVIDes) were calculated from these images (Figure 6) following the directions outlined by the American Society of Echocardiography and European Association of Cardiovascular Imaging joint consensus.[56] LVIDed and LVIDes were used to calculate LVEF from the equation LVEF% = (End Diastolic Volume - End Systolic Volume) / End Diastolic Volume *100 following the modified biplane Simpson's method.[111]

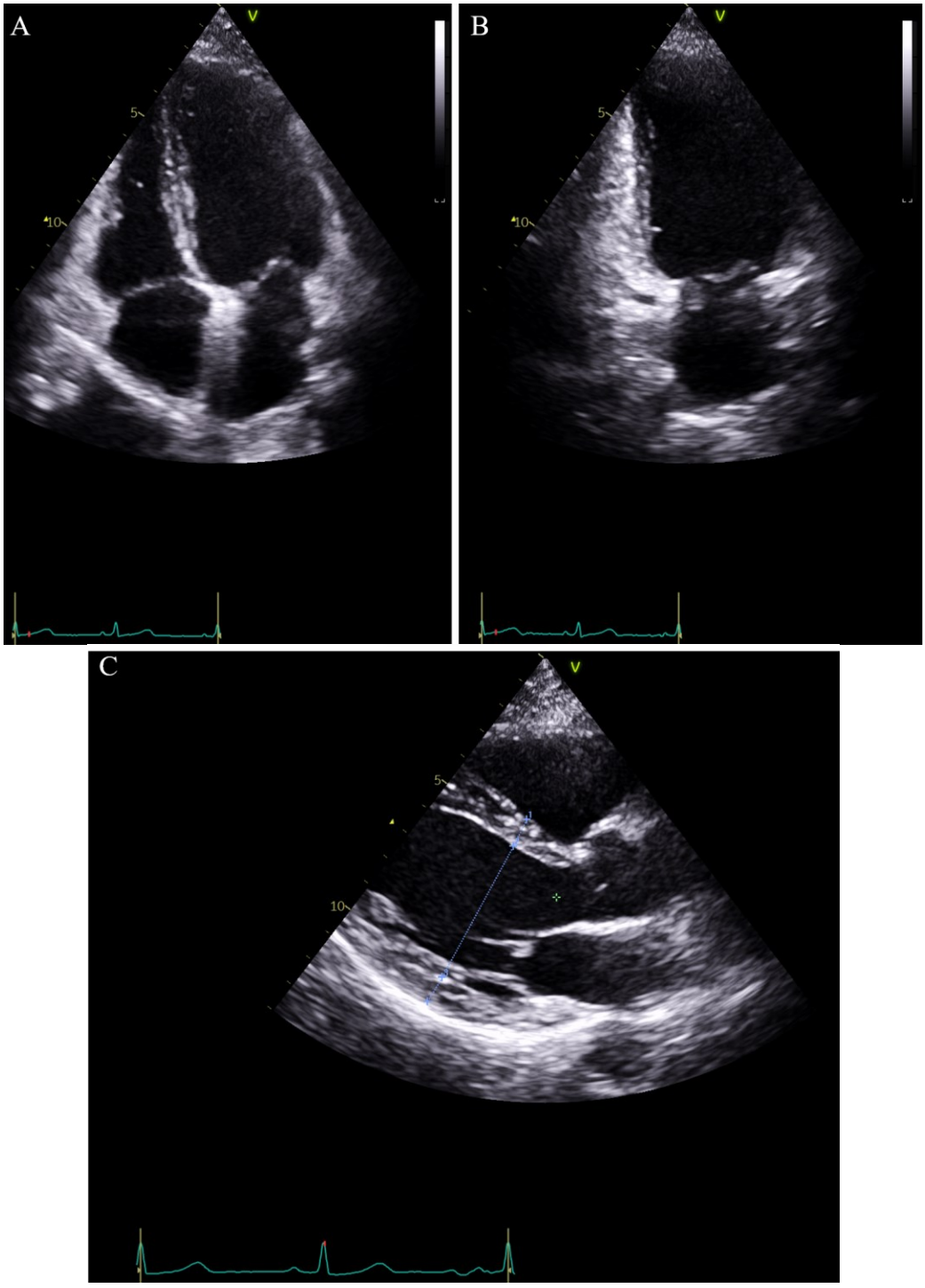


Figure 6: Example of Serial Transthoracic 2-Dimensional Echocardiographic Images

Echocardiographic images were collected in the Apical 4-chamber (A), Apical 2-chamber (B), and Parasternal Long Axis (C) views for each participant at the baseline and follow-up assessments. These images were used to calculate LVEF% following the modified biplane Simpson's method.[111]

Doppler-independent strain imaging was also completed as it can detect changes in left ventricular function earlier than LVEF%.[112,113] Doppler-independent strain imaging was conducted to measure myocardial strain in the apical 4-chamber, apical 2-chamber, and parasternal long-axis views (Figure 7). After the echocardiogram, strain was determined using semiautomated speckle tracking techniques on General Electric Healthcare's EchoPAC software (Version 20).[113] A composite bullseye plot was generated by combining data from the 3 views to produce an overall GLS% score (Figure 8). Values < -19% (i.e., more negative) indicated healthy left ventricular function.

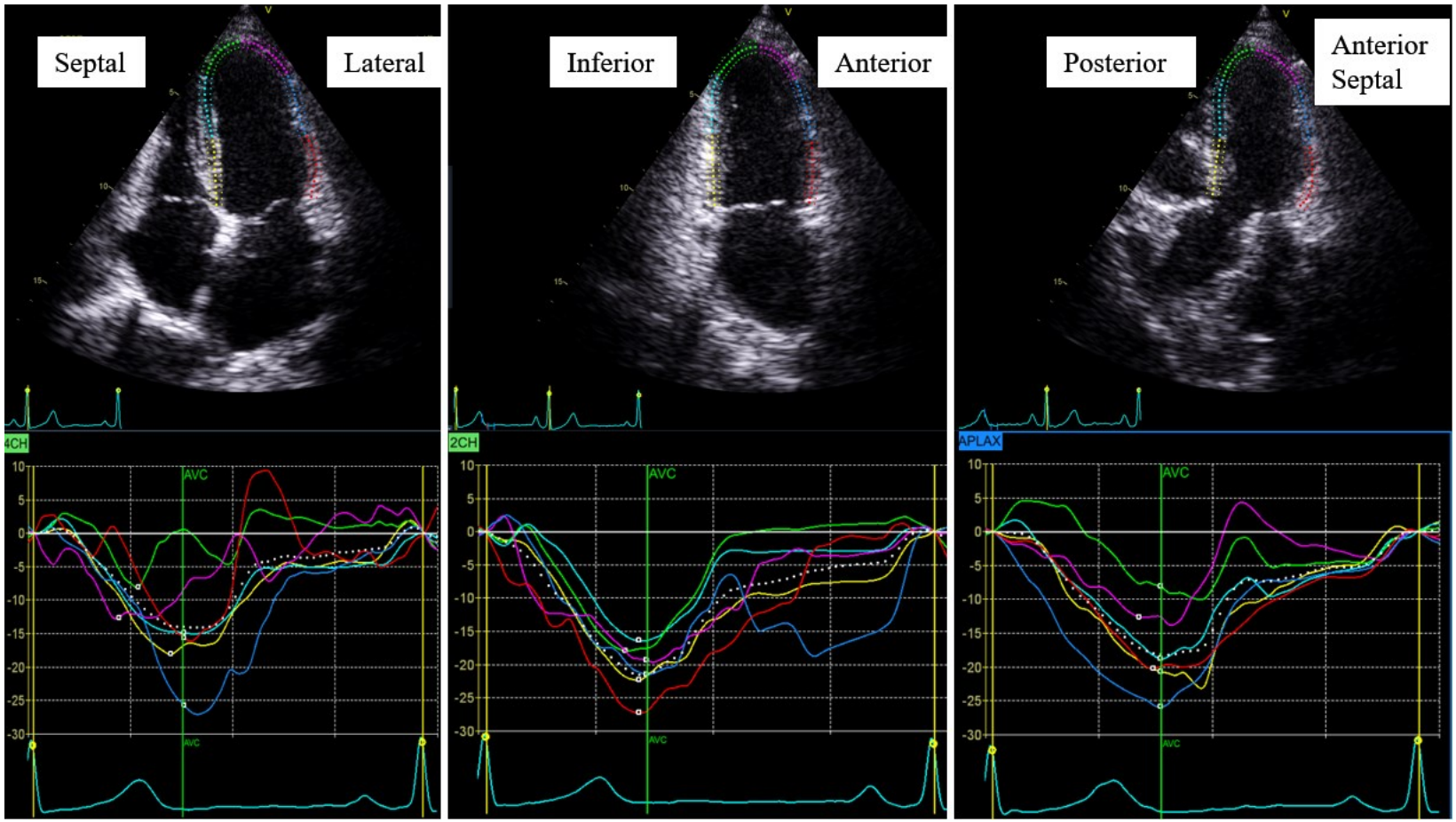


Figure 7: Example of Tissue Doppler Imaging and the Resulting Graphs from Analyses of the Myocardium

Echocardiographic images were collected in the Apical 4-chamber (A), Apical 2-chamber (B), and Parasternal Long Axis (C) views for each participant at the baseline and follow-up assessments. These images were used to calculate global longitudinal strain.

The coloured lines represent the following segments of the myocardium: basal inferior (yellow), mid-inferior (teal), apical inferior (green), apical anterior (purple), mid-anterior (blue), and basal anterior (red).
Abbreviations: APLAX, apical parasternal long axis; 2CH, 2-chamber; 4CH, 4-chamber

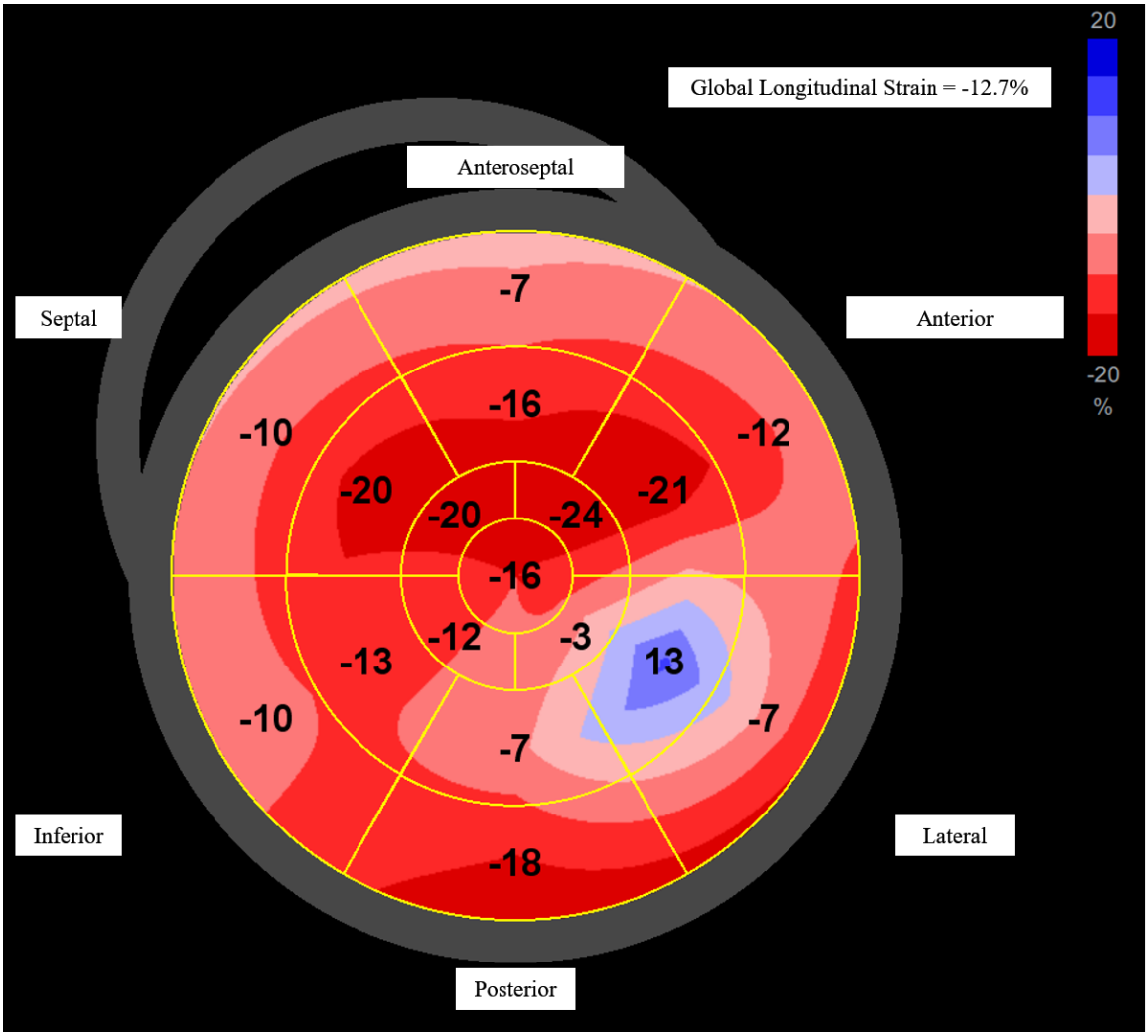


Figure 8: Example of Strain Values

Strain values were calculated from the Apical 4-chamber, Apical 3-chamber, and Parasternal long-axis views for each participant at the baseline and follow-up assessments. The resulting data are represented in a bullseye plot (above), providing strain rates for different heart segments and the overall global longitudinal strain.

Cooler colours (e.g., blue), and values closer to or > 0 indicate poor contractility.

3.4.4 Metabolomic Profiling

A nurse collected 2 tubes of 10 mL of blood in lithium heparin vacutainers from each participant. Blood samples were processed, and plasma was extracted. Plasma was analyzed because all blood was collected in an ethylenediaminetetraacetic acid or heparin tube per the EXACT 2.0 protocol. All samples were stored at -20 °C until preparation for metabolomic analysis.

Sample preparation for the targeted metabolomic profiling occurred in Dr. Grandy's laboratory (Dalhousie University, Halifax, NS) using the sample preparation kit provided by the Biological Mass Spectrometry (BMS) CORE facility. Samples were thawed over ice, and a pooled sample was established by transferring 10 µL of each sample into one tube. From the remaining plasma, 20 µL of each sample were transferred to low-protein-binding tubes (Thermo Scientific, Waltham, MA, USA), combined with 85 µL of Methanol-Isotopically Labelled Internal Standard (MeOH – ILIS). All samples were vortexed, chilled, and spun. The supernatant was transferred to a new tube (Set B), and the precipitated proteins (Set A) were set aside. The supernatant was frozen at -20 C° until liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (Figure 9).

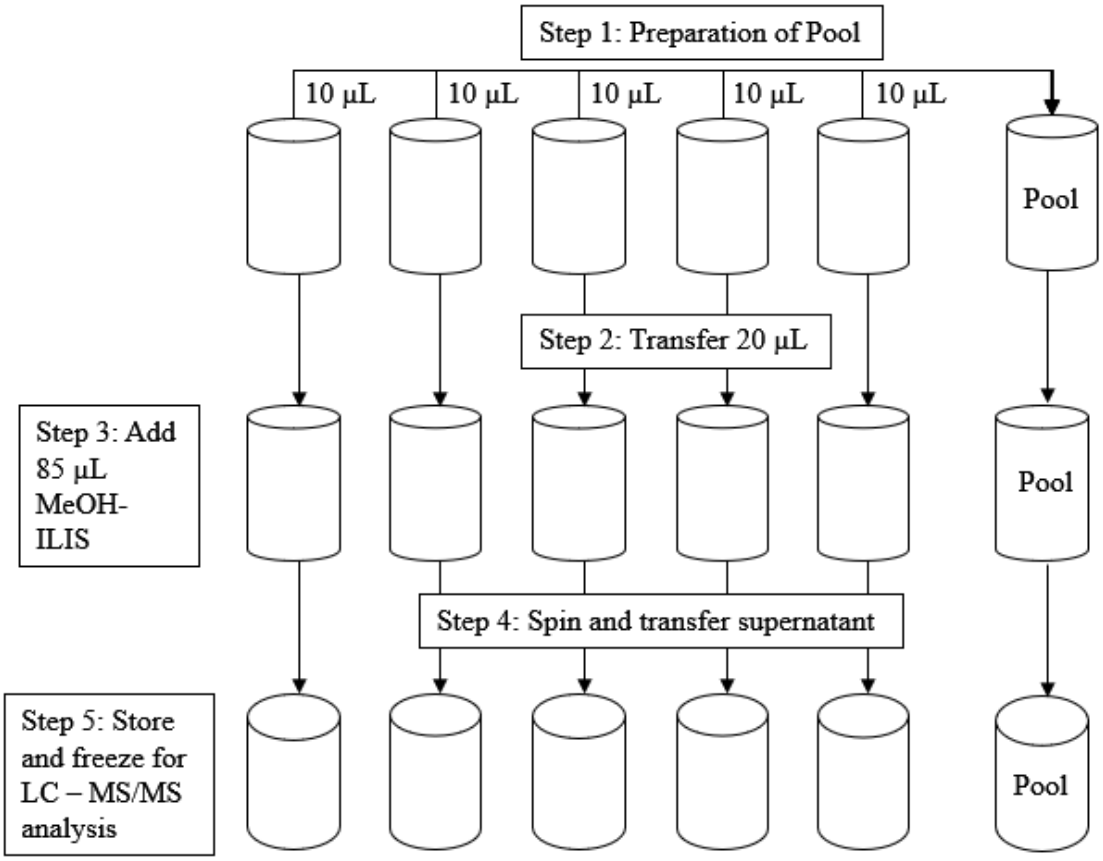


Figure 9: Sample Preparation Overview for Targeted Metabolomic Profiling

Preparation procedures for plasma adapted from the Biological Mass Spectrometry CORE facility (Dalhousie University).

Abbreviations: MeOH-ILIS, methanol-isotopically labelled internal standard; LC-MS/MS, liquid chromatography-tandem mass spectrometry

LC-MS/MS was conducted at the Biological Mass Spectrometry CORE Facility (Dalhousie University) per the CORE facility's standard procedures. The Agilent 1290 Infinity II liquid chromatograph was coupled in-line with the QTRAP 5500 (TurboIonSpray, Sciex, Framingham, MA, USA) linear ion trap quadrupole mass spectrometer. LC-MS/MS was repeated twice, once using reverse phase, then again using hydrophilic interaction liquid chromatography (HILIC) separation. Before separation, 10 µL of each sample were diluted with 180 µL of 5% acetonitrile in water + 0.1% formic acid, or 95% acetonitrile + 5% acetonitrile + 1 mmol ammonium acetate for reverse phase and HILIC separation, respectively. In both separation methods, 10 µL of each diluted sample was injected into a column heated to 40 °C then eluted across a gradient for 10 min with a maximum flow rate of 400 µL/min and a pressure of 600 bar. Five standards for the reverse phase and 4 standards for HILIC as well as 2 blanks and 1 pool for both separation methods, were also injected. Details of each separation are provided in Table 3.

Table 3: Reverse Phase Liquid Chromatography and Hydrophilic Interaction Chromatography Separation Parameters

	Reverse Phase	Hydrophilic Interaction Liquid Chromatography
Column	Waters Cortecs® T3 (2.7 µm, 2.1 x 50 mm)*	XBridge® Amide (3.5 µm, 1.0 × 50 mm)*
Solvent A	0.1 % formic acid in water	5% acetonitrile + 1 mm ammonium acetate
Solvent B	0.1% formic acid in acetonitrile	1% acetonitrile in water
Gradient Start	99% solvent A; 1% solvent B	1% solvent A; 99% solvent B

*Waters Cortecs, Mississauga, Ontario, Canada

After reverse phase and HILIC separation, the resulting analyte from each separation was ionized and injected into the mass spectrometer. Ionization was achieved

using a heat-assisted electrospray source (Sciex) set at 450 °C. Each sample was injected twice in positive mode at an ionization spray voltage of 5.5 kV, and negative mode at an ionization spray voltage of -4.5 kV. In total, the positive and negative injections contained 500 and 653 multiple reaction monitoring (MRM) target transitions in HILIC and reverse phase, respectively, acquired using Analyst (Version 1.6.3, Sciex) (Figure 10).

The BMS CORE facility processed the data using their in-house R script (Version 4.2.2). The National Research Council Canada extracted the peak heights from the MRM data using Skyline[114] (Version 22.2, MacCoss Lab, University of Washington, Seattle, Washington, USA) to screen the samples for 647 metabolites. In brief, metabolite peaks with varying retention times (> 40% coefficient of variation) and low peaks (mean pool intensity < 10 000) were excluded. Peak intensities of each metabolite were also normalized to reduce signal intensity drift, and intensities were normalized to the average of 2 quality control samples.

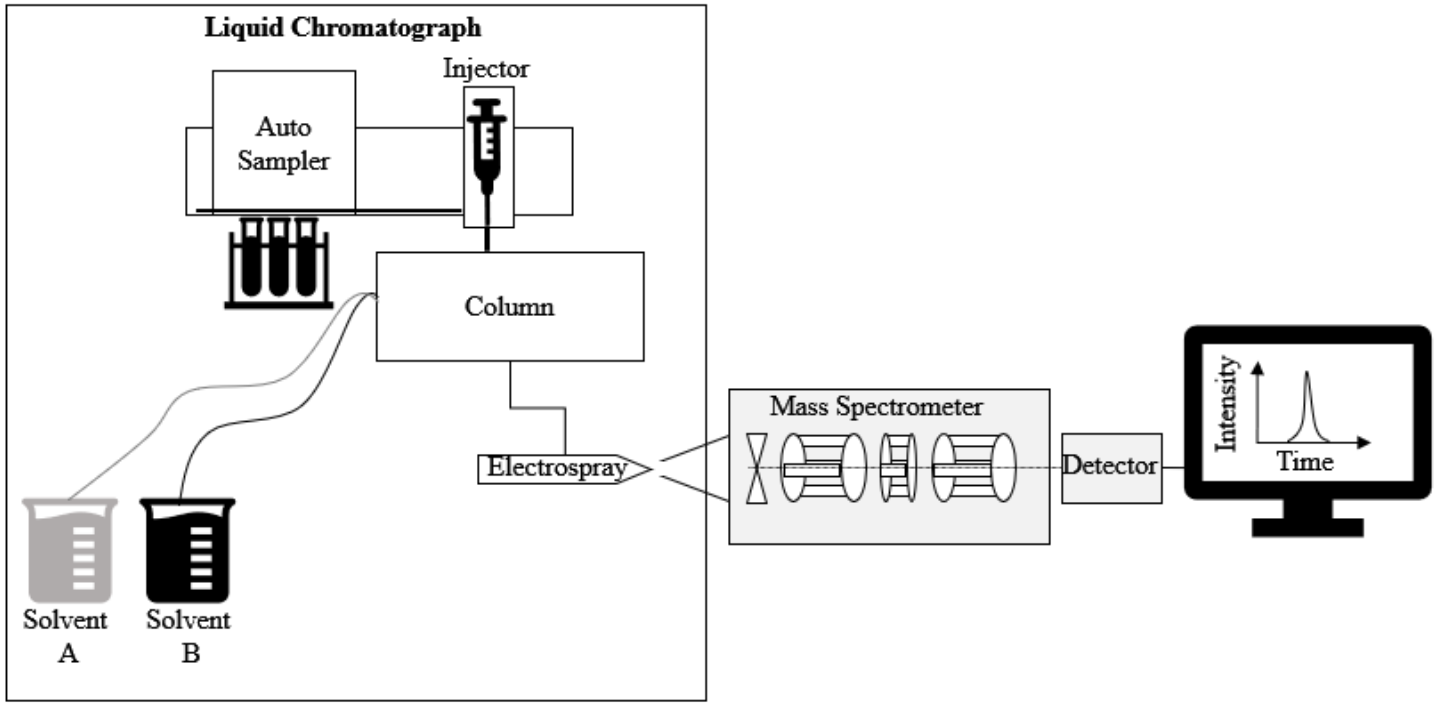


Figure 10: Overview of Liquid Chromatography Tandem Mass Spectrometry Process

3.5 Data Analysis

To describe the EXACT 2.0 participants, de-identified participant characteristics were entered into Microsoft Excel (v.2302, Microsoft Office, Redmond, Washington, USA) and loaded into SPSS (v.28.0.1.1, IBM, Armonk, New York, USA). The frequency and percentage of occurrence were determined for categorical variables; for continuous variables, the mean and SD were determined. The variables included age, body mass index (BMI), ethnicity, alcohol consumption and smoking status, presence of comorbidities (diabetes, hypertension, hyperlipidemia, family history of coronary artery disease), cancer location, hormone receptor status, treatments received (type of surgery, radiation therapy status, cumulative anthracycline dosage, trastuzumab status), LVEF%, GLS% and MET min/wk. The normalcy of LVEF%, GLS% and MET min/wk were assessed. The data were visualized using P-P plots, and a z-score of skewness and kurtosis was obtained. Non-normally distributed data were logarithmically transformed.

Exercise program adherence rates were determined by calculating the average number of exercise sessions completed following the prescribed protocol.

Differential baseline- and follow-up analyses were conducted to compare changes in LVEF% and GLS% from baseline to follow-up. A paired *t*-test was conducted using SPSS to compare the baseline and follow-up LVEF% and GLS% scores. The effect size was also determined using Cohen *d*. Linear regression models were developed using LVEF% and GLS% to assess if any significant covariates should be included in the models. The baseline measure was inputted as the predictor, and the follow-up measure was inputted as the outcome, with age and cumulative anthracycline dosage as the covariates.

Differential baseline- and follow-up analyses were also conducted to compare changes in metabolite levels. MetaboAnalyst (v5.0) was used to filter, normalize and analyze the metabolomics data.[115] The data were uploaded to MetaboAnalyst, normalized to the sum, and auto-scaled (mean centred divided by the standard deviation of each metabolite). An unsupervised principal component analysis (PCA) was conducted to identify overall metabolome differences. Orthogonal partial least squares discriminant analysis (OPLSDA) was conducted to determine overall metabolome differences from baseline to follow-up. The top 10 metabolites contributing to the within-group differences determined by the OPLSDA variable importance scores were used to inform variable selection. Figures for the PCA and OPLSDA models were developed using MetaboAnalyst. For differential analysis from baseline to follow-up, a paired *t*-test or Wilcoxon rank-sum test was conducted using the limma[116] R package in MetaboAnalyst, and significant metabolites were also used to inform variable selection.

Multiple linear regression analysis was conducted using SPSS to determine if metabolome changes predicted heart health. To create the regression model, the relative intensities of the selected metabolites and GLS% were inputted as the predictor variables, and follow-up LVEF% was the dependent variable. Baseline LVEF% was controlled, and the process was repeated using follow-up GLS% as the dependent variable. Multiple comparisons were corrected using Bonferroni's correction.

Finally, to determine if physical activity levels moderated the relationship between heart health and altered metabolites, a moderation analysis was conducted using the PROCESS (v.4.2) SPSS macro.[117] This moderation analysis was completed separately for all variables, with the baseline factor as the predictor and the follow-up

factor as the outcome. Separate moderation models were developed using baseline and follow-up physical activity data as the moderator.

Chapter 4: Results

4.1 EXACT 2.0 Patient Disposition

The disposition of patients involved in the EXACT 2.0 study as of November 2022 is shown in Figure 11.

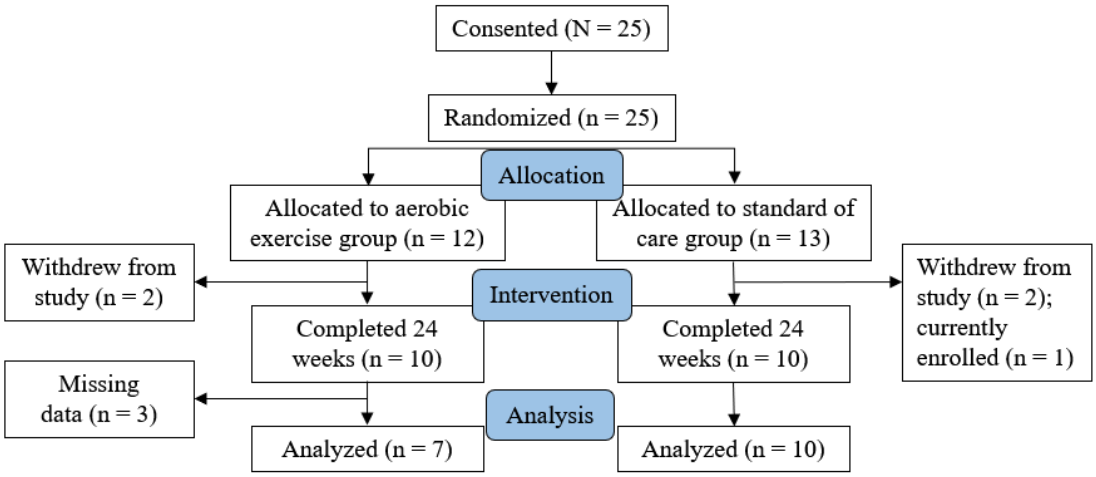


Figure 11: Patient Disposition: Enrollment, Randomization, Intervention and Analysis for the Current Thesis

4.2 Participant Characteristics

Table 4 describes participant characteristics and demographics. Most participants were middle-aged Caucasians. The majority of participants did not present with a comorbidity, but the most frequently reported was a family history of coronary artery disease. Few participants were current smokers, and around half consumed alcohol. On average, recruited participants were classified as overweight per their BMI. [118]

Participants presented with various types of, and underwent different treatments for their breast cancer. Many participants had stage III cancer (n = 8) that was estrogen receptor-positive (ER +; n = 14) and progesterone receptor-positive (PR +; n = 12), while few presented with human epidermal growth factor two-positive (HER2 +; n = 5) cancer. In addition to anthracycline treatment, most participants received surgery and around half received radiation. All participants received epirubicin.

Table 4: Characteristics of Participants

Characteristic	N = 17
Age (years); mean ± SD	51.3 ± 10.1
BMI (kg/m^2); mean ± SD	Baseline: 29.0 ± 6.1 Follow-up: 28.4 ± 6.0
Ethnicity; n (%)	Caucasian: 13 Filipino: 1 Indigenous Person of Canada: 1 African: 1 Other: 1
Alcohol Consumption Status; n (%)	Yes: 8 (47) No: 9 (53)
Smoking Status; n (%)	Yes: 4 (24) No: 13 (76)
Diabetes Status; n (%)	Yes: 0 (0) No: 17 (100)
Hypertension Status; n (%)	Yes: 3 (18) No: 14 (82)
Hyperlipidaemia Status; n (%)	Yes: 2 (12) No: 15 (88)
Family History of Coronary Artery Disease; n (%)	Yes: 9 (53) No: 8 (47)
Cancer Location; n (%)	Left: 10 (59) Right: 7 (41)
Hormone Receptor Status; n (%)	ER +: 14 (82) PR +: 12 (71) HER2 +: 5 (29)
Surgery Received; n (%)	Mastectomy: 11 (65) Lumpectomy: 6 (35)
Cancer Stage; n (%)	I: 1 (6) II: 5 (29) III: 8 (47) Missing: 3 (18)
Trastuzumab Status; n (%)	Yes: 3 (18) No: 12 (71) Missing: 2 (12)
Radiation Status; n (%)	Yes: 10 (59) No: 7 (41)
Anthracycline Dosage (mg/m^2); mean ± SD (n = 13)	377.1 ± 167.7
Total Physical Activity (MET min/wk); ± SD	Baseline: 4045 ± 3573 Follow-up: 3405 ± 2731
Leisure Physical Activity (MET min/wk); ± SD	Baseline: 1140 ± 1598 Follow-up: 768 ± 1108

Abbreviations: BMI, body mass index; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MET min/wk, metabolic equivalents of task per week; PR, progesterone receptor.

4.3 Exercise Intervention Adherence

Overall, participants in the AEX group completed a mean of 44 of the 48 exercise sessions, equating to an attendance rate of 90.53% ± 12. Self-prescribed exercise duration for the SOC group, and heart rate for the SOC and AEX groups were impossible to determine due to low-quality data from the watch. Thus, overall program adherence could not be assessed.

4.4 Changes in Cardiac Health

A paired *t*-test revealed that participants tended to exhibit reduced cardiac function at the 24-week follow-up assessment. On average, there was an insignificant trend that LVEF% ($\mu_{baseline} = 62.3 \pm 1.5$; $\mu_{follow\text{-}up} = 59.7 \pm 5.8$) moderately decreased; $t_{16} = 1.90$, $P = .08$, $d = 0.48$ (Figure 12, Panel A). Additionally, the mean GLS% ($\mu_{baseline} = -18.9 \pm 1.3$; $\mu_{follow\text{-}up} = -17.8 \pm 1.80$) moderately increased (i.e., worsened), but the changes were also not significant; $t_{16} = 1.97$, $P = .07$, $d = 0.48$ (Figure 12, Panel B). When the models were re-created with age and anthracycline dosage as covariates, no covariates were significant and, thus, were not included in any following models.

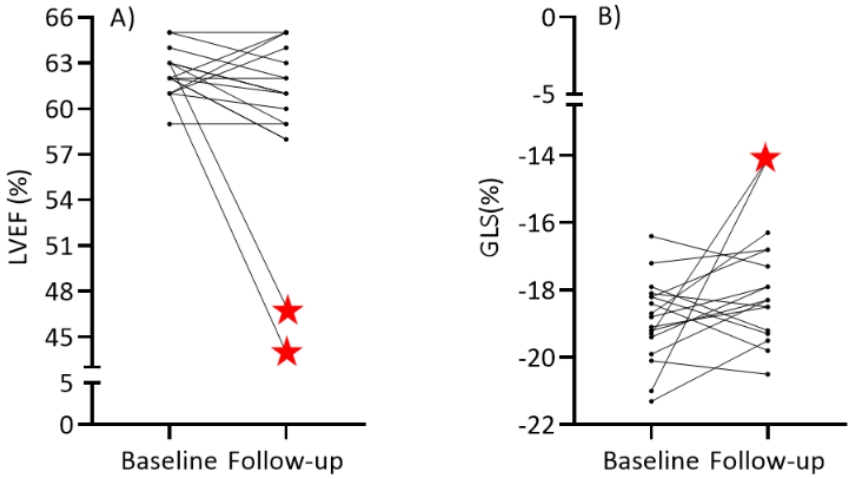


Figure 12: Change in Echocardiographic Parameters

A) Left ventricular ejection fraction (LVEF) and B) global longitudinal strain (GLS) percentages from the baseline to 24-week follow-up assessment. Neither LVEF% nor GLS% significantly changed. Red stars indicate the development of cardiotoxicity (LVEF% changed ≥ 10%).

Regarding the metabolome, 140 metabolites were identified in the HILIC LC-MS/MS samples. The reverse phase separation data could not be analyzed as there was unusual variation in the quality control sample (Appendix G). After conducting the PCA analysis, there was no apparent separation from the baseline- to follow-up assessments (Figure 13).

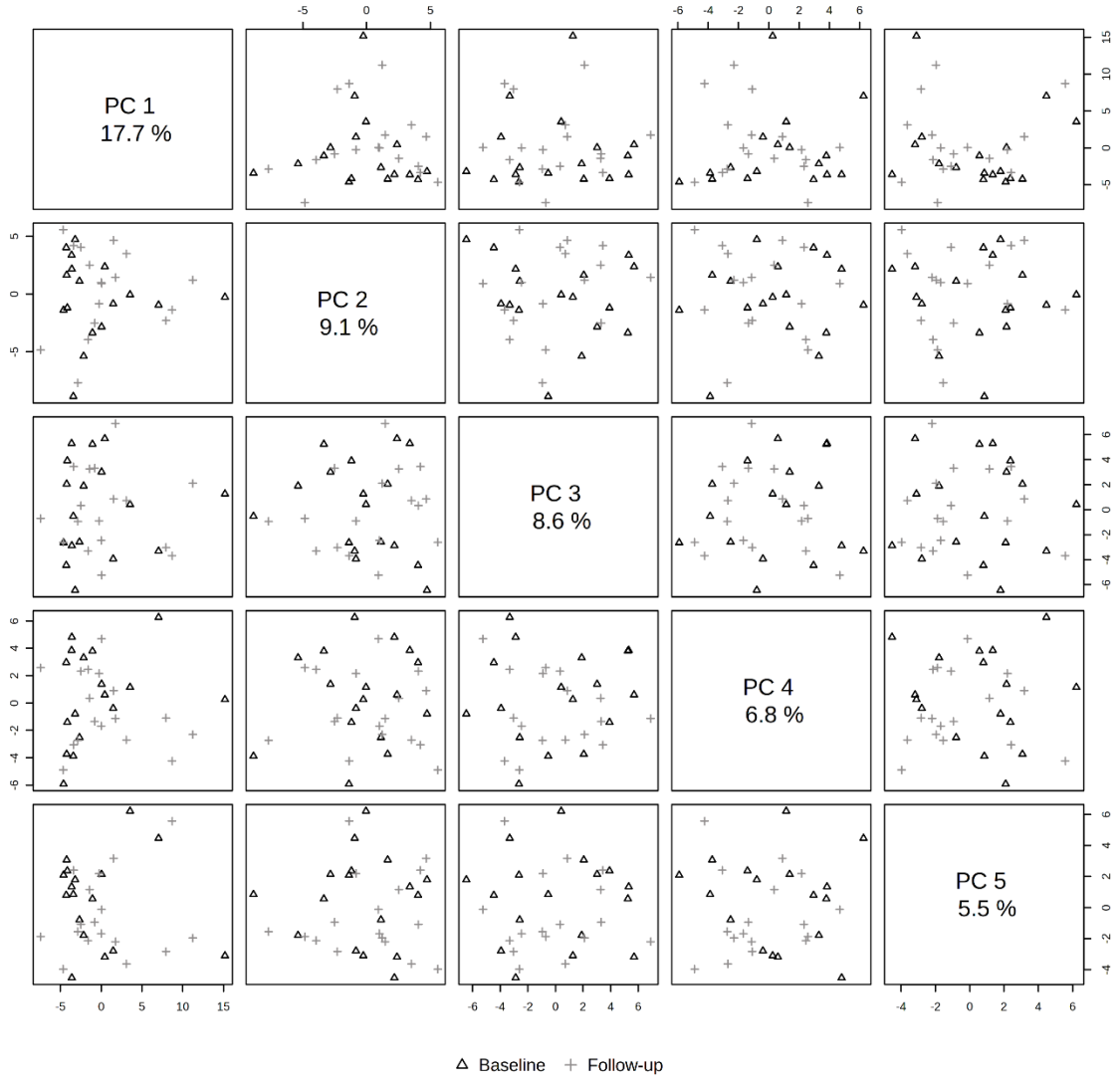


Figure 13: Unsupervised Analysis of All Detected Metabolites

The Top 5 principal components (PCs) accounting for variance in the metabolomics data are presented. Scores are presented for all metabolites detected in the targeted metabolomics analysis. The top 5 PCs do not suggest any clear separations between timepoints. The triangles represent participants at baseline, and the plus signs represent participants at follow-up.

Results from the OPLSDA analysis suggested a separation from the baseline to 24-week follow-up assessments (Figure 14).

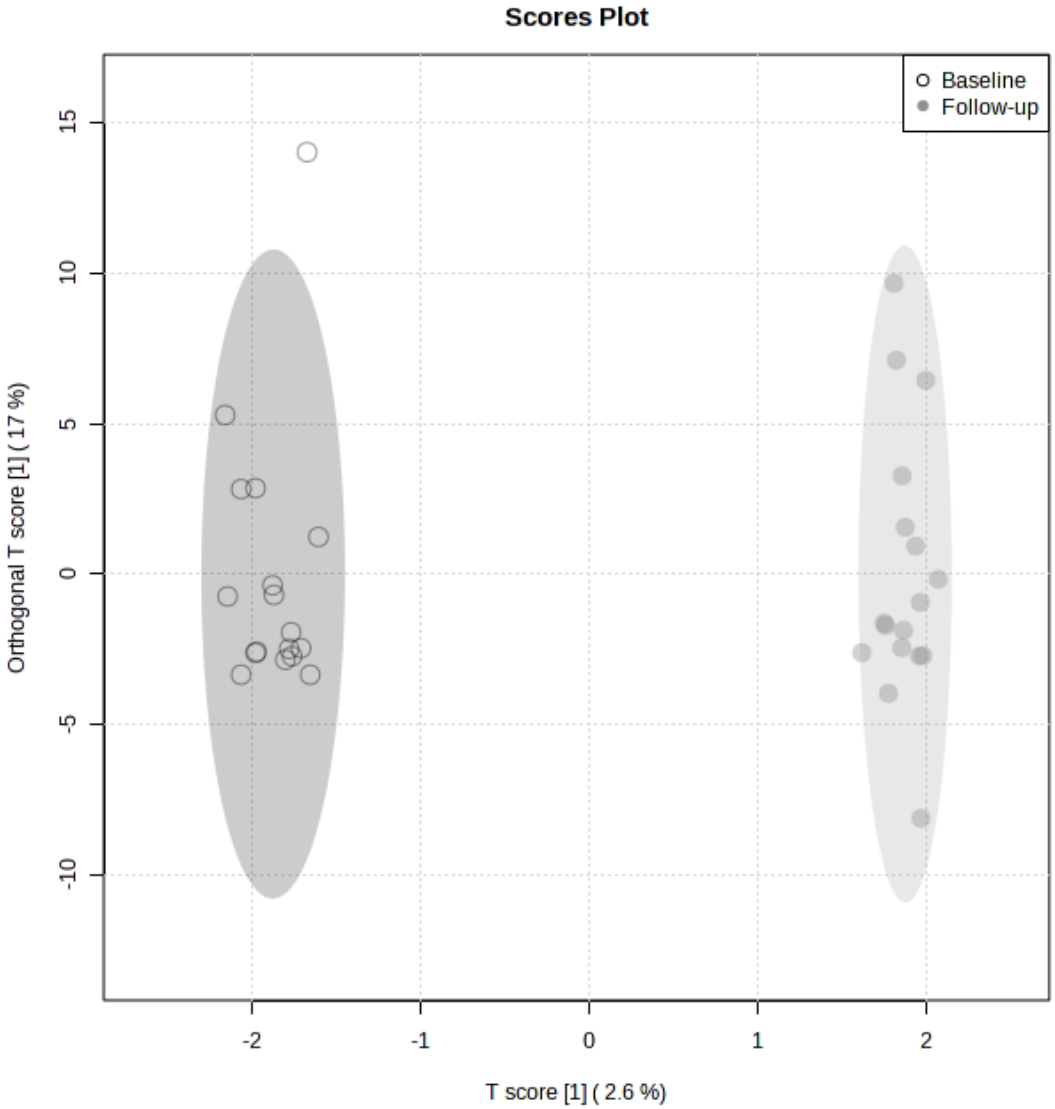


Figure 14: Between Timepoint Analysis of All Detected Metabolites at the Baseline and 24-week Follow-up Assessments

Orthogonal Partial Least Squares Discriminant Analysis model demonstrates participants' metabolome at the baseline and follow-up assessment. A higher T score indicates more substantial discriminatory power between the baseline and follow-up data, and the orthogonal T score indicates variability unrelated to timepoint differences. The open circles represent participants at baseline, and the closed circles represent participants at follow-up.

The top 10 metabolites that significantly contributed to the differences from baseline to the follow-up assessment in the OPLSDA model were SM(d18:1/22:1(13Z)), L-Carnitine, Glucosylceramide (d18:1/24:0), Linoleoyl carnitine, Urea, SM(d18:0/16:1(9Z)(OH)), PC(16:0/16:0), Oleoylcarnitine, Indoleacetic acid, and 1-Pyrroline-5-carboxylic acid (Figure 15).

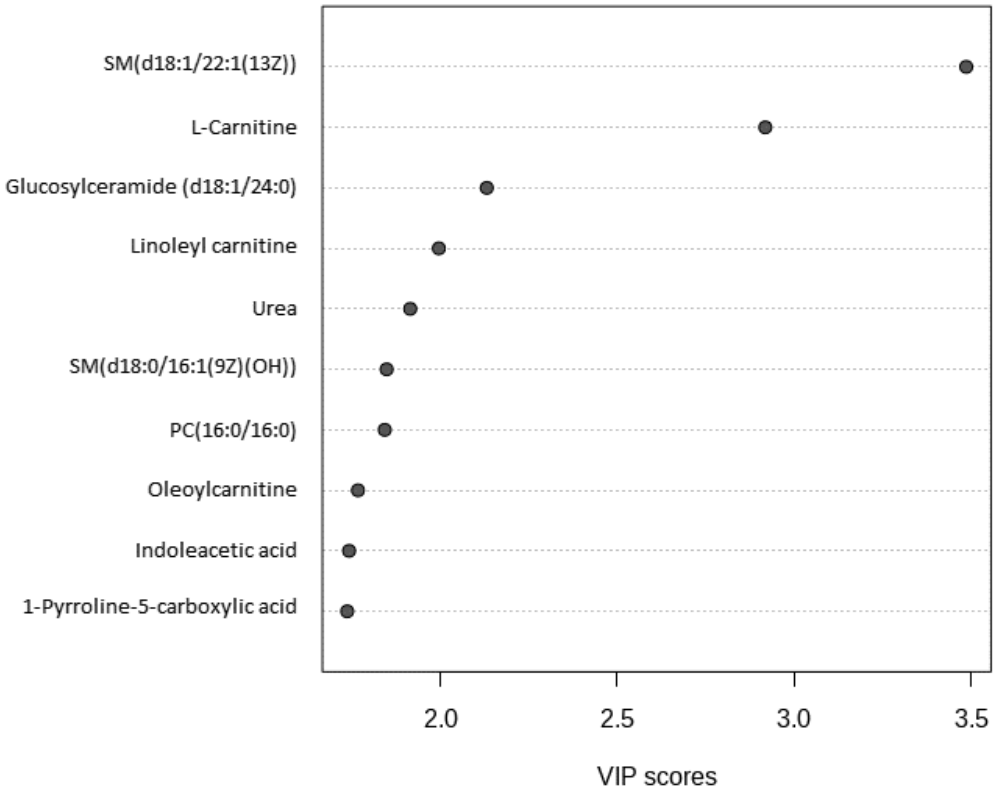


Figure 15: Top 10 Metabolites Altered at the 24-week Follow-up Assessment Determined by Participant

Top metabolites were identified as having a top 10 variable importance projection (VIP) score. VIP scores were determined using orthogonal partial least squares. Higher scores indicate metabolites contributing to more between timepoint variance.

Results from the *t*-test, or Wilcoxon sign rank sum test, indicated that the levels of SM(d18:1/22:1(13Z)), PC(18:0/18:1(11Z)), PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)), and Glucosylceramide (d18:1/24:0) were significantly elevated, and the levels of L-Carnitine and Urea were significantly reduced, on average (Figure 16).

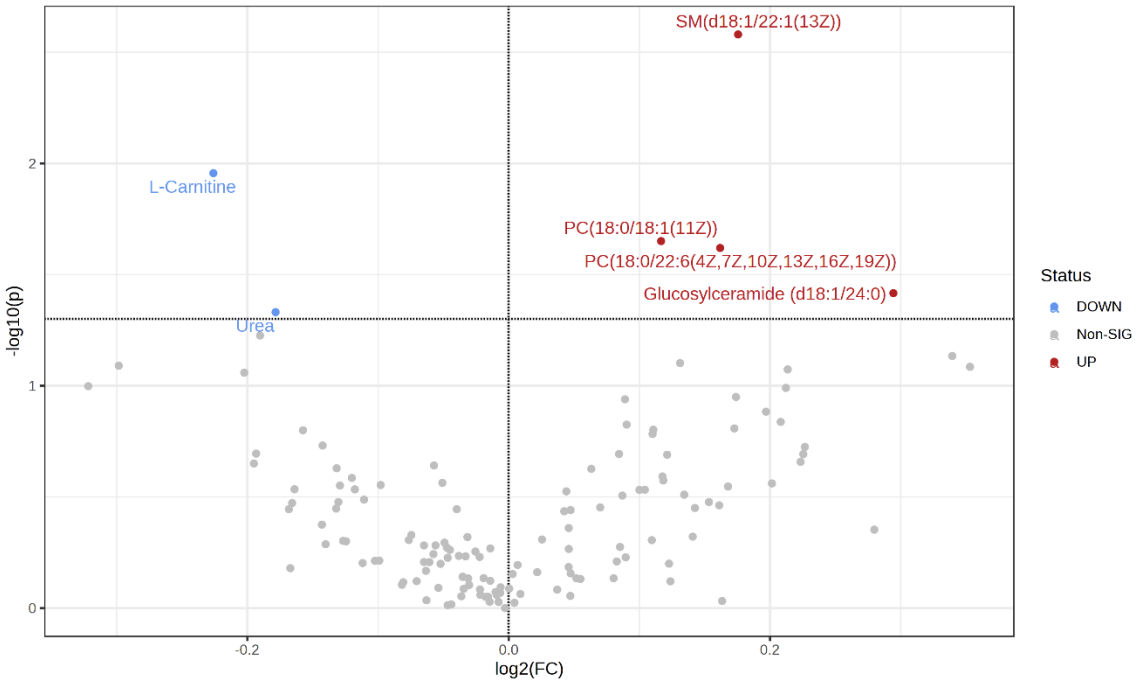


Figure 16: Significantly Altered Metabolites at the 24-Week Follow-up Assessments

Log 2-Fold Change (FC) indicates the magnitude and direction of change, and the -Log_{10} *P* value (p) indicates which metabolites significantly changed (-Log10(p) ≥ 1.30). *P*-values were determined using a paired *t*-test or Wilcoxon sign rank sum test.

12 altered metabolites were identified from the OPLSDA model and *t*-test or Wilcoxon sign rank test. The metabolites identified were 1-Pyrroline-5-carboxylic acid, Glucosylceramide (d18:1/24:0), Indoleacetic acid, L-Carnitine, Linoleoyl carnitine, Oleoylcarnitine, phosphatidylcholine (PC) (16:0/16:0), PC(18:0/18:1(11Z)), PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)), sphingomyelin (SM) (d18:0/16:1(9Z)(OH)), SM(d18:1/22:1(13Z)), and Urea (Table 5). The molecules were classified as glycerophospholipids (n = 3), SM (n = 3), fatty acyls (n = 2), carboxylic acids (n = 1), indoles (n = 1), organic carbonic acids (n = 1), and organonitrogen compounds (n = 1). These metabolites were used in the regression and moderation modelling.

Table 5: Differential Analysis of the Top Altered Metabolite Intensities

Classification	**Metabolite**	***t***	***P***	***d***
Glycerophospholipids	PC(16:0/16:0)	1.73	0.1	-0.42
	PC(18:0/18:1(11Z))	2.53	.02*	-0.61
	PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2.49	.02*	-0.61
Sphingolipids	Glucosylceramide (d18:1/24:0)	2.26	.04*	-0.55
	SM(d18:0/16:1(9Z)(OH))	1.88	0.08	-0.46
	SM(d18:1/22:1(13Z))	3.56	.003*	-0.86
Fatty Acyls	Linoleoyl carnitine	1.92	0.07	-0.47
	Oleoylcarnitine	1.86	0.08	-0.45
Carboxylic Acids	1-Pyrroline-5-carboxylic Acid	1.74	0.1	0.42
Indoles And Derivatives	Indoleacetic acid	1.86	0.08	0.45
Organic Carbonic Acids	Urea	2.16	.047*	0.52
Organonitrogen Compounds	L-carnitine	2.87	.01*	0.7

Abbreviations: PC, phosphatidylcholine; SM, sphingomyelin

*Significance at $P \leq .05$

4.5 Predicting Cardiac Health Using Various Measures

The alpha value was corrected to.025 to control for multiple comparisons.

4.5.1 Left Ventricular Ejection Fraction

Multiple linear regression analysis was used to assess if the cardiac health follow-up assessment measures significantly predicted follow-up LVEF% while controlling for baseline-LVEF% (Figure 17). The overall model was a strong fit of the data, but was not a significant predictor of LVEF%; $F_{2, 14} = 1.89$, $P = .40$, $R^2 = 0.93$. Although insignificant, ß-values revealed that the top three predictors of follow-up LVEF% were PC(18:0/18:1(11Z)), then GLS%, and finally 1-Pyrroline-5-carboxylic acid. The top three predictors were inversely associated with follow-up LVEF% (Table 6).

Table 6: Using the Follow-up Normalized Intensities of Altered Metabolites and Global Longitudinal Strain to Predict Follow-up Left-Ventricular Ejection Fraction

Predictors	**ß**	***t***	***P***
Baseline LVEF	0.15	0.37	.75
Follow-up GLS	-0.92	2.14	.17
Follow-up 1-Pyrroline-5-carboxylic Acid	-0.9	0.11	.92
Follow-up Glucosylceramide (d18:1/24:0)	0.75	1.58	.25
Follow-up Indoleacetic acid	0.43	1.01	.42
Follow-up L-carnitine	0.22	0.57	.63
Follow-up L-carnitine	0.35	0.44	.71
Follow-up Oleoylcarnitine	0.08	0.08	.95
Follow-up PC(16:0/16:0)	-1.05	1.41	.29
Follow-up PC(18:0/18:1(11Z))	0.73	1.72	.23
Follow-up PC(18:0/22:6 (4Z,7Z,10Z,13Z,16Z,19Z))	-0.30	0.44	.70
Follow-up SM(d18:0/16:1(9Z)(OH))	0.16	0.32	.78
Follow-up SM(d18:1/22:1(13Z))	-0.20	0.69	.56
Follow-up Urea	-0.13	0.25	.83

Abbreviations: GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; PC, phosphatidylcholine; SM, sphingomyelin

Significance at $P \leq 0.025$

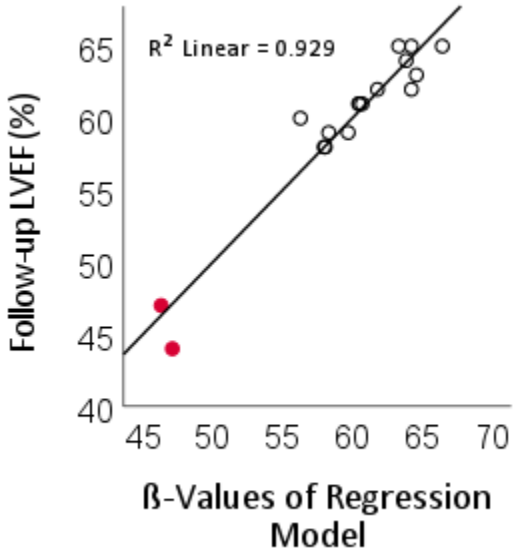


Figure 17: Predicting 24-week Follow-up Assessment Left Ventricular Ejection Fraction Using Follow-up Measures of Cardiac Health

Unstandardized ß-values of multiple linear regression model were calculated while controlling for baseline left ventricular ejection fraction (LVEF%). The overall model was statistically insignificant. Red circles show the 2 participants who developed CTX.

4.5.2 Global Longitudinal Strain

Multiple linear regression analysis was also used to assess if the follow-up assessment measures of cardiac health significantly predicted follow-up GLS% while controlling for baseline GLS% (Figure 18). The overall model was a strong fit of the data, but was not a significant predictor of follow-up GLS% $F_{2, 14} = 2.37$, $P = .34$, $R^2 = 0.94$. Although insignificant, ß-values revealed that the top three predictors of follow-up GLS% were LVEF%, then PC(18:0/18:1(11Z)), and finally PC(16:0/16:0). LVEF% was inversely associated, and the metabolites were directly associated with follow-up LVEF% (Table 7).

Table 7: Using the Follow-up Normalized Intensities of Altered Metabolites and Left-Ventricular Ejection Fraction to Predict Follow-up Global Longitudinal Strain

Predictors	**ß**	***t***	***P***
Baseline GLS	0.27	0.80	.51
Follow-up LVEF	-1.06	2.41	.14
Follow-up 1-Pyrroline-5-carboxylic Acid	0.19	0.37	.75
Follow-up Glucosylceramide (d18:1/24:0)	0.69	1.56	.26
Follow-up Indoleacetic Acid	0.50	1.51	.27
Follow-up L-carnitine	0.26	0.86	.48
Follow-up L-carnitine	0.37	0.56	.64
Follow-up Oleoylcarnitine	0.31	0.33	.78
Follow-up PC(16:0/16:0)	-0.68	1.38	.30
Follow-up PC(18:0/18:1(11Z))	0.71	1.54	.26
Follow-up PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	0.13	0.25	.83
Follow-up SM(d18:0/16:1(9Z)(OH))	0.12	0.35	.76
Follow-up SM(d18:1/22:1(13Z))	0.29	1.33	.32
Follow-up Urea	0.12	0.27	.81

Abbreviations: GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; PC, phosphatidylcholine; SM, sphingomyelin

Significance at $P \leq 0.05$

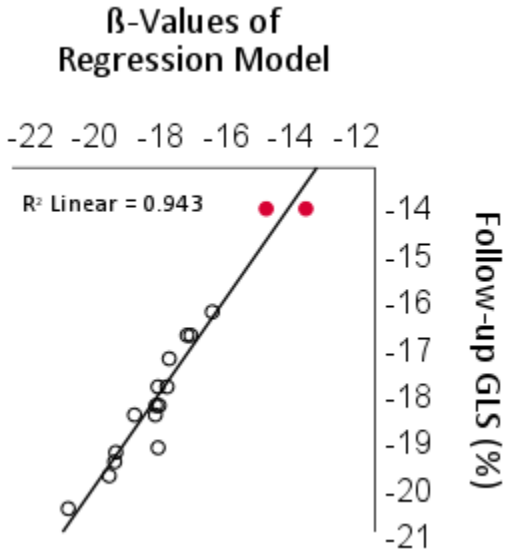


Figure 18: Predicting 24-week Follow-up Assessment Global Longitudinal Strain Using Follow-up Measures of Cardiac Health

Unstandardized ß-values of multiple linear regression model were calculated while controlling for baseline global longitudinal strain (GLS%). The overall model was statistically insignificant. Red circles show the 2 participants who developed cardiotoxicity.

4.6 The Effect of Physical Activity Levels on the Relationship Between Various Measures of Cardiac Health

Given that the same models were developed, just with an added interaction term, the alpha value remained at .025.

Moderation was used to assess whether physical activity levels impacted the relationship between baseline to follow-up for the echocardiographic parameters and metabolite relative intensities (Table 8). Separate moderation models were developed for all variables with baseline and follow-up physical activity levels as the moderating terms.

Overall, the repeated measures models of GLS% and the relative intensities of PC(18:0/18:1(11Z)), and PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) with baseline physical activity levels as the moderator were significant (Figure 19; Panels C, S, U). The repeated measures models of 1-Pyrroline-5-carboxylic acid, PC(18:0/18:1(11Z)), and PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (Figure 19; Panels F, T, V) with follow-up physical activity levels as the moderator were also significant (Table 8). Furthermore, 71% of the overall models with baseline physical activity levels as the moderator accounted for a greater proportion of the variance than those with follow-up physical activity levels as the moderator (Table 8).

The interaction terms consisting of baseline physical activity*GLS%, baseline physical activity*PC(16:0/16:0) (Figure 19; Panels C, Q) as well as follow-up physical activity*urea (Figure 19; Panel AB) were significant (Table 8). Also, the ß-values from moderation terms, including baseline physical activity, were more substantial than the ß-values from moderation terms, including follow-up physical activity in 64% of the models (Table 8).

Table 8: Impact of Physical Activity Levels on the Relationship Between Echocardiographic and Metabolomic Parameters from Baseline to Follow-up

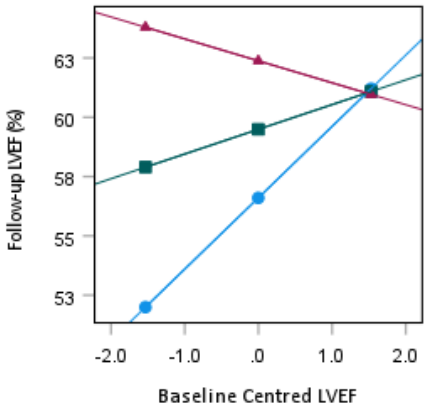
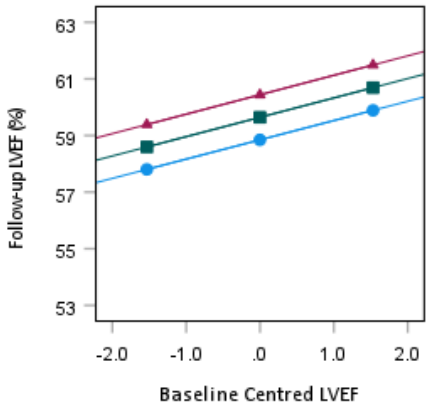
	Variable	Coefficients			ANOVA		
		ß	t	P	R^2	F	P
A	LVEF	0.01	1.24	.23	0.38	2.71	.09
	BPA	1.00	2.24	.04			
	LVEF*BPA	-1.00	1.91	.07			
B	LVEF	-0.15	0.54	.60	0.06	0.26	.85
	FuPA	1.07	0.50	.62			
	LVEF*FuPA	-0.92	0.00	> .99			
C	GLS	-0.12	1.73	.10	0.55	5.24	.01*
	BPA	-0.94	3.25	.01*			
	GLS*BPA	1.05	2.92	.01*			
D	GLS	-0.11	0.84	.41	0.16	0.84	.50
	FuPA	-0.94	1.50	.15			
	GLS*FuPA	1.05	1.13	.27			
E	1-Pyrroline-5-carboxylic acid	-0.13	0.49	.63	0.21	1.15	.37
	BPA	-0.40	1.58	.13			
	1-Pyrroline-5-carboxylic acid*BPA	-0.51	0.79	.44			
F	1-Pyrroline-5-carboxylic acid	1.14	2.06	.06	0.61	6.80	.01*
	FuPA	-0.71	4.26	<.001*			
	1-Pyrroline-5-carboxylic acid*FuPA	-0.43	1.10	.29			
G	Glucosylceramide (d18:1/24:0)	0.21	0.61	.55	0.17	0.86	.48
	BPA	0.88	1.33	.20			
	Glucosylceramide (d18:1/24:0)*BPA	-1.09	1.05	.31			
H	Glucosylceramide (d18:1/24:0)	0.01	0.49	.63	0.04	0.20	.90
	FuPA	1.00	0.57	.58			
	Glucosylceramide (d18:1/24:0)*FuPA	-1.00	0.10	.92			
I	Indoleacetic acid	0.08	0.28	.78	0.30	1.90	.18
	BPA	-1.04	2.18	.04			
	Indoleacetic acid*BPA	0.96	1.82	.09			
J	Indoleacetic acid	0.04	0.07	.95	0.09	0.40	.75
	FuPA	-0.56	0.59	.56			
	Indoleacetic acid*FuPA	1.39	0.96	.35			
K	L-Carnitine	1.14	0.23	.82	0.12	0.58	.64
	BPA	-0.74	1.27	.22			
	L-Carnitine*BPA	-0.39	0.60	.56			

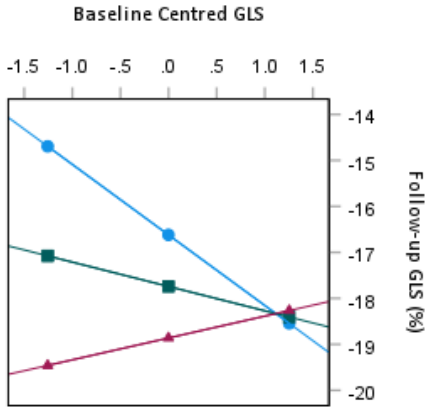
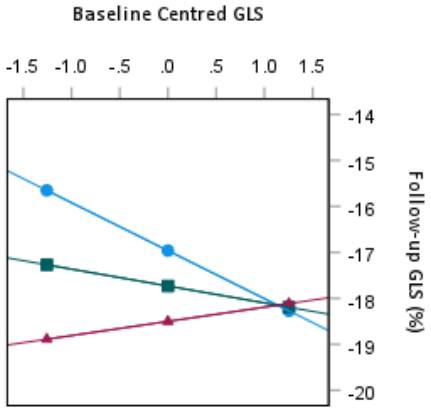
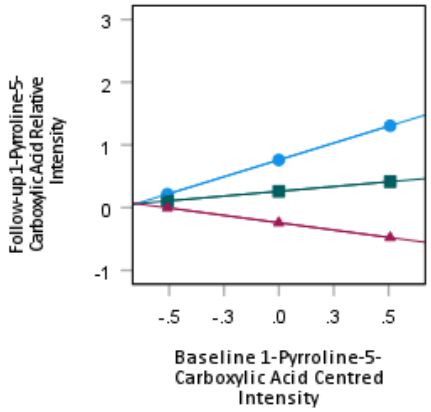
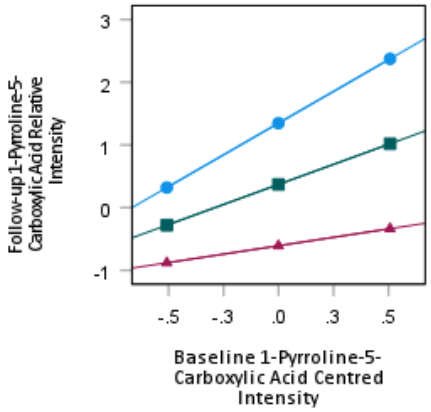
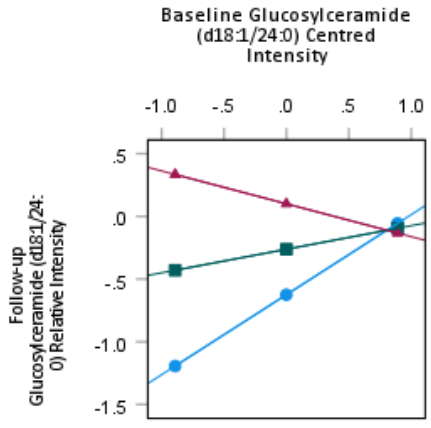
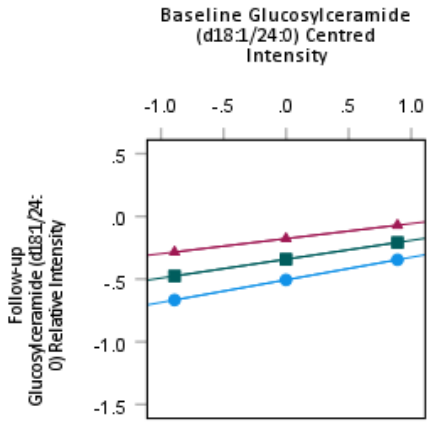
	Variable	Coefficients			ANOVA		
		ß	t	P	R^2	F	P
L	L-Carnitine	0.47	0.42	.68	0.12	0.60	.63
	FuPA	0.68	0.02	.98			
	L-Carnitine*FuPA	-1.15	1.23	.24			
M	Linoleoyl carnitine	-0.77	0.27	.79	0.06	0.28	.84
	BPA	1.13	0.78	.44			
	Linoleoyl carnitine*BPA	-0.36	0.25	.81			
N	Linoleoyl carnitine	-0.91	0.08	.94	0.11	0.55	.65
	FuPA	1.07	1.24	.23			
	Linoleoyl carnitine*FuPA	-0.17	0.42	.68			
O	Oleoylcarnitine	0.35	0.30	.77	0.11	0.51	.68
	BPA	0.78	0.58	.57			
	Oleoylcarnitine*BPA	-1.13	0.84	.41			
P	Oleoylcarnitine	0.28	0.34	.74	0.17	0.90	.47
	FuPA	0.83	0.31	.76			
	Oleoylcarnitine*FuPA	-1.11	1.10	.29			
Q	PC(16:0/16:0)	-0.96	0.00	> .99	0.46	3.75	.04
	BPA	-0.08	1.50	.15			
	PC(16:0/16:0)*BPA	1.04	2.68	.02*			
R	PC(16:0/16:0)	-1.08	0.22	.83	0.39	2.79	.08
	FuPA	0.20	0.93	.37			
	PC(16:0/16:0)*FuPA	0.89	2.01	.06			
S	PC(18:0/18:1(11Z))	0.21	2.24	.04	0.67	8.62	< .001*
	BPA	0.88	2.64	.02*			
	PC(18:0/18:1(11Z))*BPA	-1.09	0.16	.87			
T	PC(18:0/18:1(11Z))	0.43	2.29	.04	0.57	5.77	.01*
	FuPA	0.71	1.85	.08			
	PC(18:0/18:1(11Z))*FuPA	-1.14	0.18	.86			
U	PC(18:0/22:6 (4Z,7Z,10Z,13Z,16Z,19Z))	0.40	4.37	< .001*	0.67	8.95	< .001*
	BPA	-1.14	1.67	.11			
	PC(18:0/22:6(4Z,7Z,10Z,13 Z,16Z,19Z))*BPA	0.74	1.59	.13			
V	PC(18:0/22:6(4Z,7Z,10Z,13 Z,16Z,19Z))	1.15	3.70	< .001*	0.53	4.97	0.02*
	FuPA	-0.51	0.63	.54			
	PC(18:0/22:6(4Z,7Z,10Z,13 Z,16Z,19Z))*FuPA	-0.64	0.44	.67			
W	SM(d18:0/16:1(9Z)(OH))	-0.37	0.75	.47	0.19	1.03	0.41
	BPA	-0.76	0.04	.97			
	SM(d18:0/16:1(9Z)(OH))*B PA	1.13	1.33	.20			
X	SM(d18:0/16:1(9Z)(OH))	1.15	0.77	.45	0.10	0.47	0.71
	FuPA	-0.49	0.42	.68			

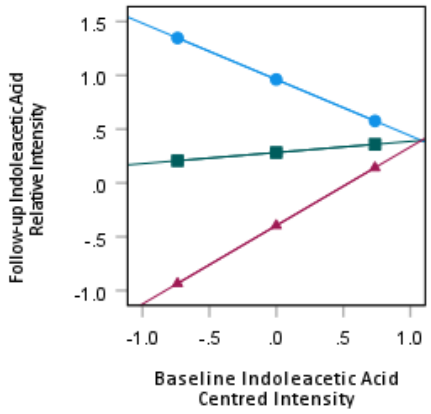
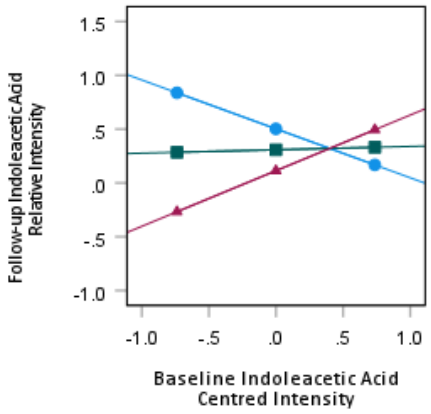
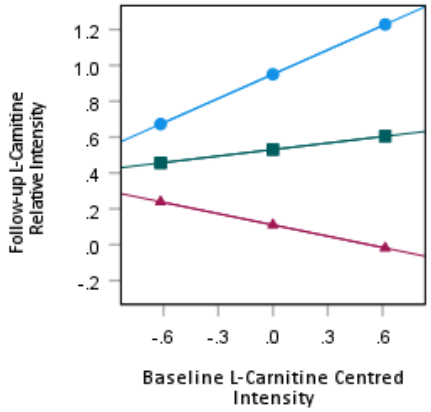
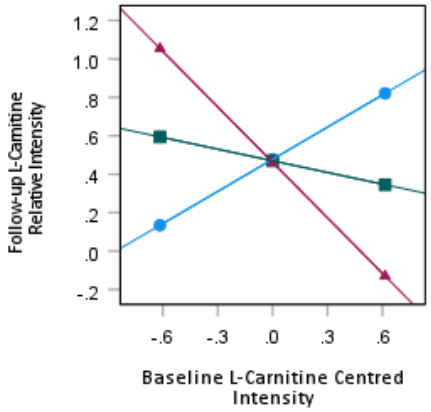
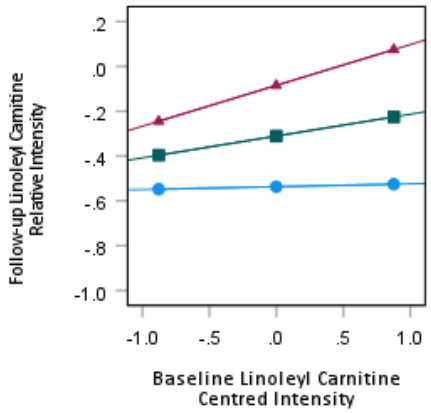
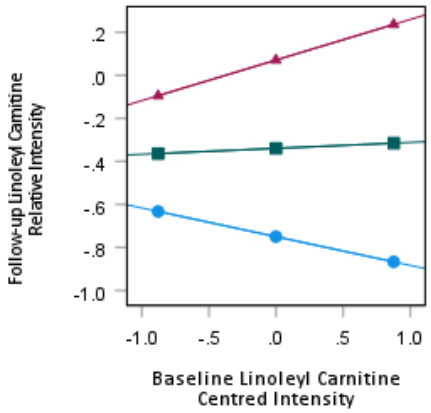
	Variable	Coefficients			ANOVA		
		ß	t	P	R^2	F	P
	SM(d18:0/16:1(9Z)(OH))*FuPA	-0.66	0.43	.67			
Y	SM(d18:1/22:1(13Z))	-0.16	0.52	.61	0.18	0.96	0.44
	BPA	1.07	1.63	.12			
	SM(d18:1/22:1(13Z))*BPA	-0.91	0.64	.53			
Z	SM(d18:1/22:1(13Z))	0.06	0.14	.89	0.15	0.77	0.53
	FuPA	0.97	1.17	.26			
	SM(d18:1/22:1(13Z))*FuPA	-1.03	1.37	.19			
AA	Urea	1.15	0.77	.45	0.06	0.27	0.85
	BPA	-0.49	0.05	.96			
	Urea*BPA	-0.66	0.04	.97			
AB	Urea	0.25	1.66	.12	0.48	4.02	0.03
	FuPA	0.85	2.79	.01*			
	Urea*FuPA	-1.10	2.71	.02*			

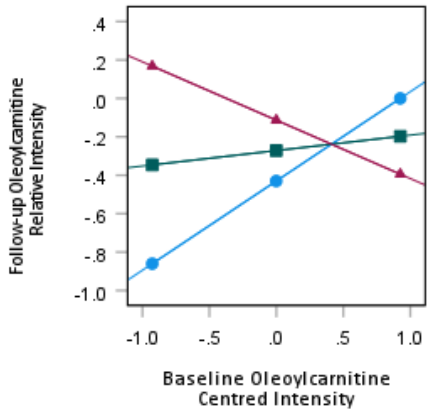
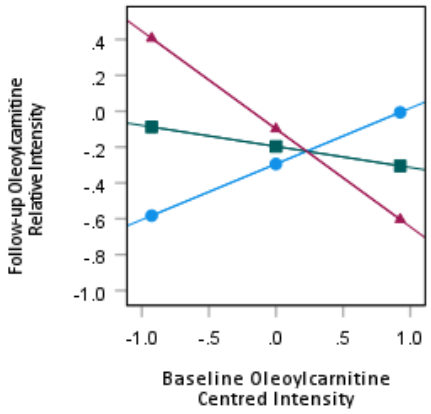
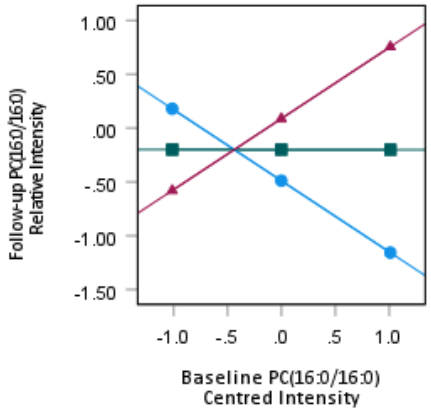
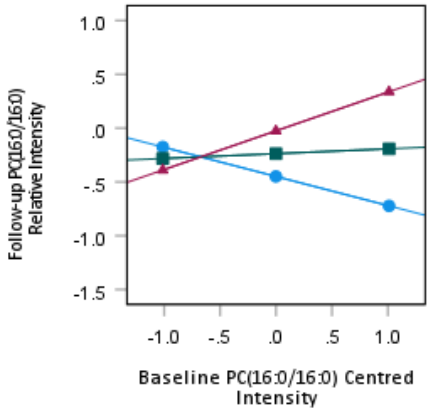
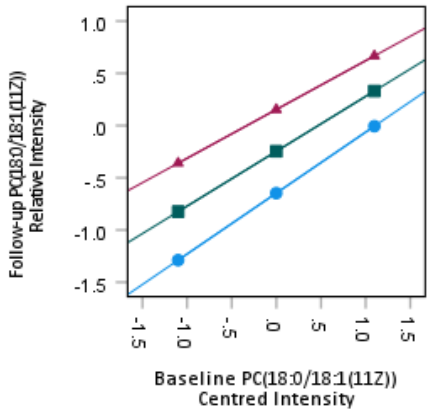
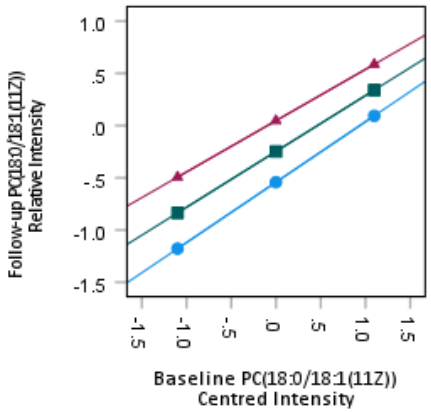
Abbreviations: BPA, baseline physical activity levels (LogMET min/wk); FuPA, follow-up physical activity levels (LogMET min/wk); GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; PC, phosphatidylcholine; SM, sphingomyelin

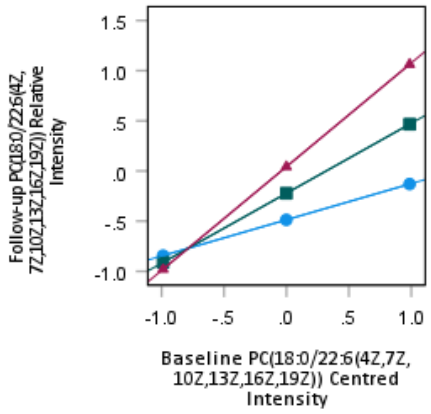
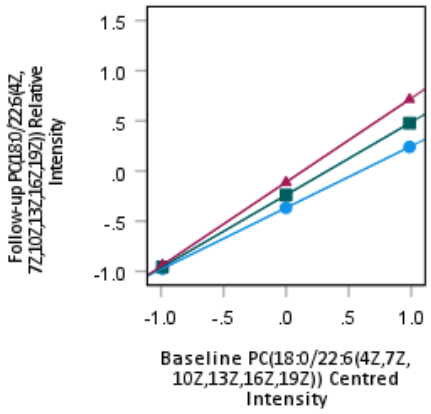
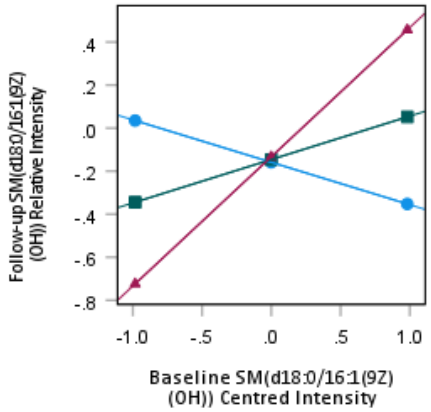
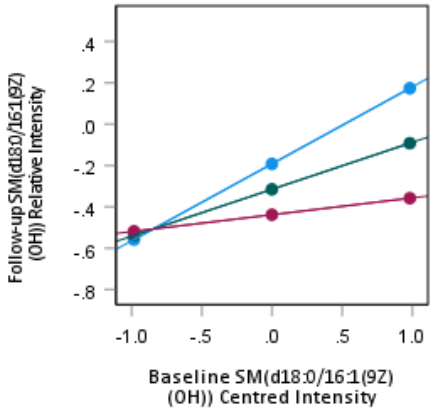
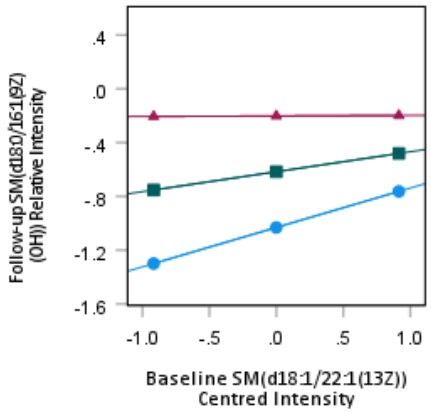
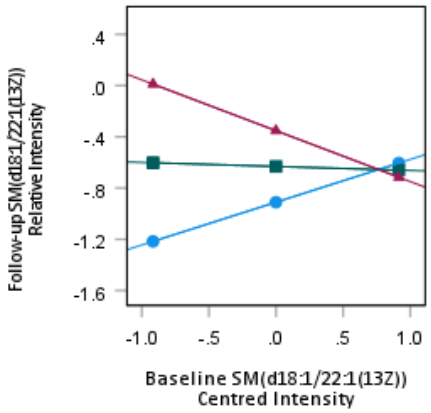
*Significance: $P \leq .025$

Moderator: Baseline Log MET min/wk	Moderator: Follow-up Log MET min/wk
	
A) Moderator: ß = -1.00, P = .07 Overall: R^2 = 0.38	B) Moderator: ß = -0.92, P > .99 Overall: R^2 = 0.06

Moderator: Baseline Log MET min/wk	Moderator: Follow-up Log MET min/wk
	
C) Moderator: ß = 1.05, *P* = .01 Overall: $R^2 = 0.55$	D) Moderator: ß = 1.05, *P* = .28 Overall: $R^2 = 0.16$
	
E) Moderator: ß = -0.51, *P* = .44 Overall: $R^2 = 0.21$	F) Moderator: ß = -0.43, *P* = .29 Overall: $R^2 = 0.61$
	
G) Moderator: ß = -1.09, *P* = .31 Overall: $R^2 = 0.17$	H) Moderator: ß = -1.00, *P* = .92 Overall: $R^2 = 0.04$

Moderator: Baseline Log MET min/wk	Moderator: Follow-up Log MET min/wk
	
I) Moderator: ß = 0.96, *P* = .09 Overall: $R^2 = 0.30$	J) Moderator: ß = 1.39, *P* = .35 Overall: $R^2 = 0.09$
	
K) Moderator: ß = -0.39, *P* = .56 Overall: $R^2 = 0.12$	L) Moderator: ß = -1.15, *P* = .244 Overall: $R^2 = 0.12$
	
M) Moderator: ß = -0.36, *P* = .81 Overall: $R^2 = 0.06$	N) Moderator: ß = -0.17, *P* = .68 Overall: $R^2 = 0.11$

Moderator: Baseline Log MET min/wk	Moderator: Follow-up Log MET min/wk
	
O) Moderator: ß = -1.13, P = .41 Overall: R^2 = 0.11	P) Moderator: ß = -1.11, P = .29 Overall: R^2 = 0.17
	
Q) Moderator: ß = -1.04, P = .02 Overall: R^2 = 0.46	R) Moderator: ß = 0.89, P = .06 Overall: R^2 = 0.39
	
S) Moderator: ß = -1.09, P = .87 Overall: R^2 = 0.67	T) Moderator: ß = -1.14, P = .86 Overall: R^2 = 0.57

Moderator: Baseline Log MET min/wk	Moderator: Follow-up Log MET min/wk
	
U) Moderator: ß = 74, P = .13 Overall: R^2 = 0.67	V) Moderator: ß = -0.64, P = .67 Overall: R^2 = 0.53
	
W) Moderator: ß = 1.13, P = .20 Overall: R^2 = 0.19	X) Moderator: ß = -0.66, P = .67 Overall: R^2 = 0.10
	
Y) Moderator: ß = -0.91, P = .53 Overall: R^2 = 0.18	Z) Moderator: ß = -1.03, P = .19 Overall: R^2 = 0.15

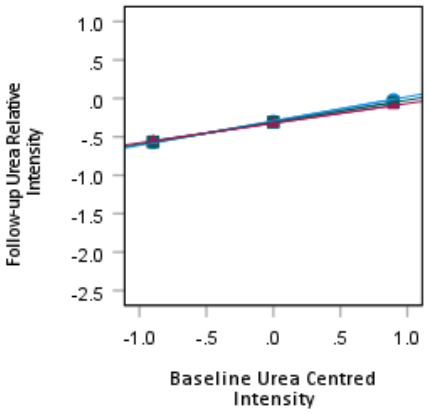
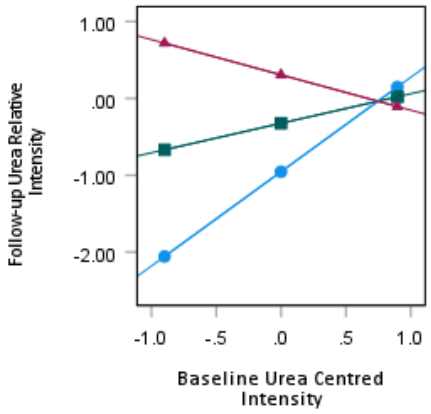
Moderator: Baseline Log MET min/wk	Moderator: Follow-up Log MET min/wk
	
AA) Moderator: ß = 1.15, *P* = .97 Overall: $R^2 = 0.06$	AB) Moderator: ß = -1.10, *P* = .02 Overall: $R^2 = 0.48$

Figure 19: Impact of Physical Activity Levels on the Relationship Between Left Ventricular Ejection Fraction and Global Longitudinal Strain or Normalized Metabolite Intensities from Baseline to Follow-up

Three separate regression lines are shown by physical activity level: ●, - 1 SD; ■, average; ▲, + 1 SD

Abbreviations: GLS, global longitudinal strain; LVEF, left ventricular ejection fraction

Chapter 5: Discussion

5.1 Summary

Anthracycline-induced CTX is a serious adverse effect many breast cancer patients develop, posing unique challenges for healthcare providers regarding effective diagnostic techniques and management strategies.[13,19,52] Metabolomic profiling offers a potential solution to such challenges, given the technique's ability to identify the earliest signs of disease. Extensive preclinical,[28,32,85,86] and some clinical research[90,92,100,105,106] suggest that higher levels of physical activity and exercise are associated with a reduced risk for anthracycline-induced CTX. Thus, this thesis aimed to determine cardiac health changes over 24 weeks in patients receiving anthracycline therapy, examine the relationships amongst LVEF%, GLS% and the metabolome, and investigate the impact of physical activity levels on the relationships between the metabolome and echocardiographic parameters.

The results of the current thesis contribute novel pieces of information to the topic of anthracycline-induced CTX. To the author's knowledge, no previous reports have investigated the impact of physical activity levels on the relationships between the metabolome and echocardiographic parameters. The findings of the current report indicated that echocardiographic parameters (i.e., LVEF% and GLS%) tended to worsen during anthracycline treatment, the metabolome changed, but changes in the metabolome were not associated with echocardiographic parameters. Additionally, baseline physical activity levels influenced the relationship between baseline and follow-up measures for GLS% and most metabolites. The thesis also identified that participants adhered to the durations prescribed in the EXACT 2.0 exercise program.

5.2 Changes in Parameters

5.2.1 Echocardiographic Parameters

Extensive clinical research[14,57,119,120] demonstrates that anthracycline therapy significantly reduces LVEF% and increases GLS% dose-dependently. However, contrary to this evidence, the current report did not find statistically significant changes in LVEF% or GLS%. Nonetheless, alterations in both measures suggested that participants may have experienced clinically significant reduced cardiac function at follow-up. Specifically, LVEF% decreased by 4.17%, and GLS% increased by 5.82%, on average, indicating a moderate effect size. Further, 2 participants developed CTX, representing 11.8% of the study sample, as they experienced ≥ 10% reduction in LVEF%. This value is higher than the expected rate (i.e., 5% for patients receiving 400 mg/m^2 of doxorubicin) of CTX[13] given the average anthracycline (doxorubicin) dosage in the current study was 377 mg/m^2. The overall study sample showed statistically insignificant changes in LVEF% and GLS%, likely because of the limited duration of the study, small sample size, and high levels of physical activity.

The 24-week follow-up period in the current report may not have been sufficient for the cardiotoxic effects of anthracyclines to manifest fully. Evidence suggests that changes in LVEF% and GLS% can occur acutely, or as early- or late-onset CTX.[13] Early-onset CTX may take up to 1 year to manifest, while late-onset CTX can develop after several years.[13] Therefore, participants in the current report might continue to experience changes in LVEF% and GLS% beyond the 24-week follow-up assessment suggesting the importance of long-term follow-up.

The current study had a small sample size, with only 17 participants in the analysis, resulting in low statistical power. The achieved power was 55% for LVEF% and 53% for GLS% (G*Power, V3.1.9.6, Universität Kiel, Germany). Increasing the sample size to 45, accounting for a 20% attrition rate, would have improved the statistical power to approximately 80% and potentially identified more evident trends. Nevertheless, the observed changes in LVEF% and GLS% were moderate effect sizes and indicated clinically relevant changes despite the low statistical power. The literature reports that a 5% increase in LVEF%, or a 1% decrease in GLS%, are associated with nearly a 5-fold,[121] or 5%[122] lower cardiac-related mortality, respectively. Accordingly, the current study identified clinically significant decreases in LVEF% and increased in GLS%. Further, participants in the current study experienced a higher magnitude of change in GLS% than LVEF%, which aligns with the current evidence that GLS% is more sensitive than LVEF% for detecting anthracycline-induced CTX.[59,112,119] Thus, participants in the current study experienced clinically significant cardiac health declines despite statistical insignificance.

Participants in the current study generally reported high baseline and follow-up leisure time physical activity levels and overall physical activity levels. They reported an average leisure time physical activity of 1140 MET min/wk at baseline, and 768 MET min/wk at follow-up, as well as an average total physical activity of 4045 MET min/wk at baseline, and 3405 MET min/wk at follow-up. On average, only 49% of healthy Canadian adults met the recommended 150 minutes of moderate-to vigorous-leisure time physical activity (around 500 MET min/wk) per week in 2019,[123] and activity levels did not significantly change during the coronavirus pandemic.[124] Magno *et al.*[125] reported that

in a population of 504 breast cancer patients (average age = 57.63 yrs ± 13.29), 239 (47.42%) were inactive (total physical activity <700 MET min/wk), 169 (33.53%) were moderately active (total physical activity 700-2519 MET min/wk), and 96 (19.05%) were active (total physical activity >2520 MET min/wk). Thus, the participants in the current study exceeded the activity guidelines, which over half of Canadians do not meet, and were more active than most breast cancer patients. Such an active sample was likely more apt to enrol in the study because of the challenges of encouraging sedentary individuals to become active. The literature suggests active individuals have improved cardiovascular health and are significantly less likely to develop anthracycline-induced CTX.[34,36,85,90,97] The studied population likely was at a reduced risk for developing CTX, and their high total physical activity levels may have attenuated declines in LVEF% and increases in GLS%. Future research should consider developing multiple study arms with different eligibility criteria based on participants' baseline physical activity levels. Such a design would allow for different exercise intensities to be prescribed for each study arm, and a stratified analysis.

5.2.2 Metabolic Parameters

The current report investigated plasma metabolic changes in a clinical setting. Notably, metabolites contributing to the most variance from baseline to follow-up were classified as carboxylic acids, sphingolipids, indoles, organonitrogen compounds, fatty acyls, glycerophospholipids, and organic carbonic acids. Lipid-based molecules (i.e., glycerophospholipids and sphingolipids) were the most frequent class of metabolite contributing to alterations (Table 5).

To the best of the author's knowledge, few reports have previously examined the metabolic profiles of plasma in breast cancer patients. Asnani *et al.*[24] identified changes in citric and aconitic acid levels between breast cancer patients with and without CTX. However, the researchers did not directly assess lipids, constituting the most altered metabolites in the current report. Furthermore, the current report aimed to identify biomarkers before CTX development, while Asnani *et al.*[24] investigated the metabolic profiles of patients already exhibiting CTX. An abstract by Cocco *et al.*[25] also sought to characterize the metabolome of breast cancer patients treated with anthracyclines. They reported that at 360 mg/m^2 of anthracycline treatment, participants with CTX had higher levels of Krebs's cycle intermediates, while patients without CTX had higher levels of cardioprotective metabolites (e.g., tryptophan). Given the limited clinical research and wide range of options for metabolomic experiments (e.g., LC-MS/MS, nuclear magnetic resonance spectrometry), it is unsurprising that the current report identified different altered metabolites.

Previous metabolomic and CTX research primarily focused on metabolomic alterations in preclinical models, such as in human-induced pluripotent stem cell-derived cardiomyocytes (hiSPC) and animals.[22,66,67,126] The findings of the current report align with some of the results from preclinical models and clinical research regarding alterations in glycerophospholipids and sphingolipids. Thonusin *et al.*[22] and Yuan *et al.*[126] also reported that serum levels of glycerophospholipids were top contributors to the within-group variance between doxorubicin-treated and non-doxorubicin-treated rats. Additionally, Tan *et al.*[127] identified lipids as the predominantly altered metabolite class, but did not specifically identify sphingolipids or glycerophospholipids. However, Palmer

et al.[67] examined the metabolome of hiSPC cell media using an untargeted approach (versus the targeted methodology used in the current report) and did not identify alterations in lipid-based molecules, although they examined numerous cardiotoxic agents and only annotated the top 6 altered features. Given ongoing discrepancies between the current study and previous reports, further research is necessary to explore the blood metabolome of breast cancer patients receiving anthracyclines.

As stated, sphingolipids and glycerophospholipids were the most frequently altered metabolite in the current report, specifically SMs and PCs (Table 5). SMs and PCs are critical constituents of sphingolipid and linoleic acid metabolism, respectively.[128] Both sphingolipids and glycerophospholipids are essential to the cell membrane structure and lipid signalling.[129,130] Dysregulated sphingolipid metabolism is associated with oxidative stress caused by the increased production of ceramides from SMs,[129,131] and dysregulated linoleic acid metabolism is associated with higher overall cholesterol levels and lower levels of high-density lipoproteins (i.e., healthy cholesterol).[132] The current report identified a moderate to high SM and PC relative intensity reduction from baseline to follow-up. This reduction may be linked to the impact of anthracyclines on sphingolipid and linoleic acid metabolism, leading to oxidative stress and dysregulated fat and cholesterol transport. Similarly, the Alberta Heart Failure Etiology and Analysis Research Team (HEART) reported that patients with heart failure exhibited lower serum concentrations of PCs and SMs than non-heart failure controls.[133]

Preclinical research suggests the impact of anthracycline treatment on SMs and PCs. Mizrachi *et al.*[134] reported that doxorubicin increased sphingomyelinase activation, increasing SM conversion to ceramide and reactive oxygen species production *in vivo*.

The same report also investigated femoral arterial blood flow in mice immediately following doxorubicin treatment, and observed reduced arterial blood flow theorized to be caused by increased reactive oxygen species production.[134] Similarly, Koleini *et al.*[135] investigated the effects of doxorubicin on phospholipid oxidation *in vivo* and observed an upregulation of oxidized PCs, selected because of their high abundance in cardiomyocyte mitochondria. The findings of the current clinical report demonstrating some similarities with preclinical studies merit further research into the possible role of PCs and SMs as biomarkers for anthracycline-induced CTX. Furthermore, any interventions impacting the levels of PCs and SMs may reduce CTX risk.

5.3 Relationship Between Echocardiographic Parameters and the Metabolome

The present study did not observe any statistically significant relationships between metabolite intensities and echocardiographic parameters (i.e., LVEF% and GLS%). While these results might suggest that metabolomic alterations are not associated with changes in echocardiographic parameters, the findings of similar studies indicate that metabolomic profiling can be a diagnostic tool for early CTX detection. The current report followed participants during anthracycline treatment, and only 2 participants developed CTX. Thus, it was impossible to complete a dichotomized analysis comparing the metabolome of participants who developed CTX to those who did not. Such an experimental design would provide better insight into the relationships between metabolite levels and echocardiographic parameters. Previously, Asnani *et al.*[24] compared the metabolome of breast cancer patients with CTX (n = 19) to patients without CTX (n = 19) and reported that citric acid levels were inversely correlated with LVEF% ($R = 0.55$, $P = .01$). Similarly, Thonusin *et al.*[22] identified correlations between changes

in LVEF% and the concentrations of 15 metabolites, including various amino acids, lipids, and Krebs's cycle intermediates in rats treated with doxorubicin. Notably, Thonusin *et al.*[22] identified a positive correlation between LVEF% and PC(34:1), and PCs were also found to be altered in the current study despite not predicting LVEF% or GLS%.

5.4 The Impact of Physical Activity

To the author's knowledge, this is the first report to investigate the impact of physical activity levels on changes in the metabolome of patients receiving anthracycline treatment. The current study identified that physical activity levels impacted changes in the metabolome and echocardiographic parameters. Notably, baseline physical activity levels appeared to be a more critical moderator of alterations in echocardiographic and metabolic parameters from baseline to follow-up than follow-up physical activity levels. The present study observed that baseline physical activity levels as a moderating variable more frequently accounted for a more significant proportion of variance and exerted a greater magnitude of effect on changes in most parameters (i.e., metabolites, LVEF% and GLS%) from baseline to follow-up. Despite baseline physical activity levels being a stronger moderator, the current report still found that follow-up physical activity levels impacted the metabolome. Such findings align with the current evidence that exercise and physical activity can attenuate anthracycline-induced CTX before, during and after treatment.[106]

Specifically, baseline physical activity levels significantly impacted the relationships between baseline and follow-up GLS% and PC(16:0/16:0) relative intensity. In the current study, high baseline physical activity was associated with a reduced risk for CTX, as participants who reported a high baseline physical activity were significantly

less likely to experience increases in GLS% (Figure 19, Panel C). In clinical research, physical inactivity and obesity are widely reported risk factors for CTX.[10,54] Preclinically, Shirinbayan *et al.*[34] investigated the impact of a prehabilitation exercise program on cardiac outcomes in an animal model. They reported that treadmill running upregulated antioxidant enzyme production resulting in cardioprotection against doxorubicin compared to sedentary rats.[34] Given that baseline physical activity levels also significantly impacted changes in PC(16:0/16:0) in the current study, perhaps one-way exercise exerts its cardioprotective effects via lipid-related pathways. Likewise, Chicco *et al.*[27] investigated the impact of voluntary wheel running prior to doxorubicin administration on CTX in rats. They identified that rats with access to a wheel had attenuated reductions in left ventricular health (attenuated declines in pressure, and increases in lipid peroxidation).[27] Given these results, future clinical research investigating the therapeutic effects of an exercise program before a patient receives anthracycline treatment, or a prehabilitation exercise program, is warranted. Many breast cancer patients receive adjuvant chemotherapy post-surgery; thus, a prehabilitation exercise program designed to increase physical fitness pre-anthracycline treatment may be beneficial. Additionally, exercise programs as short as 4 weeks have been demonstrated to improve physical fitness and postsurgical recovery times in colorectal cancer patients.[136]

While baseline physical activity levels appeared to be a more critical factor in preventing declines in cardiac health, the current research also found that follow-up physical activity levels impacted changes in metabolites (precisely, urea relative intensity). Numerous reports indicate that exercise and physical activity during

anthracycline treatment can mitigate CTX risk, although no other reports have studied this concerning the metabolome. For example, Hydock *et al.*[86] demonstrated the reduced occurrence of cardiac dysfunction in doxorubicin-treated rats with access to a running wheel compared to rats without a wheel as well as attenuated upregulation of ß-Myosin heavy chain protein – an enzyme involved in slow ATPase activity. Additionally, Chung *et al.*[93] identified that a 3-month exercise program attenuated systolic and diastolic function declines in women with breast cancer.

5.5 Adherence to an Exercise Program

As the secondary objective, this thesis also investigated participant adherence to the EXACT 2.0 exercise intervention. Given the high attendance to the exercise program duration (90.53% ± 12 of the prescribed session durations completed), the design of the EXACT 2.0 exercise program was well-received. In a study by Kirkham *et al.*, [100] participants only completed 60% of sessions, and Heinze *et al.*[37] reported that participants attended 73% of the recommended sessions. The current study had a high attendance rate, perhaps related to its individualized approach to exercise prescription. Participants were encouraged to select any mode of aerobic exercise they enjoyed (e.g., walking, swimming, biking, circuit) and the intensities were based on their baseline exercise stress test. Participants also exercised from home, so they could select 2 non-consecutive days to complete the programming. Such programming is in contrast to that of Kirkham *et al.*[100] and Heinze *et al.*[37], which utilized in-person-based exercise programming. Due to its added financial and temporal cost, travel to in-person exercise programming is a common barrier. Such factors should be considered in future exercise program designs for breast cancer patients, as program adherence and session attendance are of utmost importance.

5.6 Limitations and Future Directives

To the author's knowledge, this is the first report to explore the impact of physical activity levels on changes in metabolomic parameters during anthracycline treatment. In addition to the limitations previously mentioned, including a small sample size, short study duration, and highly active sample, there are some overall study limitations. These include the influence of unassessed factors on the metabolome, the number of time points assessed, and challenges with gathering physical activity data.

The findings of the current report may have limited generalizability because of the effects of external factors, especially liver damage, on the metabolome. Anthracycline-induced liver damage is caused by oxidative stress and occurs because the liver metabolizes anthracyclines.[137] The liver also plays an essential role in lipid metabolism,[137] so PC and SM levels could be altered via the liver, not just cardiac dysfunction. Liver damage can lead to cardiomyopathies, just as reduced cardiac output can lead to hepatitis and liver cirrhosis.[138] Thus, while the identified metabolome and cardiac health alterations may have been caused by liver dysfunction, liver dysfunction can also be caused by cardiac dysfunction. For example, reduced cardiac output can lead to hepatitis and liver cirrhosis can lead to cardiomyopathy.[138] Given the reciprocal effects of cardiac and liver damage on one another, the observed changes in the metabolome are likely indicative of cardio- and hepatotoxicity. Future research controlling for liver health using liver enzyme levels should be conducted to confirm the identity of biomarkers indicative of CTX.

Furthermore, the current study's findings contrasted the literature specifically regarding the ability of metabolomic alterations to predict cardiac health. It is possible that the current report did not identify correlations between the metabolome and LVEF%

or GLS% because of external factors, such as liver damage. Other external factors such as menopausal status/age,[139] the patient's microbiome,[140] and the characteristics of breast cancer,[141] are also known to impact the metabolome and CTX risk. Future clinical research should be conducted, controlling for as many external factors as possible, to better elucidate metabolomic profiling's ability to predict CTX.

Moreover, quantitative analyses in the current study were limited. Additional statistical inferences could be made if data were collected at > 2 timepoints. Adding a midpoint (i.e., 12 weeks) and long-term (e.g., 2 years) assessment would allow for better investigation of correlations between the metabolome and echocardiographic parameters and the impact of physical activity levels. For example, Naaktgeboren *et al.*[36] reported that higher physical activity levels are associated with improved GLS% in long-term (median 10.2 years since treatment) breast cancer survivors. Furthermore, the completed statistical modelling was not without limitations. A paired *t*-test or Wilcoxon sign rank sum test on datasets with few subjects and many variables, such as in the current study, results in a high familywise error rate and/or potential issues with multicollinearity. To manage multicollinearity, PCA and OPLSDA modelling were conducted. PCA often only indicates differences when group (i.e., timepoint) separation is extensive, which was not expected in the current study given the short study duration.[142] OPLSDA is a supervised analysis technique that identifies variance between defined groups (i.e., timepoints), which may cause the modelling to be overly optimistic in identifying differences, especially with the current sample size (N = 17).[142] However, it is interesting that lipids, specifically PCs and SMs, were identified as altered in the current study's OPLSDA and *t*-test modelling. Future research should consider periodic assessments throughout

participant treatment and long-term follow-up, as well as collecting more extensive data to validate the findings of the current study's statistical models, ideally using PCA.

Finally, the current study used the IPAQ and fitness trackers to assess physical activity levels. The IPAQ is reported to be repeatable, and has criterion validity similar to other physical activity questionnaires.[110] However, physical activity questionnaires, in general, are not as valid or reliable as laboratory measures such as peak oxygen consumption due to reporting bias.[143] Nonetheless, considering the added burden of requesting a participant to complete a maximal fitness test, such as peak oxygen consumption, is essential, which likely would reduce study feasibility. Recruiting cancer populations for an exercise study is challenging, and adding burdensome tests could drastically reduce the potential sample size. The current study also used fitness trackers to mitigate the questionnaire biases. However, some participants did not adhere to wearing the fitness trackers and/or misused them, so the tracker data could not be analyzed. Due to poor fitness tracker-wearing adherence, participants may not have reached the prescribed exercise intensity levels. Future research should consider using alternative tracking devices such as biosensor patches or phone applications.[144]

5.7 Conclusions

The current study demonstrated cardiac health and various metabolites, particularly lipids, changed during anthracycline treatment. Physical activity levels, especially baseline, impacted these changes. However, alterations in the metabolome were not predictive of echocardiographic parameters.

Thus, many unanswered questions remain regarding how physical activity can best mitigate anthracycline-induced CTX. As each patient has unique needs and often presents with numerous conditions (e.g., polypharmacy, frailty, obesity), physical

activity-based interventions to prevent CTX must be individualized. Metabolomics is a precision medicine technique that provides a snapshot of the interaction between the genome and environment and has the potential to identify the effectiveness of interventions or changes in health status prior to structural changes.[145] The current study provides early evidence supporting future research designed to develop practices to monitor the effectiveness of a physical activity-based intervention to mitigate anthracycline-induced CTX.

References

1. Sarkar S, Horn G, Moulton K, et al. Cancer development, progression, and therapy: An epigenetic overview. *Int J Mol Sci*. 2013;14(10):21087-21113. doi:10.3390/ijms141021087

2. Canadian Cancer Statistics Advisory Committee in collaboration with the Canadian Cancer Society. Canadian cancer statistics 2021. *Stat Can Public Health Agency Can*. Published online November 2021.

3. Brenner DR, Poirier A, Woods RR, et al. Projected estimates of cancer in Canada in 2022. *CMAJ*. 2022;194(17):E601-E607. doi:10.1503/cmaj.212097

4. Shields M, Wilkins K. An update on mammography use in Canada. *Health Rep*. 2009;20(3):7-19.

5. Mandelblatt J, van Ravesteyn N, Schechter C, et al. Which strategies reduce breast cancer mortality most? *Cancer*. 2013;119(14):2541-2548. doi:10.1002/cncr.28087

6. Saleh Y, Abdelkarim O, Herzallah K, Abela GS. Anthracycline-induced cardiotoxicity: Mechanisms of action, incidence, risk factors, prevention, and treatment. *Heart Fail Rev*. 2021;26(5):1159-1173. doi:10.1007/s10741-020-09968-2

7. Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of anthracyclines. *Front Cardiovasc Med*. 2020;7. Accessed February 22, 2022. https://www.frontiersin.org/article/10.3389/fcvm.2020.00026

8. Tini G, Cuomo A, Battistoni A, et al. Baseline cardio-oncologic risk assessment in breast cancer women and occurrence of cardiovascular events: The HFA/ICOS risk tool in real-world practice. *Int J Cardiol*. 2022;349:134-137. doi:10.1016/j.ijcard.2021.11.059

9. Varricchi G, Ameri P, Cadeddu C, et al. Antineoplastic drug-induced cardiotoxicity: A redox perspective. *Front Physiol*. 2018;9:167. doi:10.3389/fphys.2018.00167

10. Clark RA, Marin TS, Berry NM, Atherton JJ, Foote JW, Koczwara B. Cardiotoxicity and cardiovascular disease risk assessment for patients receiving breast cancer treatment. *Cardio-Oncol*. 2017;3(1):6. doi:10.1186/s40959-017-0025-7

11. Chen D, Kelly C, Haw TJ, et al. Heart Failure in breast cancer survivors: Focus on early detection and novel biomarkers. *Curr Heart Fail Rep*. Published online November 3, 2021. doi:10.1007/s11897-021-00535-w

12. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline chemotherapy and cardiotoxicity. *Cardiovasc Drugs Ther*. 2017;31(1):63-75. doi:10.1007/s10557-016-6711-0

13. Zamorano JL, Lancellotti P, Rodriguez Muñoz D, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines. *Eur J Heart Fail*. 2017;19(1):9-42. doi:10.1002/ejhf.654

14. Dent SF, Botros J, Rushton M, et al. Anthracycline-induced cardiotoxicity in patients with early-stage breast cancer: The Canadian Cancer Trials Group (CCTG) MA.21 experience. *Breast Cancer Res Treat*. 2020;184(3):733-741. doi:10.1007/s10549-020-05887-w

15. Cardinale D, Colombo A, Bacchiani G, et al. Early Detection of Anthracycline Cardiotoxicity and Improvement With Heart Failure Therapy. *Circulation*. 2015;131(22):1981-1988. doi:10.1161/CIRCULATIONAHA.114.013777

16. Eneh C, Lekkala MR. Dexrazoxane. In: *StatPearls*. StatPearls Publishing; 2022. Accessed February 2, 2022. http://www.ncbi.nlm.nih.gov/books/NBK560559/

17. Legha S, Benjamin R, Mackay B, et al. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. *Ann Intern Med*. Published online December 1, 2008. Accessed March 23, 2022. http://www.acpjournals.org/doi/pdf/10.7326/0003-4819-96-2-133

18. van Dalen EC, Michiels EM, Caron HN, Kremer LC. Different anthracycline derivates for reducing cardiotoxicity in cancer patients. *Cochrane Database Syst Rev*. 2010;2010(5):CD005006. doi:10.1002/14651858.CD005006.pub4

19. Virani SA, Dent S, Brezden-Masley C, et al. Canadian Cardiovascular Society guidelines for evaluation and management of cardiovascular complications of cancer therapy. *Can J Cardiol*. 2016;32(7):831-841. doi:10.1016/j.cjca.2016.02.078

20. Vejpongsa P, Yeh ETH. Prevention of anthracycline-induced cardiotoxicity: Challenges and opportunities. *J Am Coll Cardiol*. 2014;64(9):938-945. doi:10.1016/j.jacc.2014.06.1167

21. Yeung PK. Metabolomics and biomarkers for drug discovery. *Metabolites*. 2018;8(1):11. doi:10.3390/metabo8010011

22. Thonusin C, Nawara W, Khuanjing T, et al. Blood metabolomes as non-invasive biomarkers and targets of metabolic interventions for doxorubicin and trastuzumab-induced cardiotoxicity. *Arch Toxicol*. Published online November 10, 2022. doi:10.1007/s00204-022-03412-0

23. Fazzini L, Caggiari L, Deidda M, et al. Metabolomic profiles on antiblastic cardiotoxicity: New perspectives for early diagnosis and cardioprotection. *J Clin Med*. 2022;11(22):6745. doi:10.3390/jcm11226745

24. Asnani A, Shi X, Farrell L, et al. Changes in citric acid cycle and nucleoside metabolism are associated with anthracycline cardiotoxicity in patients with breast

cancer. *J Cardiovasc Transl Res*. 2020;13(3):349-356. doi:10.1007/s12265-019-09897-y

25. Cocco D, Ferro EG, Ricci S, et al. Defining the metabolomic profile associated with early cardiotoxicity in patients with breast cancer treated with anthracyclines. *Eur Heart J*. 2020;41(Supplement_2):ehaa946.3289. doi:10.1093/ehjci/ehaa946.3289

26. Varghese SS, Eekhoudt CR, Jassal DS. Mechanisms of anthracycline-mediated cardiotoxicity and preventative strategies in women with breast cancer. *Mol Cell Biochem*. 2021;476(8):3099-3109. doi:10.1007/s11010-021-04152-y

27. Chicco AJ, Schneider CM, Hayward R. Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart. *Am J Physiol-Regul Integr Comp Physiol*. 2005;289(2):R424-R431. doi:10.1152/ajpregu.00636.2004

28. Hayward R, Lien CY, Jensen BT, Hydock DS, Schneider CM. Exercise training mitigates anthracycline-induced chronic cardiotoxicity in a juvenile rat model. *Pediatr Blood Cancer*. 2012;59(1):149-154. doi:10.1002/pbc.23392

29. Hydock DS, Lien CY, Schneider CM, Hayward R. Exercise preconditioning protects against doxorubicin-induced cardiac dysfunction. *Med Sci Sports Exerc*. 2008;40(5):808-817. doi:10.1249/MSS.0b013e318163744a

30. Ascensão A, Magalhães J, Soares J, et al. Endurance training attenuates doxorubicin-induced cardiac oxidative damage in mice. *Int J Cardiol*. 2005;100(3):451-460. doi:10.1016/j.ijcard.2004.11.004

31. de Lima EA, de Sousa LGO, de S Teixeira AA, Marshall AG, Zanchi NE, Neto JCR. Aerobic exercise, but not metformin, prevents reduction of muscular performance by AMPk activation in mice on doxorubicin chemotherapy. *J Cell Physiol*. 2018;233(12):9652-9662. doi:10.1002/jcp.26880

32. Hydock DS, Wonders KY, Schneider CM, Hayward R. Voluntary wheel running in rats receiving doxorubicin: Effects on running activity and cardiac myosin heavy chain. *Anticancer Res*. 2009;29(11):4401-4407.

33. Pfannenstiel K, Hayward R. Effects of resistance exercise training on doxorubicin-induced cardiotoxicity. *J Cardiovasc Pharmacol*. 2018;71(6):332-339. doi:10.1097/FJC.0000000000000574

34. Shirinbayan V, Roshan VD. Pretreatment effect of running exercise on HSP70 and DOX-induced cardiotoxicity. *Asian Pac J Cancer Prev APJCP*. 2012;13(11):5849-5855. doi:10.7314/apjcp.2012.13.11.5849

35. Ansund J, Mijwel S, Bolam KA, et al. High intensity exercise during breast cancer chemotherapy - Effects on long-term myocardial damage and physical capacity - Data from the OptiTrain RCT. *Cardio-Oncol Lond Engl*. 2021;7(1):7. doi:10.1186/s40959-021-00091-1

36. Naaktgeboren WR, Binyam D, Stuiver MM, et al. Efficacy of physical exercise to offset anthracycline-induced cardiotoxicity: A systematic review and meta-analysis of clinical and preclinical studies. *J Am Heart Assoc Cardiovasc Cerebrovasc Dis*. 2021;10(17):e021580. doi:10.1161/JAHA.121.021580

37. Heinze-Milne SD, Keats MR, Blanchard C, et al. Exercise to Prevent Anthracycline-Based Cardiotoxicity (EXACT): A feasibility study. *Transl J Am Coll Sports Med*. 2021;6(3):1-11. doi:10.1249/TJX.0000000000000168

38. Johnston W. *Exercise to Prevent Anthracycline-Induced Cardiotoxicity (EXACT2.0) in Females with Breast Cancer*. Thesis. 2022. Accessed March 1, 2023. https://DalSpace.library.dal.ca//handle/10222/81846

39. Varghese S. *Exercise to Prevent Anthracycline-Based Cardio-Toxicity (EXACT 2.0) in Women with Breast Cancer*. master thesis. 2021. Accessed March 1, 2023. https://mspace.lib.umanitoba.ca/xmlui/handle/1993/35691

40. Kendall SJ, Johnston WJ, Arya V, et al. EXercise to prevent Anthracycline-based Cardio-Toxicity (EXACT 2.0) in women with breast cancer: A metabolic perspective. In: *2022 BHCRI/TFRI Cancer Research Conference*. ; 2022.

41. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Comparisons between different polychemotherapy regimens for early breast cancer: Meta-analyses of long-term outcome among 100 000 women in 123 randomised trials. *The Lancet*. 2012;379(9814):432-444. doi:10.1016/S0140-6736(11)61625-5

42. Zhang M, Yang H, Xu C, Jin F, Zheng A. Risk Factors for anthracycline-induced cardiotoxicity in breast cancer treatment: A meta-analysis. *Front Oncol*. 2022;12:899782. doi:10.3389/fonc.2022.899782

43. Bines J, Earl H, Buzaid AC, Saad ED. Anthracyclines and taxanes in the neo/adjuvant treatment of breast cancer: Does the sequence matter? *Ann Oncol*. 2014;25(6):1079-1085. doi:10.1093/annonc/mdu007

44. Di Marco A, Gaetani M, Orezzi P, et al. 'Daunomycin', a new antibiotic of the rhodomycin group. *Nature*. 1964;201(4920):706-707. doi:10.1038/201706a0

45. Patnaik JL, Byers T, DiGuiseppi C, Dabelea D, Denberg TD. Cardiovascular disease competes with breast cancer as the leading cause of death for older females diagnosed with breast cancer: A retrospective cohort study. *Breast Cancer Res BCR*. 2011;13(3):R64. doi:10.1186/bcr2901

46. Bernstein D. Anthracycline cardiotoxicity: Worrisome enough to have you quaking? *Circ Res*. 2018;122(2):188-190. doi:10.1161/CIRCRESAHA.117.312395

47. Lambert J, Thavendiranathan P. Controversies in the definition of cardiotoxicity: Do we care? *Am Coll Cardiol*. Accessed November 17, 2022. https://www.acc.org/latest-in-

cardiology/articles/2016/07/07/14/59/http%3a%2f%2fwww.acc.org%2flatest-in-cardiology%2farticles%2f2016%2f07%2f07%2f14%2f59%2fcontroversies-in-the-definition-of-cardiotoxicity

48. Von Hoff DD, Layard MW, Basa P, et al. Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med*. 1979;91(5):710-717. doi:10.7326/0003-4819-91-5-710

49. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin. *Cancer*. 2003;97(11):2869-2879. doi:10.1002/cncr.11407

50. Vejpongsa P, Yeh E. Topoisomerase 2β: A promising molecular target for primary prevention of anthracycline-induced cardiotoxicity. *Clin Pharmacol Ther*. 2014;95(1):45-52. doi:10.1038/clpt.2013.201

51. Vologodskii AV, Zhang W, Rybenkov VV, et al. Mechanism of topology simplification by type II DNA topoisomerases. *Proc Natl Acad Sci U S A*. 2001;98(6):3045-3049. doi:10.1073/pnas.061029098

52. Mobaraki M, Faraji A, Zare M, Dolati P, Ataei M, Manshadi HRD. Molecular mechanisms of cardiotoxicity: A review on major side-effect of doxorubicin. *Indian J Pharm Sci*. 2017;79(3):335-344. doi:10.4172/pharmaceutical-sciences.1000235

53. Zhang S, Liu X, Bawa-Khalfe T, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. 2012;18(11):1639-1642. doi:10.1038/nm.2919

54. Lyon AR, Dent S, Stanway S, et al. Baseline cardiovascular risk assessment in cancer patients scheduled to receive cardiotoxic cancer therapies: A position statement and new risk assessment tools from the Cardio-Oncology Study Group of the Heart Failure Association of the European Society of Cardiology in collaboration with the International Cardio-Oncology Society. *Eur J Heart Fail*. 2020;22(11):1945-1960. doi:10.1002/ejhf.1920

55. Singh A, DeCara JM. The current and future role of echocardiography for the detection of cardiotoxicity related to cancer therapy. *Curr Cardiovasc Imaging Rep*. 2020;13(1):4. doi:10.1007/s12410-019-9523-3

56. Lang RM, Badano LP, Victor MA, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2015;28(1). doi:10.1016/j.echo.2014.10.003

57. Díaz-Antón B, Madurga R, Zorita B, et al. Early detection of anthracycline- and trastuzumab-induced cardiotoxicity: Value and optimal timing of serum biomarkers and echocardiographic parameters. *ESC Heart Fail*. 2022;9(2):1127-1137. doi:10.1002/ehf2.13782

58. Ali MT, Yucel E, Bouras S, et al. Myocardial strain is associated with adverse clinical cardiac events in patients treated with anthracyclines. *J Am Soc Echocardiogr*. 2016;29(6):522-527.e3. doi:10.1016/j.echo.2016.02.018

59. Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: A systematic review. *J Am Coll Cardiol*. 2014;63(25, Part A):2751-2768. doi:10.1016/j.jacc.2014.01.073

60. Pudil R, Mueller C, Čelutkienė J, et al. Role of serum biomarkers in cancer patients receiving cardiotoxic cancer therapies: A position statement from the Cardio-Oncology Study Group of the Heart Failure Association and the Cardio-Oncology Council of the European Society of Cardiology. *Eur J Heart Fail*. 2020;22(11):1966-1983. doi:10.1002/ejhf.2017

61. Michel L, Mincu RI, Mahabadi AA, et al. Troponins and brain natriuretic peptides for the prediction of cardiotoxicity in cancer patients: A meta-analysis. *Eur J Heart Fail*. 2020;22(2):350-361. doi:10.1002/ejhf.1631

62. Fabiani I, Aimo A, Grigoratos C, et al. Oxidative stress and inflammation: Determinants of anthracycline cardiotoxicity and possible therapeutic targets. *Heart Fail Rev*. 2021;26(4):881-890. doi:10.1007/s10741-020-10063-9

63. McGarrah RW, Crown SB, Zhang G, Shah SH, Newgard CB. Cardiovascular metabolomics. *Circ Res*. 2018;122(9):1238-1258. doi:10.1161/CIRCRESAHA.117.311002

64. Cheng S, Shah SH, Corwin EJ, et al. Potential impact and study considerations of metabolomics in cardiovascular health and disease: A scientific statement from the American Heart Association. *Circ Cardiovasc Genet*. 2017;10(2):e000032. doi:10.1161/HCG.0000000000000032

65. Jacob M, Lopata AL, Dasouki M, Abdel Rahman AM. Metabolomics toward personalized medicine. *Mass Spectrom Rev*. 2019;38(3):221-238. doi:10.1002/mas.21548

66. Chaudhari U, Ellis JK, Wagh V, et al. Metabolite signatures of doxorubicin induced toxicity in human induced pluripotent stem cell-derived cardiomyocytes. *Amino Acids*. 2017;49(12):1955-1963. doi:10.1007/s00726-017-2419-0

67. Palmer JA, Smith AM, Gryshkova V, Donley ELR, Valentin JP, Burrier RE. A targeted metabolomics-based assay using human induced pluripotent stem cell-derived cardiomyocytes identifies structural and functional cardiotoxicity potential. *Toxicol Sci Off J Soc Toxicol*. 2020;174(2):218-240. doi:10.1093/toxsci/kfaa015

68. Andreadou I, Papaefthimiou M, Zira A, et al. Metabonomic identification of novel biomarkers in doxorubicin cardiotoxicity and protective effect of the natural antioxidant oleuropein. *NMR Biomed*. 2009;22(6):585-592. doi:10.1002/nbm.1370

69. Tan G, Lou Z, Liao W, et al. Potential biomarkers in mouse myocardium of doxorubicin-induced cardiomyopathy: A metabonomic method and its application. *PloS One*. 2011;6(11):e27683. doi:10.1371/journal.pone.0027683

70. Cong W, Liang Q, Li L, et al. Metabonomic study on the cumulative cardiotoxicity of a pirarubicin liposome powder. *Talanta*. 2012;89:91-98. doi:10.1016/j.talanta.2011.11.071

71. Li Y, Ju L, Hou Z, et al. Screening, verification and optimization of biomarkers for early prediction of cardiotoxicity based on metabolomics. *J Proteome Res*. 2015;14(6):2437-2445. doi:10.1021/pr501116c

72. Schnackenberg LK, Pence L, Vijay V, et al. Early metabolomics changes in heart and plasma during chronic doxorubicin treatment in B6C3F1 mice. *J Appl Toxicol*. 2016;36(11):1486-1495. doi:10.1002/jat.3307

73. QuanJun Y, GenJin Y, LiLi W, et al. Protective effects of dexrazoxane against doxorubicin-induced cardiotoxicity: A metabolomic study. *PLoS ONE*. 2017;12(1):e0169567. doi:10.1371/journal.pone.0169567

74. Geng C, Cui C, Wang C, et al. Systematic evaluations of doxorubicin-induced toxicity in rats based on metabolomics. *ACS Omega*. 2021;6(1):358-366. doi:10.1021/acsomega.0c04677

75. Timm KN, Perera C, Ball V, et al. Early detection of doxorubicin-induced cardiotoxicity in rats by its cardiac metabolic signature assessed with hyperpolarized MRI. *Commun Biol*. 2020;3(1):1-10. doi:10.1038/s42003-020-01440-z

76. Lin H, Meng L, Sun Z, et al. Yellow wine polyphenolic compound protects against doxorubicin-induced cardiotoxicity by modulating the composition and metabolic function of the gut microbiota. *Circ Heart Fail*. 2021;14(10):e008220. doi:10.1161/CIRCHEARTFAILURE.120.008220

77. Choksey A, Timm KN. Cancer therapy-induced cardiotoxicity—A metabolic perspective on pathogenesis, diagnosis and therapy. *Int J Mol Sci*. 2022;23(1):441. doi:10.3390/ijms23010441

78. Ruddy KJ, Sangaralingham LR, Van Houten H, et al. Utilization of cardiac surveillance tests in survivors of breast cancer and lymphoma after anthracycline-based chemotherapy. *Circ Cardiovasc Qual Outcomes*. 2020;13(3):e005984. doi:10.1161/CIRCOUTCOMES.119.005984

79. Pacciarini MA, Barbieri B, Colombo T, Broggini M, Garattini S, Donelli MG. Distribution and antitumor activity of adriamycin given in a high-dose and a repeated low-dose schedule to mice. *Cancer Treat Rep*. 1978;62(5):791-800.

80. Chow EJ, Leger KJ, Bhatt NS, et al. Paediatric cardio-oncology: Epidemiology, screening, prevention, and treatment. *Cardiovasc Res*. 2019;115(5):922-934. doi:10.1093/cvr/cvz031

81. Kopp LM, Womer RB, Schwartz CL, et al. Effects of dexrazoxane on doxorubicin-related cardiotoxicity and second malignant neoplasms in children with osteosarcoma: A report from the Children's Oncology Group. *Cardio-Oncol*. 2019;5(1):15. doi:10.1186/s40959-019-0050-9

82. He D, Hu J, Li Y, Zeng X. Preventive use of beta-blockers for anthracycline-induced cardiotoxicity: A network meta-analysis. *Front Cardiovasc Med*. 2022;9. doi:10.3389/fcvm.2022.968534

83. Ascensão A, Ferreira R, Magalhães J. Exercise-induced cardioprotection--Biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. *Int J Cardiol*. 2007;117(1):16-30. doi:10.1016/j.ijcard.2006.04.076

84. Werner C, Hanhoun M, Widmann T, et al. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. *J Am Coll Cardiol*. 2008;52(6):470-482. doi:10.1016/j.jacc.2008.04.034

85. Hydock DS, Lien CY, Jensen BT, Schneider CM, Hayward R. Exercise preconditioning provides long-term protection against early chronic doxorubicin cardiotoxicity. *Integr Cancer Ther*. 2011;10(1):47-57. doi:10.1177/1534735410392577

86. Hydock DS, Lien CY, Jensen BT, Parry TL, Schneider CM, Hayward R. Rehabilitative exercise in a rat model of doxorubicin cardiotoxicity. *Exp Biol Med*. 2012;237(12):1483-1492. doi:10.1258/ebm.2012.012137

87. Moreno-Villanueva M, Kramer A, Hammes T, et al. Influence of acute exercise on DNA repair and PARP activity before and after irradiation in lymphocytes from trained and untrained individuals. *Int J Mol Sci*. 2019;20(12):2999. doi:10.3390/ijms20122999

88. Qi Z, He J, Su Y, et al. Physical exercise regulates p53 activity targeting SCO2 and increases mitochondrial COX biogenesis in cardiac muscle with age. *PLoS ONE*. 2011;6(7):e21140. doi:10.1371/journal.pone.0021140

89. Vega RB, Konhilas JP, Kelly DP, Leinwand LA. Molecular mechanisms underlying cardiac adaptation to exercise. *Cell Metab*. 2017;25(5):1012-1026. doi:10.1016/j.cmet.2017.04.025

90. Kirkham AA, Shave RE, Bland KA, et al. Protective effects of acute exercise prior to doxorubicin on cardiac function of breast cancer patients: A proof-of-concept RCT. *Int J Cardiol*. 2017;245:263-270. doi:10.1016/j.ijcard.2017.07.037

91. Fraser S, Bigaran A, Selig S, LaGerche A. Exercise training during anthracycline-based chemotherapy for breast cancer. *J Clin Oncol*. 2017;35(15_suppl):e12110-e12110. doi:10.1200/JCO.2017.35.15_suppl.e12110

92. Ma Z. Effect of anthracycline combined with aerobic exercise on the treatment of breast cancer. *Pak J Pharm Sci*. 2018;31(3(Special)):1125-1129.

93. Chung WP, Yang HL, Hsu YT, et al. Real-time exercise reduces impaired cardiac function in breast cancer patients undergoing chemotherapy: A randomized controlled trial. *Ann Phys Rehabil Med*. 2022;65(2):101485. doi:10.1016/j.rehab.2021.101485

94. Foulkes S, Costello BT, Howden EJ, et al. Exercise cardiovascular magnetic resonance reveals reduced cardiac reserve in pediatric cancer survivors with impaired cardiopulmonary fitness. *J Cardiovasc Magn Reson*. 2020;22(1):64. doi:10.1186/s12968-020-00658-4

95. Hornsby WE, Douglas PS, West MJ, et al. Safety and efficacy of aerobic training in operable breast cancer patients receiving neoadjuvant chemotherapy: A phase II randomized trial. *Acta Oncol*. 2014;53(1):65-74. doi:10.3109/0284186X.2013.781673

96. Kerrigan DJ, Reddy M, Walker EM, et al. Cardiac rehabilitation improves fitness in patients with subclinical markers of cardiotoxicity while receiving chemotherapy: A randomized controlled study. *J Cardiopulm Rehabil Prev*.:10.1097/HCR.0000000000000719. doi:10.1097/HCR.0000000000000719

97. Howden EJ, Bigaran A, Beaudry R, et al. Exercise as a diagnostic and therapeutic tool for the prevention of cardiovascular dysfunction in breast cancer patients. *Eur J Prev Cardiol*. 2020;26(3):305-315. doi:10.1177/2047487318811181

98. Lee K, Kang I, Mack WJ, et al. Feasibility of high intensity interval training in patients with breast Cancer undergoing anthracycline chemotherapy: A randomized pilot trial. *BMC Cancer*. 2019;19(1):653. doi:10.1186/s12885-019-5887-7

99. Lee K, Kang I, Mack WJ, et al. Effects of high-intensity interval training on vascular endothelial function and vascular wall thickness in breast cancer patients receiving anthracycline-based chemotherapy: A randomized pilot study. *Breast Cancer Res Treat*. 2019;177(2):477-485. doi:10.1007/s10549-019-05332-7

100. Kirkham AA, Eves ND, Shave RE, et al. The effect of an aerobic exercise bout 24 h prior to each doxorubicin treatment for breast cancer on markers of cardiotoxicity and treatment symptoms: A RCT. *Breast Cancer Res Treat*. 2018;167(3):719-729. doi:10.1007/s10549-017-4554-4

101. Kirkham AA, Lloyd MG, Claydon VE, Gelmon KA, McKenzie DC, Campbell KL. A longitudinal study of the association of clinical indices of cardiovascular autonomic function with breast cancer treatment and exercise training. *The Oncologist*. 2019;24(2):273-284. doi:10.1634/theoncologist.2018-0049

102. Lee K, Ortiz K, Goytizolo J, Serrano T, Dieli-Conwright CM. Abstract D033: High-intensity interval training is feasible in Hispanic patients with breast cancer undergoing anthracycline chemotherapy. *Cancer Epidemiol Biomarkers Prev*. 2020;29(6_Supplement_2):D033. doi:10.1158/1538-7755.DISP19-D033

103. Kelly RS, Kelly MP, Kelly P. Metabolomics, physical activity, exercise and health: A review of the current evidence. *Biochim Biophys Acta BBA - Mol Basis Dis*. 2020;1866(12):165936. doi:10.1016/j.bbadis.2020.165936

104. Shi M, Ellingsen Ø, Bathen TF, Høydal MA, Stølen T, Esmaeili M. The effect of exercise training on myocardial and skeletal muscle metabolism by MR spectroscopy in rats with heart failure. *Metabolites*. 2019;9(3):53. doi:10.3390/metabo9030053

105. Varghese SS, Johnston WJ, Eekhoudt CR, Keats MR, Jassal DS, Grandy SA. Exercise to reduce anthracycline-mediated cardiovascular complications in breast cancer survivors. *Curr Oncol*. 2021;28(5):4139-4156. doi:10.3390/curroncol28050351

106. Tranchita E, Murri A, Grazioli E, et al. The beneficial role of physical exercise on anthracyclines induced cardiotoxicity in breast cancer patients. *Cancers*. 2022;14(9):2288. doi:10.3390/cancers14092288

107. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

108. Bruce RA. Exercise testing of patients with coronary heart disease. Principles and normal standards for evaluation. *Ann Clin Res*. 1971;3(6):323-332.

109. Gibbons RJ, Balady GJ, Bricker JT, et al. ACC/AHA 2002 guideline update for exercise testing: summary article. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1997 Exercise Testing Guidelines). *J Am Coll Cardiol*. 2002;40(8):1531-1540. doi:10.1016/s0735-1097(02)02164-2

110. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381-1395. doi:10.1249/01.MSS.0000078924.61453.FB

111. Plana JC, Galderisi M, Barac A, et al. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: A report from the

American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr Off Publ Am Soc Echocardiogr*. 2014;27(9):911-939. doi:10.1016/j.echo.2014.07.012

112. Hamdy AM. Use of strain and tissue velocity imaging for early detection of regional myocardial dysfunction in patients with beta thalassemia. *Eur J Echocardiogr J Work Group Echocardiogr Eur Soc Cardiol*. 2007;8(2):102-109. doi:10.1016/j.euje.2006.02.004

113. Fallah-Rad N, Walker JR, Wassef A, et al. The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early left ventricular dysfunction in patients with human epidermal growth factor receptor II-positive breast cancer treated with adjuvant trastuzumab therapy. *J Am Coll Cardiol*. 2011;57(22):2263-2270. doi:10.1016/j.jacc.2010.11.063

114. MacLean B, Tomazela DM, Shulman N, et al. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinforma Oxf Engl*. 2010;26(7):966-968. doi:10.1093/bioinformatics/btq054

115. Pang Z, Zhou G, Ewald J, et al. Using MetaboAnalyst 5.0 for LC–HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat Protoc*. 2022;17(8):1735-1761. doi:10.1038/s41596-022-00710-w

116. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. doi:10.1093/nar/gkv007

117. Hayes AF. *Introduction to Mediation, Moderation, and Conditional Process Analysis*. 3rd ed. Guildford Press; 2022.

118. C.S.E.P.-P.A.T.H.-Working Group. *Canadian Society for Exercise Physiology - Physical Activity Training for Health (CSEP-PATH)*. Canadian Society for Exercise Physiology; 2013.

119. Araujo-Gutierrez R, Chitturi KR, Xu J, et al. Baseline global longitudinal strain predictive of anthracycline-induced cardiotoxicity. *Cardio-Oncol*. 2021;7(1):4. doi:10.1186/s40959-021-00090-2

120. López-Sendón J, Álvarez-Ortega C, Zamora Auñon P, et al. Classification, prevalence, and outcomes of anticancer therapy-induced cardiotoxicity: The CARDIOTOX registry. *Eur Heart J*. 2020;41(18):1720-1729. doi:10.1093/eurheartj/ehaa006

121. Kramer DG, Trikalinos TA, Kent DM, Antonopoulos GV, Konstam MA, Udelson JE. Quantitative evaluation of drug or device effects on ventricular remodeling as predictors of therapeutic effects on mortality in patients with heart failure and

reduced ejection fraction: A meta-analytic approach. *J Am Coll Cardiol*. 2010;56(5):392-406. doi:10.1016/j.jacc.2010.05.011

122. Park JJ, Park JB, Park JH, Cho GY. Global longitudinal strain to predict mortality in patients with acute heart failure. *J Am Coll Cardiol*. 2018;71(18):1947-1957. doi:10.1016/j.jacc.2018.02.064

123. Statistics Canada. Table 13-10-0821-01 Household population meeting/not meeting the 2020 Canadian physical activity guidelines. doi:https://doi.org/10.25318/1310082101-eng

124. Colley R, Watt J. *The Unequal Impact of the COVID-19 Pandemic on the Physical Activity Habits of Canadians*.; 2022. Accessed June 5, 2023. https://www150.statcan.gc.ca/n1/pub/82-003-x/2022005/article/00003-eng.htm

125. Magno S, Rossi MM, Filippone A, et al. Screening for Physical Activity Levels in Non-Metastatic Breast Cancer Patients Undergoing Surgery: An Observational Study. *Integr Cancer Ther*. 2022;21:15347354221140328. doi:10.1177/15347354221140327

126. Yuan Y, Fan S, Shu L, et al. Exploration the mechanism of doxorubicin-induced heart failure in rats by integration of proteomics and metabolomics data. *Front Pharmacol*. 2020;11:600561. doi:10.3389/fphar.2020.600561

127. Tan X, Zhang R, Lan M, et al. Integration of transcriptomics, metabolomics, and lipidomics reveals the mechanisms of doxorubicin-induced inflammatory responses and myocardial dysfunction in mice. *Biomed Pharmacother*. 2023;162:114733. doi:10.1016/j.biopha.2023.114733

128. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res*. 2023;51(D1):D587-D592. doi:10.1093/nar/gkac963

129. Nikolova-Karakashian MN, Reid MB. Sphingolipid metabolism, oxidant signaling, and contractile function of skeletal muscle. *Antioxid Redox Signal*. 2011;15(9):2501-2517. doi:10.1089/ars.2011.3940

130. Montealegre C, Verardo V, Luisa Marina M, Caboni MF. Analysis of glycerophospho- and sphingolipids by CE. *Electrophoresis*. 2014;35(6):779-792. doi:10.1002/elps.201300534

131. Gaggini M, Ndreu R, Michelucci E, Rocchiccioli S, Vassalle C. Ceramides as mediators of oxidative stress and inflammation in cardiometabolic disease. *Int J Mol Sci*. 2022;23(5):2719. doi:10.3390/ijms23052719

132. Froyen E, Burns-Whitmore B. The effects of linoleic acid consumption on lipid risk markers for cardiovascular disease in healthy individuals: A review of human intervention trials. *Nutrients*. 2020;12(8):2329. doi:10.3390/nu12082329

133. Zordoky BN, Sung MM, Ezekowitz J, et al. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PloS One*. 2015;10(5):e0124844. doi:10.1371/journal.pone.0124844

134. Mizrachi A, Ben-Aharon I, Li H, et al. Chemotherapy-induced acute vascular injury involves intracellular generation of ROS via activation of the acid sphingomyelinase pathway. *Cell Signal*. 2021;82:109969. doi:10.1016/j.cellsig.2021.109969

135. Koleini N, Nickel BE, Edel AL, Fandrich RR, Ravandi A, Kardami E. Non-mitogenic FGF2 protects cardiomyocytes from acute doxorubicin-induced toxicity independently of the protein kinase CK2/heme oxygenase-1 pathway. *Cell Tissue Res*. 2018;374(3):607-617. doi:10.1007/s00441-018-2905-z

136. Li C, Carli F, Lee L, et al. Impact of a trimodal prehabilitation program on functional recovery after colorectal cancer surgery: a pilot study. *Surg Endosc*. 2013;27(4):1072-1082. doi:10.1007/s00464-012-2560-5

137. Abrahams C, Woudberg NJ, Lecour S. Anthracycline-induced cardiotoxicity: targeting high-density lipoproteins to limit the damage? *Lipids Health Dis*. 2022;21(1):85. doi:10.1186/s12944-022-01694-y

138. El Hadi H, Di Vincenzo A, Vettor R, Rossato M. Relationship between heart disease and liver disease: A two-way street. *Cells*. 2020;9(3):567. doi:10.3390/cells9030567

139. Karppinen JE, Törmäkangas T, Kujala UM, et al. Menopause modulates the circulating metabolome: evidence from a prospective cohort study. *Eur J Prev Cardiol*. 2022;29(10):1448-1459. doi:10.1093/eurjpc/zwac060

140. Lee-Sarwar KA, Lasky-Su J, Kelly RS, Litonjua AA, Weiss ST. Metabolome–Microbiome Crosstalk and Human Disease. *Metabolites*. 2020;10(5):181. doi:10.3390/metabo10050181

141. Mao C, Wang M, Li L, Tang J. Circulating metabolites serve as diagnostic biomarkers for HER2-positive breast cancer and have predictive value for trastuzumab therapy outcomes. *J Clin Lab Anal*. 2022;36(2):e24212. doi:10.1002/jcla.24212

142. Worley B, Powers R. PCA as a practical indicator of OPLS-DA model reliability. *Curr Metabolomics*. 2016;4(2):97-103. doi:10.2174/2213235X04666160613122429

143. Cooke R, Jones A. Recruiting adult participants to physical activity intervention studies using sport: a systematic review. *BMJ Open Sport Exerc Med*. 2017;3(1):e000231. doi:10.1136/bmjsem-2017-000231

144. Prieto-Avalos G, Cruz-Ramos NA, Alor-Hernández G, Sánchez-Cervantes JL, Rodríguez-Mazahua L, Guarneros-Nolasco LR. Wearable Devices for Physical

Monitoring of Heart: A Review. *Biosensors*. 2022;12(5):292. doi:10.3390/bios12050292

145. Nicholson JK, Holmes E, Kinross JM, Darzi AW, Takats Z, Lindon JC. Metabolic phenotyping in clinical and surgical environments. *Nature*. 2012;491(7424):384-392. doi:10.1038/nature11708

146. Peters MDJ, Marnie C, Tricco AC, et al. Updated methodological guidance for the conduct of scoping reviews. *JBI Evid Synth*. 2020;18(10):2119-2126. doi:10.11124/JBIES-20-00167

147. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS Peer Review of Electronic Search Strategies: 2015 guideline statement. *J Clin Epidemiol*. 2016;75:40-46. doi:10.1016/j.jclinepi.2016.01.021

Appendix A: Search Strategy

Search conducted on Aug 19, 2022 & November 22, 2022

Table 9: Search Strategy Employed in EMBase

Search	Keywords
1	'cardiotoxicity'/exp or 'cardiac toxicity'/exp or 'cardio toxicity'/exp or 'cardiotoxic effect'/exp or 'cardiotoxicology'/exp or 'heart toxicity'/exp or 'toxicity, heart'/exp
2	(((‘cardiotox*’ or heart*) near/3 (arrhyth* or death* or fail* or stress* or attack*)) or (myocard* near/1 (it* or infarc*))):ti,ab,kw
3	1 or 2
4	'anthracycline antibiotic agent'/exp/mj or 'anthracycline antibiotic'/exp or 'anthracycline antibiotic agent'/exp or 'anthracycline antibiotics'/exp or 'antibiotic agent, anthracycline'/exp or 'antibiotics, anthracycline'/exp
5	(‘anthracyc*’ or ‘doxorubicin*’ or ‘idarubicin*’ or ‘daunorubicin*’ or ‘mitoxantrone*’ or ‘cerubidine*’ or ‘adriamycin*’ or ‘ellence*’ or ‘valstar*’ or ‘idamycin*’):ti,ab,kw
6	4 or 5
7	'metabolomics'/exp or 'metabolism':ti,ab,kw
8	(‘metabolomic*’ or ‘metabolism*’):ti,ab,kw
9	7 or 8
10	3 and 6 and 9
11	'exercise'/exp OR 'exercise capacity'/exp OR 'exercise performance'/exp OR 'exercise training'/exp OR 'fitness training'/exp OR 'fitness workout'/exp OR 'physical conditioning, human'/exp OR 'physical exercise'/exp OR 'physical work-out'/exp OR 'physical workout'/exp
12	'exercis*':ti,ab,kw OR 'work out':ti,ab,kw OR 'working out':ti,ab,kw OR 'fitness*':ti,ab,kw OR ((physical NEAR/3 (move* OR activit* OR exert* OR effort* OR train* OR performance* OR practice*)):ti,ab,kw)
13	7 OR 8
14	3 AND 6 AND 13

Table 10: Search Strategy Employed in MEDLINE

Search	Keywords
1	exp *cardiotoxicity/ or cardiotoxicity.ti,ab,kf. or arrhythmias, cardiac/ or cardiomyopathies/ or heart failure/
2	((('cardiotox*' or heart*) adj3 (arrhyth* or death* or fail* or stress* or attack*)) or (myocard* adj1 (it* or infarc*))).ti,ab,kf.
3	1 or 2
4	exp *anthracyclines/ or anthracyclines.ti,ab,kf.
5	('anthracyc*' or 'doxorubicin*' or 'idarubicin*' or 'daunorubicin*' or 'mitoxantrone*' or 'cerubidine*' or 'adriamycin*' or 'ellence*' or 'valstar*' or 'idamycin*').ti,ab,kf.
6	4 or 5
7	exp *metabolomics/ or metabolomics.ti,ab,kf. or "metabolic networks and pathways"/ or metabolism/
8	'metabol*'.ti,ab,kf.
9	7 or 8
10	3 and 6 and 9
11	exp *Exercise/ or Exercise.ti,ab,kf. or Circuit-Based Exercise/ or Exercise Therapy/ or Plyometric Exercise/ or Warm-Up Exercise/ or Cool-Down Exercise/
12	(Exercis* or "work out" or "working out" or fitness* or (physical adj3 (move* or activit* or exert* or effort* or train* or performance* or practice*))).ti,ab,kf.
13	11 OR 12
14	3 AND 6 AND 13

The search strategy was developed following the Joanna Briggs Institute's three-step search strategy.[146] The search strategy was peer-reviewed by a health librarian (Dalhousie University) per the Peer Review of Electronic Search Strategies guidelines.[147] All empirical studies were considered, including experimental and quasi-experimental study designs such as randomized controlled trials, pre- and post-studies, interrupted time series, and nonrandomized controlled trials. The reference lists of reviews, meta-analyses, editorials, conference proceedings, and policy documents were also considered. Following the search, all identified citations were collated, uploaded to Covidence (https://www.covidence.org/), and assessed for their relevance to the thesis topic.

Appendix B: Brochure

EXACT 2.0

Exercise is known to improve heart health but we do not know what the benefits are when heart damage is due to chemotherapy side effects. The doctors and researchers involved in this study are interested in the following question:

- Can a 24-week exercise program help protect the heart against heart damage in patients receiving anthracycline chemotherapy?

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Halifax, NS
B3H 2Y9

Phone: 902-473-2035
Email: EXcancer@nshealth.ca

PHYSICAL ACTIVITY FOR PERSONS LIVING WITH OR AFFECTED BY CANCER

EXERCISE TO PREVENT ANTHRACYCLINE-BASED CARDIOTOXICITY

V1

NSHA REB Romeo File#: 1024489

EXACT 2.0

EXACT 2.0

TEL: 902-473-2035

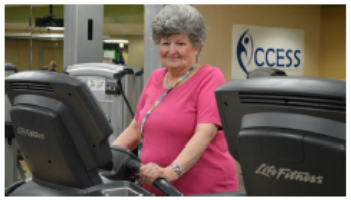
January 26, 2022

EXACT 2.0

WHO?

1.Breast cancer patients pre-treatment.

2.18-75 years old



WHAT?

- A free 6-month home-based exercise program to prevent chemo heart damage
- Equipment provided
- 2 session per week as scheduled by you.
- An individualized program created and monitored by a certified exercise professional.
- Safe, fun and effective!

WHEN?

- Before you start treatment

WHERE?

- Your home!

WHY?

Exercise is important for everyone's heart health, but it is especially important for new breast cancer patients.

Exercise:

- Increases energy levels.
- Prevents chemo side-effects.
- Improves bone and muscle mass.
- Enhances immunity.
- Improves heart function.
- Decreases mortality.

HOW?

- Ask your nurse or doctor about the EXACT 2.0 study to see how you can sign up
- Ask your nurse or doctor to be placed on a contact list and we will call you

OR

- Come visit us on the fifth floor of the Dickson building. We are just to the left as you leave the elevator and are across from the women's

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Appendix C: Informed Consent

STUDY TITLE: **EX**ercise to prevent **A**nthraCycline-based **C**ardio-**T**oxicity (EXACT 2.0) in individuals with breast cancer

CLINICAL STUDY REGISTRATION NUMBER: NCT03748550

PRINCIPAL INVESTIGATOR: Scott A. Grandy, Ph.D.

School of Health and Human Performance
Dalhousie University,
NSHA Affiliate Scientist,
Division of Cardiology
(902) 494-4303

FUNDER: Canadian Cancer Society
Canadian Institutes of Health Research

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1. Introduction

You have been invited to take part in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study. You may take as much time as you wish to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor.

Please ask the research team or the principal investigator to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

The researchers will:

- Discuss the study with you;
- Answer your questions;
- Be available during the study to deal with problems and answer questions.

You are being asked to consider participating in this study because you have been diagnosed with breast cancer and will be treated with a type of chemotherapy called anthracyclines.

If you decide not to take part or if you leave the study early, your cancer treatment and your usual health care will not be affected.

2. Why is there a need for this study?

With improvements in cancer treatments, more people diagnosed with cancer are becoming long-term survivors. However, studies have shown that the damage caused by the treatments responsible for this success can lead to other health problems. One of the most concerning problems associated with a cancer drug known as anthracyclines (type of chemotherapy) is its damaging effect on the heart, leading to an increased risk of heart disease.

Aerobic exercise (e.g., riding a bike, walking, or swimming) has been shown to be very safe and beneficial for those with cancer. Cancer patients are often encouraged to increase their levels of physical activity to help improve their fitness, health, and overall quality of life. While exercise has been shown to be beneficial for individuals receiving cancer treatments, very little is known about how exercise may protect the heart of those receiving anthracyclines. It also is not clear whether exercise performed at home leads to the same health benefits as those seen in individuals performing exercise in a supervised program. Therefore, the purpose of this study will be to collect information on the benefits of a 6-month home-based exercise program on heart health in those individuals receiving anthracycline-based chemotherapy.

3. What is being tested?

Research studies have shown that performing aerobic exercise before or during anthracycline therapy helps to prevent damage to heart as well as maintain heart function. However, these research studies have mostly been conducted on animals and it is not clear whether exercise has the same protective effects on the heart in humans. Thus, this study will examine the benefits of a home-based aerobic exercise program on heart health in patients receiving anthracycline therapy.

4. How long will I be in the study?

The first part of this study is approximately 6-months long. If you decide to participate you will be invited to complete a 6-month (e.g., walking, cycling) home-based exercise program. You will be asked to complete two exercise sessions per week which are expected last between 30 to 60 minutes (for a total of 48 training sessions over 6-months). In addition to the exercise program, you will also be asked to complete two testing sessions. The first will take place about one week before starting the exercise training. The second will take place following the completion of the 6-months program. This session will be scheduled within 4-weeks of the completion of your exercise program. Each assessment

will take approximately 1-2 hours to complete. Your total time commitment for the first part of the study would be about 52 hours.

To help us better understand the potential long-term protective effects of exercise on your heart we would like to ask your permission to contact you in the future regarding new follow-up studies. These studies will be designed to assess your health through in person visits and/or reviewing your healthcare file. At time of contact you will be provided with the specific details of the study and will have the option to participate or decline. The additional studies will take place over the next 10 years. If you indicate, you are interested in learning more about these new studies you will not be contacted more than 1-2 times per year.

5. How many people will take part in this study?

It is anticipated that about 100 people will participate in this study, 50 people in Nova Scotia and 50 people from St. Boniface Hospital (Winnipeg, MB).

6. How is the study being done?

Adult female breast cancer patients that are receiving a specific type of chemotherapy known as anthracyclines will be invited from breast cancer clinics in Nova Scotia and St. Boniface Hospital (Winnipeg, MB). Participants in this study will be randomly (by chance) placed in one of two study groups: 1) control or 2) exercise. All participants will undergo testing prior to starting the study and then will undergo the same testing again at the end of the study. Participants randomized to the control group will receive the standard treatment for their cancer. Participants randomized to the exercise group will receive standard treatment plus a 6-month home based aerobic exercise program. All data will be analyzed in Halifax, Nova Scotia, except for the heart ultrasound (echocardiogram), which will be analyzed by the team cardiologist (JS) in Winnipeg, Manitoba.

The study will involve you coming to the hospital for 1 or 2 appointments for the baseline testing and 1 or 2 appointments for the post-study testing. Each appointment will last 1-2 hours.

7. What will happen if I take part in this study?

If you agree to take part in the study, you will be asked to complete the following:

BASELINE ASSESSMENT

During the baseline assessment you will be asked to complete a survey and provide some basic information about yourself (e.g., age, sex, occupation, household income, lifestyle behaviors, quality of life, etc.). It will take you approximately 20-25 minutes to complete the survey. You may skip any questions that you are uncomfortable answering. We will also measure your height, weight, and waist size. A picture of your heart will be taken using an ultrasound machine. This procedure is described in more detail below. You

will also be asked to complete an exercise stress test and provide a blood sample (about 2 tablespoons of blood). The exercise stress test is described in more detail below. The blood sample will be drawn by a trained research nurse. The collection of the blood sample and completion of the stress test and ultrasound is necessary for study participation. All blood samples will be taken to Dr. Scott Grandy's secure research lab (Dalhousie University) where it will be stored in a secured freezer until it is analyzed. Approximately 1 tablespoon of blood will be sent to Winnipeg for analysis and 1 tablespoon of blood will be analyzed in Halifax.

Echocardiogram

An echocardiogram uses sound waves to produce an image of your heart. This image allows us to see your heart beating and pumping blood. For this procedure the technician will place gel on a transducer which looks like an electric shaver. The technician will then press the transducer against your chest over your heart in order to get the picture. The technician may have to try different positions in order to get the best picture. This procedure takes place in a private room. This procedure is not likely to cause any discomfort, but if it does please let the technician know immediately. Echocardiograms will be sent to Winnipeg for analysis. All files will be deidentified scans and sent via secure file transfer.

Exercise Stress Test

The exercise stress test will be supervised by a cardiologist. Prior to beginning the test, electrocardiograph (ECG) or heart monitoring stickers will be placed on your chest (a private room will be available for the application the stickers). The electrode stickers will then be attached to cables which link to an ECG machine (allowing us to monitor your heart while exercising). You will then be asked to perform a graded exercise test by walking on a treadmill. You will begin at a very slow pace. The speed and incline (slope) of the treadmill will increase every three minutes until you feel that you are no longer able to continue. If you feel unwell during the test, you will be asked to tell the doctor and technician at once. The test will be stopped if you feel severe chest pain or become very tired or short of breath.

6-MONTH EXERCISE PROGRAM

The aerobic exercise training program will be developed by a specially trained member of the research team, a certified exercise physiologist (CEP). The intensity (i.e., how hard you will work) of the exercise program will be determined by an assessment of your current fitness level and abilities. This will be determined using the results from your baseline stress test. To ensure that you are not working too hard, we will provide you with a heart rate monitor (worn around your wrist) so that we can monitor how hard you are exercising. At the end of your baseline assessment you will be given your 6-month home based exercise program. The CEP will explain how to perform the program as well as how to use the heart rate monitor. The CEP or a member of the research team will follow-up with you on a weekly basis to see if you have any questions or if your exercise program needs to be adjusted. You will also be given a number that you can call at any time if you have questions about your exercise program.

POST TRAINING ASSESSMENT

Following the completion of the exercise program, you will be asked to complete a survey assessing your lifestyle behaviors and overall quality of life. We will repeat the measures of weight and waist girth. You will also be asked to complete another exercise stress test and provide a final blood sample as well as the ultrasound of your heart. This assessment will be scheduled with 4-weeks of completing your exercise program. To make sure that you maintain the benefits that you gained from the exercise program we ask that you continue to exercise 2 times per week at a moderate intensity until you come in for the final assessment. A member of the research team will explain the specifics of what you need to do when they call to schedule your post-training assessment.

OPTIONAL FOLLOW-UP

If you agree, we will follow your health for up to 10 years. The bulk of this follow-up will be done "behind the scenes" by accessing your hospital records and other health databases. However, you may also be contacted in the future to provide additional information on your health and lifestyle behaviors (e.g., physical activity) or to return to our assessment center to provide additional health data (e.g., blood samples, fitness assessments, heart scans). You may indicate your consent for long-term follow-up on the signature page.

ADDITIONAL RESPONSIBILITIES

Throughout your participation in the study, it is important that you tell the research team about any new treatment therapies, drugs or medicines you are taking or wish to take. You must also tell the research team about anything unusual that is happening with your health. This includes any medical problems that seem to be getting worse. If you have to see another doctor or have to go to a hospital, you should let the doctors know that you are in a research study. You should also tell your own doctor as quickly as possible, for your safety.

NOTE: You may decide not to take part in any of these activities and to stop participating in the study at any time by contacting the research team.

8. Are there risks to the study?

As with any physical activity program or study there are some risks. To give you the most complete information available, we have listed the *possible* risks, which may appear alarming. We do not want to alarm you, but we do want to make sure that you have had a chance to think about all the risks carefully before you choose to participate. Please also be aware that there may be risks in participating in this study that we do not know about yet.

Physical activity studies have shown that a very common side-effect of training for both those with and without cancer is mild fatigue, shortness of breath, increased body temperature, muscle soreness and/or stiffness. These symptoms will vary depending on your level of fitness. For example, if you have not exercised for a long time, it is likely that you will experience greater muscle stiffness at the beginning of the program than at the end. These side-effects typically go away within 1-2 days. However, if they last longer or

you are concerned you can contact one of the members of the research team to discuss your concerns. Study staff will check in with you on a weekly basis to ensure that you are doing your exercise correctly and not doing too much exercise. This will decrease your risk of experiencing unnecessary fatigue or muscle soreness. If you experience an injury during training, please seek the necessary medical treatment and then report the injury to the research team. We also ask that you to report any other injuries or illnesses that occur during the time of the study.

Exercise stress testing has been shown to be a safe procedure with the risk of a serious adverse event occurring (e.g., life threatening complication) is very rare (i.e., less than 5 per 100,000 tests). Given that many cancer patients receive treatments that may impact exercise tolerance, the risk of an adverse event may be elevated. A cardiologist will be present for all testing and we will closely monitor your response to the test to maximize your safety.

In addition to the exercise program, you will be asked to complete two surveys (one at each assessment) and provide two blood samples. These surveys will ask you questions about your lifestyle behaviors and overall quality of life. If you are uncomfortable in responding to any of these questions you can leave them blank or you are free to withdraw from the study at any time without penalty. There is a possibility of pain, bruising, swelling or infection related to giving blood. These discomforts are minimal and brief.

To protect your information, we will not keep your name or other information that may identify you with the sample; only a code number. Files that link your name to the code number will be kept in a secure place. Although no one can absolutely guarantee confidentiality, using a code number makes the chance much smaller that someone other than the research staff or other authorized groups or persons (discussed later in the consent form) will ever be able to link your name to your sample or to any test results.

The effects or discomforts of tests/procedures that are part of this study but are also part of your normal clinical care (e.g., heart scan, additional blood tests) will be reviewed by your treating physician. In order to ensure your safety, a copy of this consent form, outlining the study details and contact information, will be sent to your primary oncologist.

You will be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the research team.

9. Are there benefits of participating in this study?

You may or may not benefit directly from participating in this study. However, possible benefits include improved fitness and quality of life. Your participation may or may not help other people with cancer receiving treatment in the future.

10. Are there other choices?

You are free to seek other opinions or choices if you wish. You do not have to participate in this trial to begin an exercise program or to become more physically active. You may choose to speak with your physician, oncologist, or a qualified fitness expert about physical activity.

11. What happens at the end of the study?

If you would like a summary of the results, please notify the research team and a summary will be mailed or emailed to you upon completion of the study. Should you be interested in learning more about the physical activity options in your area, we encourage you to speak to your physician, oncologist, or the research team CEP.

12. What are my responsibilities?

As a study participant you will be expected to:

- Read and sign the consent form;
- Follow the directions of the research team;
- Complete the 6-month, home based, biweekly exercise program;
- Complete the testing at the beginning and end of the study;
- Report any problems that you experience that you think might be related to participating in the study; and
- Report any changes to your health during the time of the study (even those occurring outside of the study).

13. Can my participation in this study end early?

The Nova Scotia Health Authority Research Ethics Board and the principal investigator have the right to stop patient recruitment or cancel the study at any time.

The principal investigator may decide to remove you from this study without your consent for any of the following reasons:

- There is new information that shows being in this study is not in your best interest;
- You are experiencing side-effects that are harmful to your health or well-being;
- You are not following the directions of the Principal Investigator or research team;
- The Principle Investigator or Nova Scotia Health Authority Research Ethics Board decides to stop the study.

If you are withdrawn from this study, a member of the research team will discuss the reasons with you and plans will be made for your continued care outside of the study.

You can also choose to end your participation at any time. If you choose to withdraw from this study by providing notice to the research team, your decision will have no effect on your current or future medical treatment and healthcare.

If you withdraw your consent, the information about you, including all completed assessments (e.g., exercise stress test, questionnaires, blood samples) that were collected before you left the study will still be used. No new information about you will be collected (and no further testing of your blood samples will be done without your permission). If you wish to withdraw from the study, please inform the study staff.

14. What will happen to my sample after the study is over?

After this study is over, we will dispose of all the samples we collected as part of the study by burning them.

15. What about new information?

It is possible that new information may become available while you are in the study that might affect your health, welfare, or willingness to stay in the study. You will be told about the new information and then asked whether you wish to continue taking part in the study or not.

16. Will It Cost Me Anything?

Compensation

We will reimburse you in accordance with the NSHA travel policy for study-related expenses resulting from significant travel including mileage, lodging and meals. Mileage will be reimbursed for a rate of $0.46 CAD per kilometer, lodging will be reimbursed upon receipt of original supporting invoices at a rate not to exceed $200 CAD plus tax per night of lodging, and patient meal will be reimbursed up to $43 per day, per patient. Travelling caregivers will also be reimbursed up to $43 per day.

If needed, patient parking and public transportation will be reimbursed at $15 CAD per day and taxi services not exceeding $75 CAD per one-way trip.

17. What about my privacy and confidentiality?

Protecting your privacy is an important part of this study and every effort to protect your privacy will be made. However, complete privacy cannot be guaranteed. For example, the principal investigator may be required by law to allow access to research records. Also, as your physician/oncologist has reviewed your medical history to ensure your fit with this study he/she will be aware that you are taking part in the study.

If the results of this study are presented to the public, nobody will be able to tell that you were in the study.

If you decide to participate in this study, the research team will collect personal health information from you and your health record. The research team will collect and use only the information they need for this study and to judge the safety and usefulness of the study.

"Personal health information" is health information about you that could identify you because it includes information such as your;

- Name,
- Information from the study surveys;
- New and existing medical records; or
- The types, dates and results of various tests and procedures.

Access to Records

Other people may need to look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines. These people might include:

- The Nova Scotia Health Authority Research Ethics Board and people working for or with the Nova Scotia Health Authority Research Ethics Board because they oversee the ethical conduct of research studies within the Nova Scotia Health Authority.

These people will view your study records at this institution and will not take identifying information away with them.

Use of Your Study Information

To protect your information, we will not keep your name or other information that may identify you with any of the study measurements; only a code number. Files that link your name to the code number will be kept separately from any of the measurements, samples or other information about you. Although no one can absolutely guarantee confidentiality, using a code number makes the chance much smaller that someone other than the research staff or other authorized groups or persons will ever be able to link your name to your sample or to any test results.

Information collected for this study will be kept for 25 years. Information will be stored in a databank at Nova Scotia Health Authority in Halifax. Information may be shared with other researchers for the purposes of health research. Any study data about you that is sent outside of the Nova Scotia Health Authority will have a code and will not contain your name or address, or any information that directly identifies you.

The REB and people working for or with the REB may also contact you personally for quality assurance purposes.

Your Access to Records

You may ask the study researchers to see the information that has been collected about you.

18. Declaration of Financial Interest

The Canadian Cancer Society and Canadian Institutes of Health Research are reimbursing the principal investigator and/or the principal investigator's institution to conduct this study. The amount of payment is sufficient to cover the costs of conducting the study.

19. What About Questions or Problems?

For further information about the study you may call the principal investigator who is the person in charge of this study:

The principal investigator is Dr. Scott Grandy.
Telephone: 902-494-4303
Email: Scott.Grandy@dal.ca

If you experience any symptoms or possible side effects or other medical problems, please let the principal investigator or research coordinator know as soon as possible.

If you can't reach the principal investigator or research coordinator, or it is after regular business hours, speak to the physician on call. The after hour's number is **(902) 473-2222.**

This doctor may not be the one you usually see while in this study. Please call the principal investigator or research coordinator the next business day to tell them about the possible side effects or other medical problems you experienced.

20. What Are My Rights?

You have the right to all information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction before you make any decision. You also have the right to ask questions and to receive answers throughout this study. You have the right to withdraw your consent at any time.

If you have questions about your rights as a research participant, and/or concerns or complaints about this research study, you can contact the Nova Scotia Health Authority Research Ethics Board manager at 902-473-8426 or Patient Relations at (902) 473-2133 or 1-855-799-0990 or healthcareexperience@nshealth.ca.

In the next part you will be asked if you agree (consent) to join this study. If the answer is "yes", please sign the form.

21. Consent Form Signature Page

I have reviewed all of the information in this consent form related to the study called:

EXercise to prevent **A**nthraCycline-based **C**ardio-**T**oxicity (EXACT 2.0)
in individuals with breast cancer

I have been given the opportunity to discuss this study. All of my questions have been answered to my satisfaction.

I authorize access to my personal health information, and research study data as explained in this form.

This signature on this consent form means that I agree to take part in this study. I understand that I am free to withdraw at any time without affecting my future care.

☐ I **agree** to permit the researchers to re-contact me to consider participation in future related research studies. (If yes, please provide contact information: _____)

☐ I **do not agree** to permit the researchers to re-contact me to consider participation in future related research studies.

_____ _____ ____/____/___

Signature of Participant Name (Printed) Year Month Day*

_____ _____ ____/____/___

Signature of Person Conducting Consent Discussion Name (Printed) Year Month Day*

_____ _____ ____/____/___

Signature of Investigator Name (Printed) Year Month Day*

Note: Please fill in the dates personally

I will be given a signed copy of this consent form.
Thank you for your time and patience!

Appendix D: Health Habits Questionnaire

GETTING TO KNOW YOU

The information within this section is needed to help understand the characteristics of the people participating in this study. For this reason, it is very important information. **Be assured that it will remain confidential.**

1. Your date of birth: DD _____ MM _____ YYYY _____

2. Sex (tick the box): □ Female □ Male

3. What is your marital status? (tick the box that best describes you):
□ Never married □ Divorced □ Living common law □ Married □ Separated
□ Widowed

4. Ethnicity: (tick the box that best describes you):
□ White □ Korean □ Latin American
□ Black □ Filipino □ Aboriginal people of North America
□ Chinese □ Arab □ Japanese
□ South Asian □ Other: _____

5. What is the highest level of education that you have completed?
□ Elementary school
□ High school
□ Trade, technical or vocational school
□ Diploma from a community college or non-university certificate
□ University certificate below Bachelor's level
□ Bachelor's degree
□ Graduate degree (MSc, MBA, MD, PhD, etc.)
□ None

6. What is your employment status? (tick the box that best describes you):
□ Full-time □ Unemployed □ Retired □ Part-time □ Homemaker
□ Presently on disability leave □ Doing unpaid or volunteer work □ Student

7. The next question asks about your household income. We understand that this information is very private, but the question is important as it helps us to understand whether the study includes a wide variety of participants. All answers will be kept anonymous and strictly confidential. Which category best describes the total income of all household members, before taxes, for last year?

□ Less than $10,000
□ $10,000-$24,999
□ $25,000-$49,999

□ $50,000-$74,999
□ $75,000-$99,999
□ $100,000-$149,999
□ $150,000-$199,999
□ $200,000 or more
□ Don’t know
□ Prefer not to answer

Appendix E: International Physical Activity Questionnaire

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the ***last 7 days***.

- Please answer each question even if you do not consider yourself to be an active person.
- Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.
  Think about all the **vigorous** and **moderate** activities that you did in the ***last 7 days***.

- **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.
- **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. *Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family.* These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No ***Skip to PART 2: TRANSPORTATION*** →

The next questions are about all the physical activity you did in the ***last 7 days*** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the ***last 7 days,*** on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for ***at least 10 minutes at a time*.**

   _____ **days per week.**

   No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for ***at least 10 minutes at a time***. During the ***last 7 days,*** on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the ***last 7 days***, on how many days did you **walk** for ***at least 10 minutes at a time*** **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the ***last 7 days***, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

No travelling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the ***last 7 days***, on how many days did you **bicycle** for ***at least 10 minutes at a time*** to go **from place to place**?

_____ **days per week**
No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the ***last 7 days***, on how many days did you **walk** for ***at least 10 minutes at a time*** to go **from place to place**?

_____ **days per week**

No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the ***last 7 days*** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the ***last 7 days***, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for ***at least 10 minutes at a time***. During the ***last 7 days***, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for ***at least 10 minutes at a time***. During the ***last 7 days***, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the ***last 7 days*** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the ***last 7 days***, on how many days did you **walk** for ***at least 10 minutes at a time*** **in your leisure time**?

_____ **days per week**

No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for ***at least 10 minutes at a time***. During the ***last 7 days***, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for ***at least 10 minutes at a time***. During the ***last 7 days***, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the ***last 7 days***, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the ***last 7 days***, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

Appendix F: Echocardiogram Protocol

Transthoracic Echocardiography		
Parasternal Long Axis	2-D	Left Ventricle
	Colour Flow Imaging	Aortic Valve, Mitral Valve
	Zoom	Left Ventricular Outflow Tract
Right Ventricular Inflow Tract	2-D	Right Ventricle
	Colour Flow Imaging	Tricuspid Valve
Parasternal Short Axis	2-D	Right Ventricular Outflow Tract
	Pulsed Wave	Right Ventricular Outflow Tract
	Continuous Wave	Pulmonary Valve
	Colour Flow Imaging	Tricuspid Regurgitation
	Continuous Wave	Tricuspid Regurgitation
	2-D 30-70 Fps	*Base Left Ventricle
	2-D 30-70 Fps	*Mid Left Ventricle
	2-D 30-70 Fps	*Apex (Important)
	M-Mode	Left Ventricle
	Colour Tissue Doppler Imaging	Left Ventricle
Apical 4-Chamber	2-D	All 4 Chambers
	2-D	Left Ventricle for Biplane Ejection Fraction
	2-D 30-70 Fps	*LV For Speckle Strain
	Colour Flow Imaging	Mitral Valve
	Pulsed Wave	Mitral Valve (Align with Flow)
	Continuous Wave	Mitral Regurgitation
	Continuous Wave	Mitral Valve (Align with Flow)
	Colour Tissue Doppler Imaging	Left Ventricle
	Colour Tissue Doppler Imaging	Right Ventricle
	Tissue Doppler Imaging	Med Annulus
	Tissue Doppler Imaging	Lateral Annulus
Apical 3-Chamber	2-D	Left Ventricle
	2-D 30-70 Fps	*Left Ventricle for Speckle Strain
	Colour Flow Imaging	Aortic Valve, Mitral Valve
	Colour Tissue Doppler Imaging	Left Ventricle
	Pulsed Wave	Left Ventricular Outflow Tract
Apical 2-Chamber	2-D	Left Ventricle, Left Atrium
	2-D	Zoom Left Atrium (Volumes)
	2-D	Zoom Left Atrium

	Colour Tissue Doppler Imaging	Left Ventricle
Apical 4 Chamber (Right Ventricle Focused View)	2-D 30-70 Fps	*Left Ventricle for Speckle Strain
	2-D 30-70 Fps	*Right Ventricle for Speckle Strain
	2-D	Right Ventricle, Right Atrium
	Pulsed Wave	Tricuspid Valve Inflow
	Tissue Doppler Imaging	Right Ventricle Annulus
	M-Mode	Tricuspid Annular Plane Systolic Excursion
	Continuous Wave	Tricuspid Regurgitation
Subcostal	2-D	Inferior Vena Cava
	Colour Flow Imaging	Hepatic Veins
Suprasternal Notch	Colour Flow Imaging	Descending Aorta
	Pulsed Wave	Superior Vena Cava

Abbreviations: 2-D, 2-dimensional; FPS, frames per second.

Appendix G: Reverse Phased Chromatographic Separation