

Cardiovascular Function in HER2-positive Breast Cancer Survivors

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Abstract

Background: Trastuzumab, a HER2 antagonist, has been shown to improve rates of overall survival in patients with HER2-positive breast cancer. However, cardiotoxic effects associated with treatment limits these benefits. Due to an increase in survival years, women with a history of HER2-positive breast cancer might be at increased risk of developing cardiotoxicity (i.e., LVEF < 55% with or without accompanying symptoms of heart failure) during survivorship. Exercise (“stress”) conditions is an established method to examine comprehensively impairments in cardiovascular function that may go undetected during resting conditions providing insight into the risk for future cardiac events.

Purpose: We sought to assess cardiac and vascular function of HER2-positive breast cancer survivors at rest and under exercise conditions. **Hypothesis:** 1) At rest, vascular dysfunction would be evident in breast cancer survivors. 2) Exercise would reveal cardiac dysfunction in HER2-positive breast cancer survivors, which was not apparent under resting conditions.

Methods: Eight HER2-positive breast cancer survivors and eight age-matched healthy controls underwent the following measures: 1) resting vascular function (Applanation tonometry, pulse wave velocity and baroreceptor sensitivity) and cardiac function (2D-echocardiography), 2) exercise haemodynamics (heart rate, stroke volume, cardiac output) during an incremental exercise test, and 3) ventricular-vascular coupling (left ventricular elastance and arterial elastance) at rest and during incremental exercise.

Results: At rest, there were no differences between groups in any echocardiographic measures. No differences were detected in vascular function, although baroreceptor sensitivity was reduced in the breast cancer group. Resting ventricular-vascular coupling was also similar between groups. With exercise, there was evidence of reduced LV performance with a blunted response to increase LV elastance. No differences were observed in arterial elastance or ventricular-vascular coupling. All haemodynamic responses were similar between groups. **Conclusion:** In HER2-positive breast cancer survivors there are indicators of impairments in cardiac function during exercise conditions, but limited differences during resting conditions. Exercise echocardiography may be clinically relevant for detecting impairments in left ventricular function in HER2-positive breast cancer survivors that appear to have normal cardiac function at rest.

Preface

I, Alis Bonsignore, completed the writing and conception of this thesis. I was also responsible for collection of data, data acquisition from varying techniques and data analysis. An additional PhD student assisted with data collection. My committee members including Dr. Darren Warburton, Lee Jones and Shannon Bredin also provided support for conception and implementation of research.

A version of Chapter 3 will be submitted for peer review. Alis Bonsignore was responsible for writing the manuscript, while the co-authors made significant intellectual contributions for the systematic review as well as edits to the manuscript.

A version of Chapter 4 will also be submitted for peer review. Alis Bonsignore will be responsible for writing the manuscript, while the co-authors made significant contributions to collection of the data and will play an active role in providing edits for the manuscript.

The data from this research investigation was obtained with approval from the UBC Clinical Research Ethics Board under Certificate H13-02916.

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List of Abbreviations

Abbreviation

LVEF
LV
SEER
VO₂peak
2D
VVC
E_{LV}
Ea
SV
Q
PWV
BRS
HER2/ERBB2
IHC
FISH
HERA
NSABP
NCCTG
BCIRG
TCH
ACT
ACT-T

FEC
D/V
CREC
SOLD
NYHF
PI3K
AKT
PTEN
PDK1
PDK2
mTOR
MAPK
ERK
JNK
VEGF
NRG1
ROS
AMPK
PGC-1 α PGC-1b

Definition

Left ventricular ejection fraction
Left Ventricle
Surveillance, Epidemiology and End Results-Medicare
Peak aerobic power
2-dimensional
Ventricular-vascular coupling
Ventricular elastance
Arterial elastance
Stroke volume
Cardiac output
pulse wave velocity
Baroreceptor Sensitivity
Human epidermal growth factor receptor 2
Immunohistochemistry
Fluorescence
Herceptin Adjuvant
National Surgical Adjuvant Breast and Bowel Project
North Central Cancer Treatment Group
Breast Cancer International Research Group 006
Docetaxel, carboplatin, and trastuzumab
Anthracyclines, cyclophosphamide and taxanes
Anthracyclines, cyclophosphamide, taxanes,
and trastuzumab
Fluorouracil, epirubicin and cyclophosphamide
Docetaxel or vinorelbine
Cardiac Review and Evaluation Committee
Synergism Or Long Duration
New York Classification of Heart Failure
Phosphatidylinositol 3-kinases
Ak-transforming factor
Phosphatase and tensin homolog
phosphoinositide-dependent protein kinase 1
phosphoinositide-dependent protein kinase 1
Mammalian target of rapamycin
Mitogen-activated protein kinase
Extracellular regulated kinase
C-Jun N-terminal kinase
Vascular endothelial growth factor expression
Neuregulin-1
Reactive oxygen species
AMP-activated kinase
Peroxisome-proliferator-activated receptor γ co-
activator

FOXO	Forkhead box O
FMD	Flow mediated dilation
eNOS	nitric oxide synthase
NO	Nitric oxide
VSMC	Vascular smooth muscle cells
ESV	End systolic volume
TDI	Tissue Doppler imaging
STE	Speckle tracking imaging
Echo	Echocardiography
PL-DOX	Pegylated liposomal doxorubicin
EDV	End diastolic volume
ESV	End systolic volume
PWTs	Posterior wall thickness systole
EDD	End diastolic diameter
IVS	Interventricular septum
PV	Pulmonary venous
VTI	Velocity time integral
IVRT	Isovolumetric relaxation time
PARQ+	Physical Activity Questionnaire for Everyone
MAP	Mean Arterial Pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
BSA	Body surface area
HRV	Heart rate variability
SBV	Systolic blood pressure variability
RRI	RR interval
HF	High frequency
LF	Low frequency
NN	Mean normal-to-normal interval
SDNN	Standard deviation of NN intervals
SDSD	The standard deviation of differences of NN intervals
RMSSD	Root mean square successive difference of intervals
ER	Estrogen receptor
PR	Progesterone receptor
IVSd	Interventricular septum in diastole
IVSs	Interventricular septum in systole
LVd	Left ventricular diameter in diastole
LVs	Left ventricular diameter in systole
PwTd	Posterior wall thickness in diastole
MV	Mitral valve

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Chapter 1: Rationale and Introduction

This chapter provides background and rationale for this thesis investigation. Specific aims and expected hypotheses are also highlighted.

1.1: INTRODUCTION AND RATIONALE

Breast cancer is the most common cancer among women and the second leading cause of cancer death in Canada [1]. With nearly 90% of women alive 5 yr following diagnosis, the focus has shifted from breast cancer free-survival to long-term treatment-related cardiotoxicity [2]. This is important, because cardiovascular disease is recognized as one of the leading cause of death in breast cancer survivors and one in five women are expected to develop heart failure following a breast cancer diagnosis [3], [4]. Cardiotoxicity is well established as an adverse and potentially life threatening late event of treatment for breast cancer. The cardiotoxic effects of treatment are characterized by a decline in left ventricular ejection fraction (LVEF) potentially leading to asymptomatic or symptomatic heart failure during treatment or within 1 to 10 yr following a breast cancer diagnosis [5]. However, the long-term risk of developing cardiac dysfunction, which may progress to heart failure in women who do not experience a decline in LVEF during treatment, is not well characterized. Moreover, subclinical markers with prognosis information regarding long-term risk of cardiac dysfunction and associated adverse cardiac events in asymptomatic breast cancer survivors remain elusive. Indeed, more individuals might be at higher risk of co-morbid disease and cytotoxic ailments associated with treatment potentially leading to reduced survivorship (with an increased risk to succumb to heart failure) in the breast cancer population [6].

An increased understanding of the biology of breast cancer has led to the identification of novel therapeutic targets including trastuzumab (Herceptin™), a new recombinant DNA-derived humanized monoclonal antibody against the proto-oncogene, HER2/neu gene product. The use of trastuzumab is shown to improve rates of disease free survival and overall survival among the breast cancer population [7]–[10]. However, the use of trastuzumab treatment is limited by its high incidence of cardiotoxicity, especially when treated in combination with anthracyclines [5]. Trastuzumab-induced cardiotoxicity was first characterized in the metastatic setting and was described in 3% to 7% of patients who received trastuzumab monotherapy and in up to 27% of patients receiving combination therapy with anthracyclines [5]. Similarly, the use of trastuzumab is associated with a 13% risk of cardiotoxicity when treated with paclitaxel and trastuzumab, while recent studies have shown a higher risk of nearly one in four breast cancer patients developing trastuzumab-mediated cardiac dysfunction [5], [11]. Given that trastuzumab therapy is associated with an increase in rates of survival [7]–[10], women treated with trastuzumab therapy may be at an increased risk of developing cardiotoxicity during survivorship due to an increase number of survival years following therapy. As such, the long-term health risk associated with cardiovascular injury due to HER2 directed therapies are important in the management of breast cancer survivors. These clinical ramifications, in addition to modifiable risk factors (smoking status, weight gain, physical inactivity) and non-modifiable risk factors (hypertension, family history, age, diabetes) among the breast cancer population may markedly increase the patient's risk of developing cardiac dysfunction and progressive heart failure following the completion of therapy [12].

In early-stage breast cancer, the risk of dying from cancer-related causes decreases

over time [13]. However, the risk of dying from cardiac related causes increases with follow-up time [14]. Importantly, cardiovascular disease has been identified as the leading cause of death in the breast cancer population (15.9%) followed by breast cancer as a close second (15.1%) after 12 yr of follow up [15]. It is suggested that these trends will continue to rise due to continued improvements in breast cancer-specific mortality in combination with the rapidly aging population. For example, data from the Surveillance, Epidemiology and End Results-Medicare linked database (SEER) suggests that, the risk of cardiovascular related death increases in women of older age for women age ≥ 85 compared to women aged 75-84 yr with stage II breast cancer (42.2% and 32.2% died of cardiovascular disease, respectively) [4]. Similarly, evidence has shown that despite a decline in LVEF during treatment, patients with breast cancer have a substantial increase in long-term risk of heart failure, especially for women older than 65 yr of age, which may carry a long-term risk of mortality [16]. Moreover, circulatory system disorders are important causes of death in women with breast cancer, particularly among women over 65 yr, and an increase risk is observed with longer-term follow up [17]. Thus, attention is warrant to identify early markers of cardiovascular dysfunction in breast cancer survivors in order to design effective treatment strategies to potentially mitigate the development of cardiovascular dysfunction that may lead to long-term risk of developing heart failure in the latent years following therapy.

The use of noninvasive techniques that are reliable and sensitive to the early identification of cardiovascular dysfunction with prognostic information regarding risk of developing cardiovascular related morbidity and mortality is needed. Maximal aerobic power ($VO_2\text{max}$) is the gold standard for assessing cardiovascular fitness and is correlated inversely with cardiovascular related mortality [18], [19]. In clinical settings it is often not feasible to

meet the criteria for a VO_2max and as such the peak oxygen consumption (VO_2peak) is often recorded during an incremental stress test. Recent data has demonstrated that women with breast cancer experience VO_2peak outcomes that are consistently 30% below that of age- and sex-matched inactive individuals without a history of breast cancer [20]. Exercise intolerance is usually considered as a sign of cardiovascular dysfunction because the first symptoms of heart failure often occur during exercise [21].

Exercise measures of cardiovascular function are acceptable for assessment of left ventricular (LV) contractile reserve and other systems that govern VO_2peak (blood, vascular, muscle, lungs), which may in turn influence cardiac function [22]. To our knowledge, only one study has assessed LV contractile reserve in breast cancer survivors [23] and outcomes of this investigation demonstrated that these measures are superior to resting measures of systolic function using 2 dimensional (2D) echocardiography in the identification of cardiac dysfunction in breast cancer. However, this investigation did not include women treated with trastuzumab. Accordingly, it is unknown whether the assessment of LV contractile reserve in HER2-positive treated breast cancer survivors successfully identifies cardiac dysfunction in this subset of cancer survivors. Accordingly, the purpose of this research is to identify early markers of cardiac and vascular dysfunction at rest in asymptomatic HER2-positive breast cancer survivors, and whether exercise measures of cardiac and vascular function improve the identification of cardiovascular dysfunction when employed in the HER2-positive breast cancer population.

1.2: AIMS

We sought to determine the utilization of resting and exercise measures (assessment of LV contractile reserve) of cardiovascular function in HER2-positive breast cancer

survivors. We explored vascular function at rest among HER2-positive breast cancer survivors. Moreover, we assessed changes in cardiovascular function from rest to peak exercise in breast cancer using resting and stress echocardiography to determine if cardiovascular impairment was evident in asymptomatic HER2-positive breast cancer survivors that may not be detected under resting conditions. Each of these measures was compared to age-matched apparently healthy controls to measure changes in cardiovascular function among HER2-positive breast cancer survivors.

Specific Aim 1: The primary aim of our investigation was to evaluate LV contractile reserve through assessment of ventricular-vascular coupling in asymptomatic HER2-positive breast cancer survivors compared to age matched apparently healthy controls. This information provides insight regarding the magnitude of change in LV performance and vascular function as well as the interaction between LV elastance (E_{LV}) and arterial elastance (E_a) on cardiovascular function in breast cancer survivors at rest and during exercise (“stress”) conditions.

Specific Aim 2: The secondary objective of our investigation was to measure cardiovascular haemodynamics (stroke volume (SV), cardiac output (Q), and heart rate) at rest and during exercise conditions in women with a history of HER2-positive breast cancer. Additional information was gained regarding limitations to exercise tolerance in women with breast cancer, a global marker of cardiovascular performance.

Specific Aim 3: We also sought to compare resting measures of arterial distensibility (pulse wave velocity (PWV)) and arterial compliance (measures of vascular function) in patients with a history of HER2-positive breast cancer survivors. Measures of vascular function are modulated by autonomic function, thus these responses were assessed by

spontaneous baroreflex sensitivity. Moreover, we assessed resting cardiac function using conventional 2D-echocardiographic measures currently utilized to monitor cardiac function in the breast cancer population.

1.3: HYPOTHESIS

We hypothesize that:

- A) Differences in measures of vascular function would be evident between HER2-positive breast cancer survivors and age-matched healthy controls with an increase in vascular stiffness (increase in PWV and a decrease in vascular compliance) and reduced baroreceptor sensitivity (BRS) under resting conditions.
- B) Women with a history of HER2-positive breast cancer would present with normal systolic and diastolic function at rest using conventional 2D echocardiography; however cardiovascular dysfunction would be evident under exercise conditions with an inability to decrease the ventricular-vascular coupling ratio compared to apparently healthy age-matched controls.
- C) Women with a history of HER2-positive breast cancer would have a blunted SV response to incremental exercise in comparison to apparently healthy matched controls.

Chapter 2: Review of the Literature

The purpose of this chapter is to provide a detailed introduction regarding cardiotoxicity in HER2-positive breast cancer and factors that contribute to the increased risk of cardiac related morbidity and mortality in this patient population. Moreover, concepts of resting measures of cardiovascular function and stress echocardiography are highlighted in the identification of cardiac dysfunction.

2.1: INTRODUCTION

Breast cancer is a heterogeneous disease that can be classified by molecular profiles that include the expression of HER2 receptors and their respective activated pathways. Discovery of the HER2 receptor and its role in cancer prognosis has lead to the development of trastuzumab (Herceptin), a monoclonal antibody targeting HER2 receptors (also known as ERBB-2) in breast cancer malignancies. Over-expression of this receptor occurs in 20-30% of women with early-stage breast cancer and is associated with aggressive disease and poor prognosis [24], [25]. The use of trastuzumab and was approved in 1998 by the food and drug administration board for treatment in the metastatic setting for patients with HER2-positive breast cancer leading to a reduction in recurrence and mortality [26]. However, these clinical benefits are often associated with adverse cardiovascular events associated with treatment. Traditionally, the cardiotoxic effects of treatment are characterized by a decline in LVEF potentially leading to asymptomatic or symptomatic heart failure [5]. However, LVEF is an imperfect parameter for detecting cardiotoxicity related to trastuzumab therapy, because LVEF fails to detect subtle changes in cardiac function, and when reduced, it reflects a marker of advanced myocyte damage accompanied with poor prognosis [27]. Accordingly, subclinical markers of cardiovascular dysfunction with prognostic information regarding the

long-term risk of adverse cardiac events are more clinically important for detecting treatment-related cardiotoxicity among the breast cancer population. Accordingly, the clinical use of trastuzumab in treatment for HER2-positive breast cancer and the prevalence of cardiotoxicity in the adjuvant setting is highlighted in the following sections. Moreover, the potential reasons for increased risk of developing cardiotoxicity in breast cancer (direct and indirect consequences of treatment) are discussed. Also, subclinical measures of cardiovascular function that might be useful in predicting the early onset of cardiac and vascular dysfunction associated with treatment (as seen in other patient populations) are outlined.

2.2: TRASTUZUMAB IN THE ADJUVANT SETTING

Treatment for HER2-positive early-stage breast cancer is focused on cure and prevention of relapse of disease usually consists of chemotherapy, radiation therapy, surgery, hormone therapy, HER2 directed therapies or a combination of these treatments.

Overexpression of the HER2 protein can be indirectly assessed by quantifying HER2 cell surface receptors using immunohistochemistry (IHC), directly by measuring the number of HER2 gene copies using fluorescence (FISH) or bright field in situ hybridization [28].

Appropriate HER2 receptor identification leads to the initiation of targeted therapies against the HER2 receptor either as a monotherapy or in addition to other cytotoxic therapy combinations. Currently, chemotherapy and trastuzumab combination therapy is approved for use in the adjuvant setting given concurrently or sequentially followed by one year of trastuzumab [7], [8]. Recent clinical evidence suggests that trastuzumab may be more effective when incorporated as a concurrent regimen rather than sequential therapy [29]. The use of trastuzumab for shorter durations (e.g., six months) is currently under investigation in

long-term follow up trials, however disease-free survival is seemingly less favorable compared to longer durations [30]. Currently, trastuzumab is the only HER2 directed therapy approved for use in the adjuvant setting [31] while the administration of newer HER2 directed agents, including lapatinib and pertuzumab are currently being investigated in clinical trials [32], [33]. Early investigations for the use of trastuzumab demonstrates clinical benefits of disease progression and overall disease-free survival, which is improved upon recipient of anthracyclines including doxorubicin and epirubicin in the adjuvant setting [7], [8]. Identification of newer agents that are also approved for use in the adjuvant setting in combination with trastuzumab includes alkylating agents such as cyclophosphamide and taxane therapies including docetaxel, carboplatin and paclitaxel.

2.3: OUTCOMES OF THE LARGE CLINICAL TRIALS

The Herceptin Adjuvant (HERA) (Breast International Group [BIG] 01-01) Trial [7] is a international, phase III randomized trial involving women with HER2-positive early-stage invasive breast cancer treated with surgery with or without radiotherapy in combination with chemotherapy. The chemotherapy regimen consisted of at least four cycles of anthracyclines (epirubicin or doxorubicin) and/or taxane therapies (paclitaxel or docetaxel). A total of 5,102 patients with HER2-positive, node-positive, or node-negative disease with tumour >1 cm were enrolled. Women were randomized into three groups including women treated with trastuzumab given as adjuvant treatment (at a dose of 8 mg per kg of body weight intravenously once, then at a dose of 6 mg per kg every 3 wk) for 2 yr, women treated with trastuzumab at the same dose and on the same schedule for one year and an observation group (chemotherapy without trastuzumab). At a median follow up of 4 yr, treatment with trastuzumab resulted in an increase in disease free survival (78% versus 72% in the

trastuzumab groups and the observation group respectfully) supporting the notion of trastuzumab use following adjuvant chemotherapy.

The National Cancer Institute sponsored two trials to monitor treatment with trastuzumab in the adjuvant setting, which was comprised of the National Surgical Adjuvant Breast and Bowel Project (NSABP) and the North Central Cancer Treatment Group (NCCTG) trial. These trials addressed the use of trastuzumab in combination with adjuvant chemotherapy for women with HER2-positive breast cancer as appose to sequentially in the HERA trial. The NSABP B-31 trial compared the use of combination therapy including doxorubicin and cyclophosphamide followed by paclitaxel (group one) with the same chemotherapy plus 52 wk of trastuzumab beginning on day one of paclitaxel therapy (group two). The NCCTG trial compared three regimens that were nearly identical to the NSABP B-31 trial with only small differences in terms of the scheduling of paclitaxel treatment and some aspects of hormonal therapy and radiotherapy. The joint analysis consisted of 1,679 women in the control group (chemotherapy without trastuzumab) and 1,672 women in the chemotherapy plus trastuzumab group. With a median follow-up of 3 yr, the disease-free survival rates were 75.4% in the control group and 87.1% in the trastuzumab group. The rate of overall survival at 3 yr was 94.3% in the trastuzumab group and 91.7% in the control group [8]. This was the first large clinical trial to demonstrate the benefit of concurrent treatment of trastuzumab with chemotherapy compared to adjuvant chemotherapy alone for women with HER2-positive breast cancer.

The Breast Cancer International Research Group 006 (BCIRG-006) trial evaluated the use of trastuzumab during adjuvant therapy in combination with chemotherapy, which did not include an anthracycline due to the high rates of cardiotoxicity observed in the clinical

trials including trastuzumab and anthracycline combination regimens [9]. The use of docetaxel, carboplatin, and trastuzumab (TCH) was selected due to positive preclinical results in phase II and III trials in the metastatic setting for patients with advanced HER2-positive disease [34]. There were 3,222 women randomly assigned to three groups with HER2-positive, invasive, high-risk, node-negative or node-positive breast cancer. The first group of women received doxorubicin and cyclophosphamide followed by docetaxel excluding the use of trastuzumab (ACT). The second group of women received ACT plus trastuzumab (ACT-T), beginning with the first dose of docetaxel and continuing for 1 yr. In the third group, patients received TCH followed by trastuzumab for an additional 34 wk (no anthracyclines). At a median follow up of 65 mo, disease-free survival was improved in the ACT-T group compared to the ACT group, although ACT-T was associated with improved outcomes compared to the TCH group (the 5 yr rate of disease-free survival was 75% for the ACT, versus 84% for the ACT-T versus 81% for the TCH group) [9]. Similar results were observed for the incidence rates of overall survival, which were also indicated at 65 mo of follow up (87% for the ACT group, versus 92% for the ACT-T group, versus 91% for the TCH group) [9]. These results propose the possible benefits of trastuzumab therapies in addition to non-anthracycline regimens for treatment of HER2-positive breast cancer in the adjuvant setting.

Lastly, the FINHER trial [10] was a smaller prospective, multicenter phase III trial including 1,010 women with axillary-node positive or high-risk axillary node-negative early-stage breast cancer. This trial sought to evaluate the use of docetaxel or vinorelbine followed by three cycles of 5-fluorouracil, epirubicin and cyclophosphamide (FEC). A sub-analysis (n = 232) of women with HER2-positive breast cancer was also included where these women

were randomized to receive trastuzumab for 9 wk together with docetaxel or vinorelbine or in the absence of trastuzumab. Sub-analysis of the women with HER2-positive early stage breast cancer revealed that trastuzumab was superior for the treatment of HER2-positive breast cancer regarding rates of disease free survival compared to those treated with chemotherapy alone. Of those treated with trastuzumab, the increased rate of disease free survival was higher for those treated with vinorelbine compared to those treated with docetaxel suggesting the efficacy of docetaxel in the treatment of HER2-positive breast cancer in combination with trastuzumab therapies.

Table 2.1: Outcomes of the Large Adjuvant Clinical Trials

Trial	Treatment	Disease-free survival	Overall Survival
HERA (n = 5,102)[7]	Group one: Anthracyclines, no trastuzumab Group two: ACT-T (1 yr) Group three: ACT-T (2 yr) *Sequential administration of anthracyclines and trastuzumab	Group one: 78.6% Group two: 72.2% *Group two and three were analyzed together (indicated as group two)	Group one: 89.3% Group two: 87.7%
BCIRG-006 (n = 3,222) [8]	Group one: ACT Group two: ACT-T (1 yr) Group three: TCH and trastuzumab (34 wk)	Group one: 75% Group two: 84% Group three: 81%	Group one: 87% Group two: 92% Group three: 91%
NCCTG trial and NSABP B-31 (n = 3,351)[8]	Group one: ACT-T (1 yr) Group two: ACT *Concurrent administration of anthracyclines and trastuzumab	Group one: 67.1% Group two: 85.3 %	Group one: 86.6% Group two: 91.4%
FINHER (n = 1,010) [35]	Group one: D/V and FEC Group two: D/V and FEC and trastuzumab (9 wk)	Group one: 73.3% Group two: 80.9%	Group one: 81.9% Group two: 89.6%

Outcomes of the large clinical trials for the use of trastuzumab in the adjuvant setting for HER2-positive breast cancer. HERA- Herceptin Adjuvant; BCIRG- Breast Cancer International Research Group 006 NSABP- National Surgical Adjuvant Breast and Bowel Project; NCCTG- North Central Cancer Treatment Group; TCH- docetaxel, carboplatin, and trastuzumab; FEC- fluorouracil, epirubicin and cyclophosphamide; D/V- docetaxel or vinorelbine; ACT- anthracyclines, cyclophosphamide and taxanes; ACT-T- anthracyclines, cyclophosphamide, taxanes and trastuzumab

2.4: TRASTUZUMAB RELATED CARDIOTOXICITY

Due to the incidence of cardiovascular dysfunction experienced during the clinical trials for the use of trastuzumab in the metastatic setting, the blinded Cardiac Review and Evaluation Committee (CREC) was developed. The CREC standardized the criteria for diagnosing cardiac events/dysfunction and categorized the events in accordance with the New York Heart Association (NYHA) standards. Accordingly, treatment related cardiotoxicity was defined by the CREC as: 1) a decline in LVEF of 5% to < 55% with

accompanying signs or symptoms of congestive heart failure or a decline in LVEF of at least 10% to < 55% without accompanying signs or symptoms, 2) symptoms of congestive heart failure, 3) associated signs of congestive heart failure including S3 gallop and/or tachycardia, or 4) cardiomyopathy characterized by a decline in LVEF that either was global or more severe in the septum [5]. This criterion was therefore used to assess the cardiotoxic effects of trastuzumab treatment in clinical trials for early- stage breast cancer. From these trials, it is apparent that the onset of cardiotoxicity can occur early within 1 yr of beginning chemotherapy or latent effects, which are observed 1 to 10 yr following therapy [3]. However, cardiotoxicity in the acute phase such as the occurrence of conduction abnormalities including electrocardiographic QT-interval changes, supraventricular and ventricular arrhythmias or acute coronary syndromes, pericarditis and/or myocarditis-like syndromes may also occur [36]. These changes are often observed from the initiation of therapy and up to two weeks after termination of treatment.

2.5: PREVALENCE OF CARDIOTOXICITY IN CLINICAL TRIALS

Recent investigations regarding the adverse cardiac events observed in the HERA trial raise concerns about limitations to the use of ACT-T versus ACT in the adjuvant setting. Patients treated with ACT-T compared to those treated with ACT alone were at increased risk of severe heart failure (NYHF stage III or IV), (0.6% versus 0% respectively), asymptomatic heart failure (NYHF stage I or II), (2.15% versus 0.12% respectively) and a significant reduction in LVEF during treatment (3.04% versus 0.53% respectively) [37]. A subsequent analysis of the HERA trial suggests that the prevalence of cardiac endpoints is also evident with 8 yr follow up [38]. Outcomes of this investigation suggested that the incidence of heart failure (0.8% versus 0.8% versus 0.0%, respectively) and reduction in

LVEF (7.2% versus 4.1% versus 0.9%, respectively) was significantly higher in the 2 yr and 1 yr trastuzumab arms compared with the group treated with anthracyclines alone.

Importantly, outcomes of these investigations also identified that patients who were treated with higher cumulative doses of doxorubicin ($287 \text{ mg}\cdot\text{m}^{-2}$ versus $257 \text{ mg}\cdot\text{m}^{-2}$) or epirubicin (480 versus $422 \text{ mg}\cdot\text{m}^{-2}$), who had a lower LVEF at initial screening and those with a higher body mass index during treatment demonstrated a greater risk for the development of trastuzumab related cardiotoxicity [37]. These analyses did not include patients with known cardiovascular disease; as such the risk of cardiac end points in patients with known cardiovascular disease is not highlighted in these investigations.

Sequential use of trastuzumab and anthracycline therapy is also associated with adverse cardiotoxic effects similar to those observed in the HERA trial. The assessment of cardiac safety concerns from the NSABP B-31 trial was conducted at a median 3 yr of follow up. The incidence of cardiac safety end points were characterized by the NYHA classification of stage III or IV symptoms of heart failure or a decrease of 10% or to less than 55% consistent with the CREC criteria. The cumulative incidence of cardiotoxicity was 0.8% for the ACT group compared to 4.1% in the treatment group (ACT-T). Discontinuation of trastuzumab also occurred in 19% of the patients due to cardiac-related reasons including asymptomatic declines in LVEF, symptoms of cardiovascular disease or other cardiac related symptoms [39]. More recent data reported from Romond colleagues (2012) [40] demonstrates that the rate of cardiotoxicity is merely the same with the cumulative incidence of cardiotoxicity of 1.3% in the treatment group without trastuzumab compared 4.0% in the group treated with trastuzumab at 7 yr of follow-up. Further, patients with known diagnoses

of cardiovascular disease were also excluded from these investigations as seen earlier in the HERA trial.

During the BCIRG-006 trial, intensive cardiac monitoring was conducted with a median follow up of 4 yr. The rate of cardiotoxicity characterized by the NYHA classification of stage III or IV symptoms of heart failure was highest in the two groups receiving ACT and ACT-T compared to TCH and trastuzumab (0.7% versus 2.0% versus 0.4% respectively). [41]. A reduction in the mean LVEF (defined as >10% relative loss) followed the same trend with an incident rate of 18.6% in the ACT-T group, 11.2% in the ACT group and 9.4% in the TCH plus trastuzumab group [41]. At a median 4 yr follow up, LVEF continued to decrease in 33% of patients who received the ACT-T regimens that experienced a decline in LVEF during treatment [41]. Indeed, ACT-T therapy increases the risk of cardiotoxicity in breast cancer, which was not alleviated following therapy, suggesting the need for long-term follow up in this population.

Given the short duration of trastuzumab exposure in the FINHER trial it was expected that the cardiotoxic effects would remain limited. However, within 1 to 5 yr following therapy, 6.8% of patients treated with chemotherapy and trastuzumab and 10.5% of patients treated without trastuzumab experienced a decrease in LVEF of at least 20% [42]. Moreover, the rate of heart failure was 0.9% for those who received trastuzumab and 1.7% who received chemotherapy without trastuzumab [42]. These data render the possibility that trastuzumab exposure in the short term may decrease the likelihood of cardiotoxic effects. Importantly, results of the FINHER trial demonstrate similar outcomes in overall survival from trastuzumab exposure between 9 wk and 12 mo. Ongoing investigations including the Synergism Or Long Duration (SOLD) trial are currently underway to determine the efficacy

and safety of trastuzumab for 9 wk compared to 12 mo, although the results of these investigations are currently unknown.

Table 2.2: Trastuzumab Mediated Cardiotoxicity in the Adjuvant Setting

Trial	Treatment	Heart Failure	>LVEF
HERA[38]	Group one: Anthracyclines	Group one: 0.0%	Group one: 0.9%
	Group two: ACT-T (1 yr)	Group two: 0.8%	Group two: 4.1%
	Group three: ACT-T (1 yr)	Group three: 0.8%	Group three: 7.2%
BC-106[43]	Group one: ACT	Group one: 0.7%	Group one: 11.2%
	Group two: ACT-T	Group two: 2.0%	Group two: 18.6%
	Group three: TCH and trastuzumab (34 wk)	Group three: 0.4%	Group three: 9.4%
NSBA[43]	Group one: ACT-T (1 yr)	Group one: 4.0%	15.5% of patients discontinued therapy due to cardiac related causes in the ACT-T group
	Group two: ACT	Group two: 1.3%	
	*Concurrent administration of anthracyclines and trastuzumab		
FINHER[42]	Group one: D/V and FEC	Group one: 1.7%	Group one: 10.5%
	Group two: D/V and FEC and trastuzumab	Group two: 0.9%	Group two: 6.8%

Cardiotoxicity outcomes of the large clinical trials for the use of trastuzumab in the adjuvant setting for HER2-positive breast cancer. HERA- Herceptin Adjuvant; BCIRG- Breast Cancer International Research Group 006 NSABP- National Surgical Adjuvant Breast and Bowel Project; NCCTG- North Central Cancer Treatment Group; TCH- docetaxel, carboplatin, and trastuzumab; FEC- fluorouracil, epirubicin and cyclophosphamide; D/V- docetaxel or vinorelbine; ACT- anthracyclines, cyclophosphamide and taxanes; ACT-T- anthracyclines, cyclophosphamide, taxanes and trastuzumab

2.6: TRASTUZUMAB-MEDIATED CARDIAC DYSFUNCTION

Indeed, direct as well as indirect consequences of trastuzumab treatment may impede the cardiac and vascular systems leading to increased risk of cardiac and vascular dysfunction among women with a history of HER2-positive breast cancer [44]. These clinical outcomes in addition to an increasing age in the population may result in increased rates of cardiac and vascular dysfunction potentially increasing the risk of heart failure among breast

cancer survivors. Herein, we highlight the direct mechanisms of action that may potentiate the risk of cardiac dysfunction among the HER2-positive breast cancer population.

In order to understand the direct mechanisms associated with trastuzumab related cardiotoxicity, it is first important to highlight the biological function of the therapy. Selective binding of the trastuzumab antibody to the HER2 receptor down regulates the expression of HER2 and blocks ligand independent HER2–HER3 (ErbB2/ ErbB3) dimerization [45], [46]. Resultant outcomes include inhibition of phosphatidylinositide 3-kinases (PI3K)/Akt-transforming factor (Akt) signaling responsible for cell growth, proliferation and survival. Specifically, the loss of tumour suppressor gene phosphatase and tensin homolog (PTEN) is common in breast malignant tumours [47]. PTEN functions to mediate cell growth suppression and inhibition of cell migration through inhibition of PI3K/Akt-mediated signaling [48]. Trastuzumab treatment is associated with increased PTEN membrane localization and phosphatase activity leading to rapid Akt dephosphorylation inhibiting cell proliferation and results in cell cycle arrest in the G1 phase [49]. Furthermore, recent data indicates that phosphoinositide-dependent protein kinase 1 (PDK1) is overexpressed in many breast carcinomas, and that alterations of PDK1 are critical in the context of oncogenic PI3K activation [50]. PDK1 phosphorylates Akt and recruits a second kinase (PDK2) for full activation of Akt/PI3K/ mammalian target of rapamycin (mTOR) signaling leading to an increase in nuclear trafficking of proteins responsible for cell growth [51], [52]. Inhibition of the HER2 receptor results in tumour suppression mediated in part by reduced PDK1 and Akt/PI3K activity ultimately reducing the expression of genes responsible for cell maintenance and/or growth [53]. Moreover, anti-apoptotic B-cell lymphomas (Bcl-2 and Bcl-X) overexpression has been reported in human breast tumour cell

lines suggesting that they are key mediators in tumourgenesis [54]. HER2 inhibition down-regulates Bcl-2 protein expression and induces apoptosis via Akt/PI3K signaling, reducing tumourgenesis through the regulation of cell survival pathways [55].

In addition to PI3K signaling, HER2 activates mitogen-activated protein kinase (MAPK) signaling cascades including extracellular regulated kinase (ERK)1/2 pathway, the c-Jun N-terminal kinase (JNK) pathway, the p38 pathway and the ERK5 pathway [56]. MAPK signaling is activated in response to growth factors and/or stress stimuli ultimately leading to cell proliferation, growth and survival [57]. Trastuzumab rapidly reduces MAPK signaling, thus promoting regression of tumourgenesis through reduced rates of cellular proliferation and survival [58]. Although the role of MAPK/ERK signaling has been identified in the progression on breast cancer, the effect of trastuzumab on cell growth and survival mediated through MAPK/ERK signaling is not fully understood. However, it seems that tight regulation between PI3K/Akt and MAPK/ERK signaling may mediate these results. For example, in HER2-positive breast cancer cells, trastuzumab inhibited HER2 phosphorylation resulting in consequential effects on downstream signaling molecules including MAPK and PI3K/Akt which reduced rates of cell proliferation by greater than 50% [58]. Moreover, the efficacy of trastuzumab may also depend upon its ability to induce an immune response. HER2 targeted antibodies, including trastuzumab, were shown to promote apoptosis in multiple breast cancer cell lines via antibody-dependent cellular cytotoxicity [59]. The immune cell-modulated activity is mediated in part by an increase in number of natural killer cells that infiltrate the tumour site following cytotoxic treatment [60].

Trastuzumab has also been shown to inhibit angiogenesis, a complex process in which new blood vessels are formed to promote growth and progression of human solid

tumours. Inhibition of HER2 receptors is associated with reduced vascular endothelial growth factor expression (VEGF) [61], a potent stimulus of angiogenesis, thus suggesting its role in reduced angiogenesis associated with trastuzumab therapy. For example, trastuzumab treatment in human breast cancer cells resulted in approximately 50% down-regulation of VEGF, transforming growth factor α , plasminogen activator inhibitor 1, and angiopoietin 1 (mRNA) [62]. Moreover, MAPK/ ERK and PI3K/Akt signaling is induced by angiogenic growth factors [63]. Thus, MAPK activation by growth factors initiate's tumour angiogenesis, whereby promoting the migration, proliferation, differentiation, and survival of endothelial cells [64]. Accordingly, inhibition of MAPK/ERK and PI3K/Akt signaling may reduce angiogenic factors leading to regression of tumourgenesis following treatment with trastuzumab.

The emerging use of HER2 directed therapies has transformed the management of breast cancer, however unexpected off target cardiotoxic events have limited their clinical use. The role epidermal growth factor receptor (ErbB1, ErbB2, ErbB3, and ErbB4) signaling and its associated ligands including neuregulin-1 (NRG1) and downstream targets Akt/PI3K signaling cascades in the heart have been recognized as major pathways mediating HER2 directed cardiotoxicity [65]. ErbB2 signaling and ligands (NRG1 and EGF) form a stringently regulated network that controls critical cellular functions that regulate cell proliferation, migration, differentiation and apoptosis [66]. Equally, within the heart, the NRG1/ErbB signaling network plays a role in the function and/or survival of cardiomyocytes including regulation of survival pathways [67], [68] energy metabolism [69] protein synthesis [70], [71] and prevention of dilated cardiomyopathy [68], [72].

The important role of NRG1/ErBb myocardial signaling was first recognized for its

role in cardiac development in utero. ErbB2/ErbB4 knockout mice die during embryonic development as a result of deficient cardiac development and compromised function due to a lack of myocyte proliferation [73], [74]. Moreover, knockout of NRG1/ErBb signaling in adult heart results in ventricular chamber dilation manifested by reductions in LV wall thickness, enlarged ventricles, reduced contractility and conduction abnormalities [72], [75]. Measures of the heart pumping- function including reduced fractional shortening [72] and an increase in dp/dt [75] demonstrate unfavorable changes in cardiac function. ErbB2 [72] and ErbB4 [75] receptor signaling has also been shown to protect against dilated cardiomyopathy and cardiomyocytes response to stress when subjected to aortic banding in the adult heart [65], [68] . Similarly, isolated cardiac cells are more sensitive to doxorubicin-induced cardiotoxicity suggesting the role of ErbB2 signaling to withstand stress conditions [68]. Accordingly, in vivo evidence suggests for the critical role of ErbB2/ErbB4 signaling in remodeling of cardiomyocytes and maintenance of the contractile function of the myocardium with the ability to respond to stress.

A number of processes appear to be mediated by ErbB/NGF1 signaling including regulation of survival pathways in the adult heart. NRG-1/ErbB signaling has been heavily implicated in the activation of PI3K/Akt signaling responsible for cell survival of cardiomyocytes [67]. For example, rat cardiomyocytes treated with an inhibitory ErbB2 antibody display a dose-dependent increase in reactive oxygen species (ROS) production and cell death [76]. Mouse embryonic fibroblasts lacking Bcl2 pro-apoptotic family members Bax and Bak are resistant to these adverse effects suggesting that the effects of trastuzumab on the heart occur through a mitochondrial pathways and are mediated by ROS production [76]. Moreover, ErBb2 inhibition has been shown to cause mitochondrial dysfunction and

caspase activation while overexpression of BcL-xL results in partial rescue to chamber dilation and contraction [68], [77]. As such, cell survival of cardiomyocytes may be in part mediated by activation of the PI3K/Akt pathways, while inhibition of ErbB2 receptors may down regulate important survival pathways.

Regulation of NRG1/ErbB/Akt activity and downstream targets in cardiomyocytes also plays a significant role in energy metabolism. ErbB/Akt induction via NRG1 signaling leads to the translocation of glucose transporter four required for glucose metabolism [78]. In cardiac cells, NRG1-stimulated glucose uptake was completely abolished by PI3K inhibition in vitro suggesting its role in glucose metabolism regulation [79]. Altered rates of glycolysis and reduced plasma membrane translocation of the glucose transporter four precedes the development of systolic heart failure [80] and may have implications in trastuzumab-induced cardiomyopathy. Moreover, AMP-activated kinase (AMPK) is a well-conserved metabolic regulator responsible for activation of glucose and oxidative metabolism in the heart [81]. Trastuzumab fails to lead to the activation of AMPK potentially altering energy reserves of the heart responsible for cardiac function and response to stress. Newer HER2 tyrosine kinase inhibitor therapies including lapatinib are showing reduced cardiotoxic effects which is postulated (in part) from their ability to activate AMPK suggesting its important role reduced cardiac function observed with trastuzumab [82]. Lastly, peroxisome-proliferator-activated receptor γ co-activator 1a and 1b (PGC-1 α PGC-1b) are recognized as regulators of mitochondrial biogenesis and oxidative metabolism [83]. Gene expression of PGC-1a is regulated by AMPK activation while PGC-1b activity is mediated by P13K activation [84], [85]. As such, inhibition of AMPK or PI3K activity impedes PGC-1a and PGC-1b activity in the regulation of mitochondrial biogenesis in the heart. For example, a reduction in

transcription of PGC-1 α in the heart is accompanied by a reduction in mitochondrial gene expression and ATP content, which lead to a decrease in cardiomyocyte size [86]. Together these data suggest the important role of NRG1/ErbB/Akt signaling in the regulation of energy metabolism and the development of cardiotoxicity.

In the normal heart, NRG1/Akt signaling is responsible for the regulation of cellular process including muscle protein synthesis, protein hypertrophy and reduced rates of protein degradation [87]. Specifically, induction of mTORC1 complex from circulating growth factors leads to increases in myofilament protein synthesis via the PI3K/Akt/mTOR pathway [88]. As such, suppression of total and phosphorylated Akt from HER2 directed therapies [76] might potentially lead to reduced rates of cardiomyocyte protein synthesis. Moreover, reduced levels of PGC-1 α [89], increased circulating cytokines such as TNF- α and increased activation of NF κ B potentially inhibit the Akt/PI3K/mTOR pathways [90], thus inhibiting protein synthesis in the heart, however this relationship is still unclear. Moreover, suppression of protein hypertrophy via the MAPK/ERK pathway may be involved in trastuzumab mediated cardiac injury. The transcription factor GATA4 plays a direct functional role as a regulator of cardiomyocyte hypertrophic growth and gene expression through direct activation from MAPK signaling [91]. Trastuzumab reduces signaling mediated by the MAPK cascades potentially leading to reduced rates of hypertrophic growth of cardiomyocytes. Lastly, Forkhead box O (FOXO) transcription factors are implicated in the regulation of cell survival, growth and proliferation that is tightly regulated through Akt phosphorylation [92]. In cardiomyocytes, FOXO transcription factors are responsible for protein degradation downstream Akt signaling. Phosphorylation of AKT inhibits induction of FOXO3 proteins reducing rates of protein degradation [93]. Accordingly, reduced rates of

phosphorylated Akt from HER2 directed therapies might potentiate the rate of protein degradation via FOXO signaling leading to impaired cardiomyocyte function and changes in structure.

2.7: TRASTUZUMAB MEDIATED VASCULAR INJURY

While cardiotoxicity is well established as an adverse event associated with HER2 directed therapies, clinical studies addressing the effects of these treatments on vascular (endothelial) function and structure is not well characterized. In other populations, such as advanced age, or diabetes, alterations in vascular function have been implicated in the progression of heart failure [94], [95]. Therefore changes in vascular function following treatment for HER2-positive breast cancer may mediate the onset of late cardiac events including the development of cardiotoxicity. To our knowledge, only one study to date has assessed endothelial function in HER2-positive breast cancer survivors via flow-mediated dilation (FMD) [96]. Outcomes of this investigation demonstrated no changes in FMD in a small sample of HER2-positive breast cancer survivors (n = 17) however, the effects of HER2 directed therapies on arterial stiffness and modulation of autonomic function responsible for fluctuations in heart rate and blood pressure is unknown, warranting further investigation.

Basic science research provides some insight to the possible mechanisms responsible for change in vascular structure and function following inhibition of HER2 proteins. Animal models have shown that blockade of HER2 activation results in mitochondrial dysfunction and subsequent ROS accumulation [97]. This is important, because an excess accumulation of ROS production has been shown to cause alterations in endothelial cell growth and remodeling [98]. These vascular responses subsequently lead to an increase in levels of pro-

inflammatory cytokines and cellular adhesion molecules responsible for initiating the atherosclerotic cascade. Furthermore, it is imperative to highlight that HER2 receptors are expressed by endothelial cells [99]. HER2 activation directly induces endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) production which leads to dilation of arterioles via P13K/Akt signaling [100]. Consequently, inhibition of endothelial HER2 receptors may mediate a reduction in eNOS and increase ROS production with subsequent endothelial dysfunction. Indeed, endothelial dysfunction and inflammatory markers are associated with greater arterial stiffness and the pathological development of atherosclerosis, however whether these alterations occur due to treatments for HER2-positive breast cancer requires further investigation [101].

Vascular smooth muscle cells (VSMCs) have also been reported to express HER2 proteins and are responsible for their function [102]. VSMCs are located in the tunica intima and operate to stabilize the vessel by inhibiting cellular proliferation and migration, stimulating production of components of the extracellular matrix, and providing survival factors [103]. The interaction between vascular endothelial cells and smooth muscle cell is essential for the formation of mature vascular structures. The interaction between endothelial cells to recruit VSMCs is mediated by signaling via HER1 and HER2 receptors located on VSMCs. Whereas, if these interactions to recruit VSMCs are blocked, vessels become dilated or start to regress leading to poorly functioning vessels [104]. Moreover, trastuzumab mediated cardiac injury may be mediated by increased activation of angiotensin II signaling [105]. Transactivation of HER2 by angiotensin II involves ROS-dependent activation leading to the induction of and pro-growth signaling via MAPK/ERK pathways. Consequently, these mechanisms lead to VSMC proliferation, migration and cell growth [106]. Replication of

VSMCs in the media is followed by migration into the intima and further replication. These morphological alterations together with leukocyte infiltration and lipid accumulation are associated with induction of the atherosclerotic cascade [107]. This shifting in VSMC phenotype progressively leads to an increase in arterial stiffness due to an increase in collagen deposition, VSMC hypertrophy and a decrease in elastin content [95]. Whether these alterations contribute to vascular injury and increase the risk of heart failure in women treated with a history of HER2-positive breast cancer is unknown.

Although data regarding HER2 directed therapy on vascular function is insufficient, few studies have shown adverse vascular toxicity following anticancer therapies that are used in combination with HER2 directed therapies. In breast cancer, a single dose of anthracyclines results in a rapid depletion of systemic NO levels resulting in endothelial dysfunction measured non-invasively by FMD [108]. Moreover, treatment with anthracyclines is associated with increases in aortic stiffness measured by PWV with short-term follow up (> 1 yr) [109]. In a recent report from Mizia-Stec and colleagues (2013) [44] arterial remodeling following treatment with anthracyclines was observed. Outcomes of their investigation showed an increase in carotid intima-media thickness, accompanied by a decrease in aortic compliance and an increase in aortic stiffness indices 1 yr following therapy. This is consistent with other reports who demonstrate an increase in carotid intima media thickness and unfavorable alterations in lipid profiles following treatment with anthracyclines [110]. Accordingly, the increased oxidative stress associated with HER2 directed therapies might mediate alterations in vascular structure and/or function, similar to the mechanisms mediating alterations observed with anthracycline treatment.

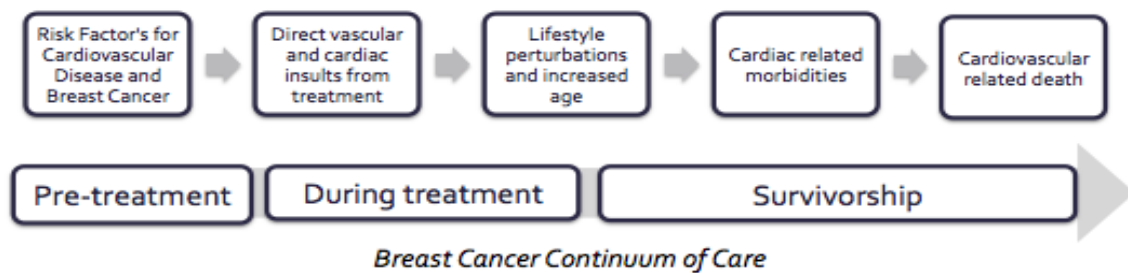
2.8: CARDIAC RISK FACTORS IN BREAST CANCER

Cardiovascular disease is mediated in part, by risk factors that can be treated or modified including high blood pressure, cholesterol, overweight/obesity, tobacco use, lack of physical activity and diabetes. Given that breast cancer and cardiovascular disease share common risk factors, these factors may contribute to increased risk of cardiotoxicity following treatment for early stage-breast cancer (see figure 2.1). For example, risk factors associated with cardiovascular disease among cancer survivors have been identified including 62.0% who were overweight or obese, 55.0% who reported hypertension, 20.7% reported diabetes, 18.1% were inactive, and 5.1% were current smokers [111]. Further, among the breast cancer population, Irwin and colleagues (2005) [112] reported that women increase their weight on average by 1.7 -4.7 kg from 1 to 3 yr following a diagnosis of breast cancer. This weight gain is often associated with a decline in physical activity. On average, breast cancer patients decrease their physical activity levels by approximately 2 hr per wk from before treatment and those who gain weight are more likely to reduce their levels of physical activity [113]. Moreover, cardiac risk factors were assessed and reported in a 7-yr follow up of the NSABP B-31 large clinical trial. Women receiving antihypertensive medications at baseline were at increased risk of developing heart failure [40]. Lastly, a number of women develop degrees of glucose intolerance during chemotherapy [114], and those with impaired glucose tolerance at baseline have poorer outcomes following treatment for breast cancer [115]. Accordingly, these risk factors may potentiate the long-term risk of developing cardiotoxicity among the breast cancer population.

Non-modified risk factors that constitute the development of cardiovascular disease include age, race, family history, and gender. Nearly 81% of women who are diagnosed with

breast cancer are over the age of 50 [1]. Given that the risk of cardiovascular disease increases with age, elderly breast cancer survivors might be at increased risk of developing heart failure following therapy [40]. Moreover the lifetime risk of developing heart failure is almost equivocal between men and women [116], [117], however women might be at an increased risk of developing heart failure following breast cancer due to cardiac and vascular injury associated with treatment.

Figure 2.1: Progression of Cardiac Related Mortality in Breast Cancer



Represents proposed reasons for the increased risk of cardiovascular related mortality in breast cancer, including direct and indirect results of treatment.

2.9: SUBCLINICAL ASSESSMENT OF CARDIOVASCULAR FUNCTION

The development of cardiotoxicity in breast cancer can occur during therapy, however it is often apparent years following the completion treatment. Indeed, the early detection of treatment related cardiotoxicity in breast cancer is increasingly recognized as an important part of management in breast cancer. With these efforts, recent guidelines put forth from the European Society for Medical Oncology society recommends evaluation of initial signs of LV dysfunction at rest that might occur before reduction of LVEF with assessment 4 to 10 yr following therapy [118]. Although the early detection of myocardial changes using resting indices of cardiac function appears to be conceptually important [119], these measures fail to assess the reserve capacity of the heart and may not identify women with

asymptomatic LV dysfunction [120]. Because the first symptoms of heart failure often occur during exercise, exercise intolerance is usually considered as a sign of cardiovascular dysfunction [21]. Exercise measures of cardiac function also assess VO_2peak , which provides the gold standard assessment of cardiopulmonary function (or aerobic capacity). The measurement of VO_2peak incorporates not only assessment of cardiac function, but also other organs (blood, blood vessels, lungs and muscle) that may limit exercise tolerance, and in turn influence cardiac function. Such strategies are established for standard of care including incremental cardiopulmonary exercise testing in the cardiac rehabilitation setting, and may be clinically useful in the breast cancer population. Moreover, echocardiographic quantification of cardiac haemodynamics during exercise has been shown to be a sensitive and reliable marker of cardiovascular dysfunction due its role in measuring LV contractile reserve [120]. As such, exercise measures of cardiovascular function might be useful in identifying cardiovascular impairment in women who are otherwise asymptomatic at rest.

2.9.1: Exercise Haemodynamics

Across the cancer trajectory, there is a profound reduction in exercise tolerance (particularly during chemotherapy) [20]. However, resting LVEF and other resting indices of LV function have been shown to correlate poorly with exercise tolerance and not all women present with impaired LVEF during and following treatment [120], [121]. It has been argued that the lack of relationship between resting indices of cardiac function and exercise tolerance suggesting that other myocardial changes under exercise conditions are major determinants of exercise tolerance in women with breast cancer. As such, evaluations of exercise measures of cardiac function are more likely to determine the relationship with exercise intolerance and impaired cardiac function in breast cancer (similar to other chronic conditions) [122].

The measurement of exercise haemodynamics provides powerful insight to examine the response of the cardiovascular system to stress and to assess its functional reserve. During exercise, the goal of the cardiovascular system is to maintain blood flow to working tissues by altering Q (heart rate*SV). To achieve this, the cardiovascular system must adequately regulate heart rate, afterload, enhance diastolic filling and achieve more-complete emptying during systole (contractility) [120]. In the healthy heart, under exercise stress conditions, LV end-diastolic volumes (EDV) increase slightly, while end-systolic volumes (ESV) decrease significantly. The increase in Q during mild exercise is achieved by an augmentation of both SV and heart rate, whereas the further increases in Q during maximal exercise is achieved primarily from an increase in heart rate [123]. In the presence of LV dysfunction the heart fails to pump blood at a rate required by the metabolizing tissues [124]. In patients with heart failure, Q may be normal at rest, but fails to rise normally during exercise. Failure to increase Q can be caused by an increase in mitral regurgitation fraction, an isolated abnormality in systolic function causing a defect in the ejection of blood during systole, or by an isolated abnormality in diastolic function causing to a defect in ventricular filling [120]. Sympathetic modulation of the cardiovascular system to increase heart rate is also blunted in the presence of LV dysfunction during exercise, reducing the contractile efficiency of the myocardium [125]. Accordingly, assessing hemodynamics during exercise among the breast cancer population may enhance the early diagnosis of cardiovascular dysfunction.

2.9.2: Ventricular-Vascular-Coupling

The dynamic relationship between the LV and the arterial system can be assessed through measurement of ventricular-vascular coupling, a key determinant of cardiovascular

performance and cardiac energetics. Because blood flow is pulsatile, changes in SV leads to alterations in the arterial pulse wave amplitude and pressure. Thus, arterial load influences LV performance and in turn, arterial properties are dependent on LV performance [126]. LV performance can be characterized by E_{LV} , which is represented as the intercept and slope of the pressure volume relationship. The net arterial load can be defined by E_a and is represented by the negative slope of the end systolic pressure and end diastolic volume relationship. [127]. The interaction between E_a and E_{LV} is termed ventricular-vascular coupling (E_a/E_{LV}). Ventricular-vascular coupling can be described further as:

$$E_a = \text{end-systolic pressure} / \text{SV} \text{ divided by } E_{LV} = \text{end-systolic pressure} / \text{ESV}$$

where end-systolic pressure is characterized as systolic blood pressure*0.9.

Accordingly, alterations in the determinants of ESV (heart rate, stiffness component, restrictive component) or SV (sympathetic modulation of ionotropy, afterload or EDV) influence changes in E_a/E_{LV} [128]. In healthy men and women in the resting state, ventricular-vascular-coupling is maintained in a range that maximizes the efficiency of the heart (0.6-1.2 represents the optimal interaction between the LV and arterial system). When the cardiovascular system is stressed (during exercise conditions), energy efficiency is sacrificed in favor of cardiac efficiency. This is manifested by a decrease in the coupling index with a greater relative increase in ventricular contractility than arterial load to ensure sufficient cardiac performance to meet the increased energy demands of the body [129].

In patients with moderate cardiac dysfunction, under resting conditions, patients have an augmented E_a accompanied by a disproportional reduction in the E_{LV} resulting in a three to four-fold marked increase in the E_a/E_{LV} ratio [130]. Under exercise conditions E_a generally increases due to a decrease in SV with a matched increase in pressure, while E_{LV}

will be blunted due to an increase in ESV (a marker of reduced LV systolic function) or manifested by an increase in pressure. With this, cardiac performance is compromised reflected by a blunted response to decrease the ventricular-vascular coupling ratio under stressed conditions (due to an increase in E_a and blunted ability to increase E_{LV}) [131]. Indeed, resting echocardiographic measures demonstrate that women with HER2-positive breast cancer experience alterations in LV systolic and diastolic functions despite a change in LVEF following chemotherapy [132]. Changes in LV function at rest may in turn attenuate the increase in E_{LV} during exercise compromising cardiac efficiency, or be coupled with an increase E_a . Importantly, the E_a/E_{LV} ratio is useful to gauge the prognosis of heart failure and predict cardiovascular related mortality in other clinical populations [133], [134]. Whether optimal ventricular-vascular coupling is compromised during exercise in women with breast cancer (due to an increase in E_a or a blunted response to increase E_{LV}) and can provide prognostic information regarding cardiac dysfunction in breast cancer survivors requires further investigation.

2.9.3: Vascular Function

The cardiovascular system is designed to provide ample pressure and flow to the body at rest and over broad ranges of stress. Arterial stiffness can be defined as the change in vessel diameter for a given volume change. Indices to describe arterial stiffness include arterial distensibility defined as the relative diameter change due to an increment change in pressure or arterial compliance characterized as an absolute change in vessel diameter for a given pressure [135]. In a normal elastic aorta, blood is ejected from the heart during systole and travels along the arterial tree generating a pressure wave that reflects from the periphery and returns to the heart during diastole. This reflected wave helps increase pressure during

diastole causing an increase in coronary blood flow. With an increase in aortic stiffness, the velocity of the pressure wave increases, and the reflected pressure wave reaches the heart at systole rather than diastole, resulting in augmentation of the systolic blood pressure [136]. The diminished elastic recoil of the stiff aorta, combined with the absence of diastolic augmentation from the reflected pressure wave, has the potential to reduce coronary filling and cardiac energetics. As such, increased arterial stiffness may attenuate coronary filling and compromise cardiac function, especially under stressed conditions in women with HER2-positive breast cancer. Measurement of arterial stiffness can be accomplished using sophisticated measures including PWV and arterial compliance using Applanation tonometry. Seemingly, these measures are important, because changes in vascular stiffness (or vascular load on the LV) may sacrifice cardiac performance progressively leading to cardiac dysfunction; and have been shown to predict adverse cardiac events in other clinical populations [137].

Blood pressure is under autonomic control of the central nervous system, and arterial stiffness is dependent on blood pressure. Accordingly, it is important to assess baroreceptor sensitivity in combination with assessment of arterial stiffness. The baroreflex is made up of two independent systems that operate in the short-term regulation of blood pressure [138]. The low pressure baroreceptors, are cardiopulmonary stretch receptors located in the atria, great veins and ventricles, which augments sympathetic nervous system activity in response to reductions in central venous pressure and volume [139]. A decrease in blood pressure causes the deactivation of baroreceptors with enhancement of sympathetic activity and vagal nerve inhibition, leading to increased heart rate, vascular resistance, SV and venous return [140]. The high pressure baroreceptors are stretch-receptors located in the tunica adventitia of

the aortic arch and carotid sinus which can be assessed through measures of BRS [139]. An increase in blood pressure leads to an increase in vagal tone, and sympathetic inhibition, which consequently results in decreased vascular tone, venous return, cardiac contractility and heart rate [141]. These processes are regulated through a negative feedback loop. The highly sensitive receptors in the carotid sinus send action potentials through the glossopharyngeal nerve (cranial nerve IX); while the baroreceptors in the aortic arch are sent through the vagus nerve (cranial nerve X) back to the nucleus of the solitary tract embedded in the medulla oblongata thus activating or inhibiting sympathetic activity. Alterations of these processes and BRS can contribute to the development and progression of cardiovascular disease as a result of changes in cardiac haemodynamics and vasomotor tone [142]. Thus, measurement of BRS will provide insight to the role of trastuzumab therapy on alterations in central nervous system activity, which may contribute to the development of latent cardiac morbidity.

2.10: CONCLUSION

Overall, it is apparent that trastuzumab therapy has led to increased rates of survival in women HER2-positive breast cancer, although these women may succumb to heart failure as a result of treatment during survivorship [143]. However, literature to support subclinical markers of cardiac dysfunction and their ability to predict the early onset of cardiotoxicity, even in women who are asymptomatic during survivorship, remains limited. Moreover, the long-term effects of trastuzumab treatment on changes in vascular function, which often precedes the development of heart failure is not well characterized in the HER2-positive breast cancer population. Given the increase in survival years associated with trastuzumab treatment, and the limited knowledge regarding long-term risk of cardiotoxicity in this

population, further investigation is warranted to determine changes in cardiovascular function and the associated cardiac prognosis in HER2-positive breast cancer survivors. Such research is important to clinicians, patients and physicians alike in order to detect changes in cardiac function earlier in order to reduce the risk to succumb to heart failure during survival years.

Chapter 3: Cardiovascular Function Measured with Echocardiography in Breast Cancer Patients Treated with Systemic Adjuvant Therapy: A Systematic Review of the Literature

The purpose of this chapter is to review systematically the existing literature concerning the use of subclinical markers of cardiac dysfunction in breast cancer through the use of echocardiography. A version of this will be submitted for peer review.

3.1: INTRODUCTION

Breast cancer remains one of the leading causes of cancer in Canadian women [1]; however with improved rates of disease free survival, focus on treatment-related cardiac toxicity is important for the management of breast cancer patients [2]. The most widely accepted definition of cardiac toxicity is defined as a reduction in LVEF of $>5\%$ with signs and symptoms of heart failure or a decline in LVEF of $>10\%$ without signs and symptoms of heart failure [5]. Cardiovascular disease (specifically the development of heart failure) is recognized as one of the leading cause of morbidity and mortality in the breast cancer population with one in five women expected to develop heart failure and a 29% increased risk to succumb to heart failure following a breast cancer diagnosis [3], [143]. Moreover, both treatment-related cardiac damage and the prevalence of cardiovascular disease risk factors (dyslipidemia, family history, diabetes mellitus, and hypertension) may play a role in the development of cardiac toxicity following a diagnosis of breast cancer. As such, it is pertinent to consider careful cardiac monitoring for the management of breast cancer patients who undergo cytotoxic treatment with a focus on early detection and understanding of potential cardiac changes that may occur as a result of treatment.

Currently, serial assessment of LVEF with 2D echocardiography, 3D echocardiography or radionuclide ventriculography is the most practical means of monitoring cardiac toxicity. Importantly, the measurement of LVEF to monitor the development of cardiac toxicity has important limitations. First, the measurement of LVEF is subject to intra-observer and inter-observer measurement error between different measurement techniques that are equivocal to the change to detect the onset of cardiotoxicity [144]. Moreover, changes in LVEF occur when substantial damage has already occurred and systolic dysfunction might not be reversible [27]. Further, changes in LVEF only represent a change in systolic function (contractile properties of the heart), while changes in diastolic function (relaxation of the heart) may also be altered as a result of treatment [145]. As such, the measurement of LVEF does not provide adequate information regarding the extent of change in cardiac function that occurs as a result of treatment for breast cancer.

Newer investigations are using echocardiography measures that are more sensitive to change in systolic and diastolic function of the heart in order to detect changes in cardiac function that occurs as a result of cancer therapies [146]. This is important, because many of the large clinical trials that define the incidence of breast cancer patients who develop cardiac dysfunction as a result of treatment only represent the population who experience a decrease in LVEF [3]. These data may inadequately represent the extent and incidence of cardiac dysfunction that occurs as a result of treatment. Therefore, the purpose of this systematic review is to summarize the existing data regarding the use of subclinical echocardiography measures to assess changes in cardiac function that occur as a result of potentially cytotoxic therapies both during and following treatment for breast cancer. Due to the role of cardiovascular disease risk factors that may increase the risk of cardiac related morbidity and

mortality in breast cancer patients, the prevalence of cardiovascular risk factors among the patient population(s) will also be discussed.

3.2 METHODS

3.2.1: Inclusion and Exclusion Criteria

All human studies that used echocardiography to assess cardiac function in the breast cancer population were deemed eligible. In order to be included in this review, studies had to include: all female subjects, early or locally advanced breast cancer patients (lymph node involvement but not distant metastasis), data regarding chemotherapy regimens, no prior exposure to chemotherapy and/or radiation, limited to English language and two time points or a control group to assess changes that occur in echocardiography parameters as a result of adjuvant therapy for breast cancer. Studies were also excluded if they only assessed LVEF or included mixed cancer types.

3.2.2: Search Strategy

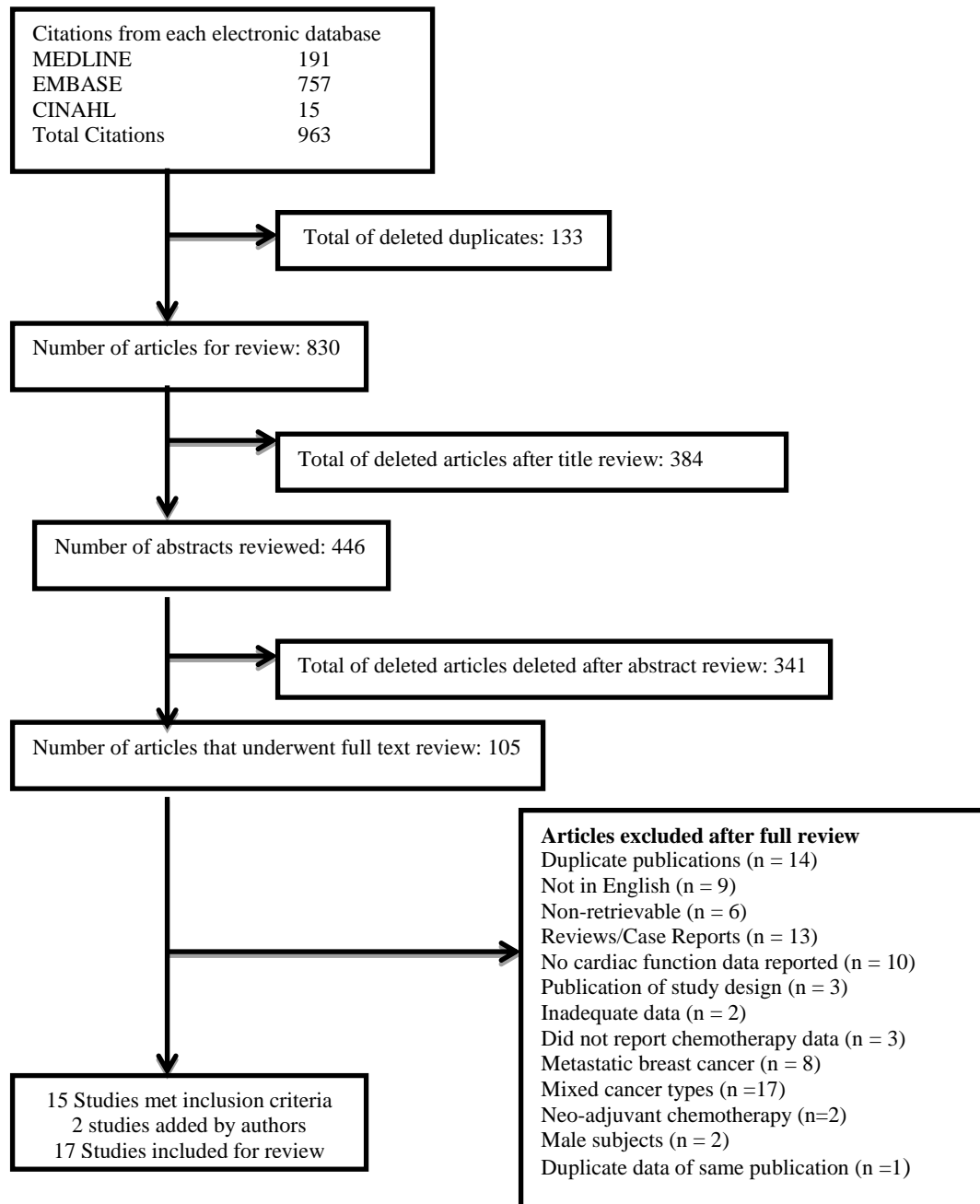
The search strategy adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for reporting of systematic reviews [147]. A rigorous search strategy assessing the use of echocardiography to measure cardiovascular function in early stage or locally advanced breast cancer was conducted using the following databases: MEDLINE (1950–March 2014, OVID Interface), EMBASE (1972–March 2014, OVID Interface) and (CINAHL (1982–March 2014, OVID Interface). The following MESH terms and key words were used for the systematic review search: echocardiography or Doppler and LV function or heart disease or cardiomyopathy and breast neoplasms or breast cancer or breast carcinoma with limits of human studies and adults (age 19 or older) were also applied if available within the database. All results from each database

were downloaded and stored on an online referencing system RefWorks (Bethesda, MD, USA). The yields of studies from each of the individual database searches are outlined in figure 3.1.

3.2.3: Data Extraction

All studies identified in the literature search underwent vigorous screening in a multi-step process by two separate reviewers (A.B and A.P) and the numbers of excluded studies were recorded at each step (figure 3.1). First, all duplicated citations were deleted from the compiled citation list. Each reviewer then separately screened all titles and abstracts upon first review for inclusion and exclusion criteria and reason for exclusion or inclusion were agreed upon between reviewers. Abstracts and titles that were not related to breast cancer, included only metastatic breast cancer, review articles, conference abstracts, articles not in English, letters to the editors, case reports, and articles that did not report echocardiographic data were excluded. Once all abstracts were screened, each full text version underwent full review for inclusion criteria with 91.5% concusses between reviewers. All discrepancies were mutually reviewed and resolved by consensus between the two reviewers. If the article was excluded upon the full text review, the reason for exclusion was recorded. All relevant data was then extracted by one reviewer (A.B.) and verified by a second reviewer (A.P.) using a standardized data extraction form. The following data were extracted for aggregate: author, year of publication, number of patients, breast cancer type (stage, grade, lymph node involvement), age, sex, type of treatment used (chemotherapy, endocrine therapy, targeted therapies, radiation etc.), doses, cardiac risk factors among the study group(s), type and timing of imaging, change in systolic parameters, change in diastolic parameters, and change in structure parameters.

Figure 3.1: Diagram of Systematic Review Process



3.2.4: Quality and Level of Evidence

The quality of investigations was determined using the downs and black scoring system by each reviewer and consensus was made through discussion when appropriate [148]. A modified version of the downs and black tool was used due to wording of the last question to a scale of 0 to 1 where a 1 was scored if a power calculation or sample size calculation was present and a score of 0 was assigned if there was no power calculation [149]. The level of evidence was also assessed using a five-point scale with one indicating the highest level of evidence and five indicating a low level of evidence. The scoring ranges from a randomized control trial (level one), a prospective control trial or cohort study (level two), a case-control study design (level three), a case series study or pre to post testing or post testing design (level four) and a case report, observational, or clinical consensus design (level five) [149].

3.3 RESULTS

3.3.1: Literature Search Findings

A total of 963 citations were identified and 17 peer-reviewed publications met all inclusion with a total of 14 studies identified during treatment involving 679 subjects (see table 3.1) [44], [150]–[162] and three studies identified following treatment involving a total of 275 subjects (see table 3.2) [23], [145], [163]. The mean age (when reported as mean age) of the women ranged from 42 ± 7 to 69.8 ± 3.1 yr. The prevalence of at least one cardiovascular disease risk factor (dyslipidemia, hypertension, family history, smoking, type two diabetes) was reported among the patient population in a total of six studies [132], [153], [154], [156], [162], [164] and use of cardiovascular medications was reported in three of the studies [132], [153], [154] conducted during chemotherapy (see table 3.3). All of the studies

conducted following treatment excluded subjects with known cardiovascular disease or those with contraindications to cardiopulmonary testing. Moreover, all studies were conducted using resting echocardiography images with the exception of one study that used dobutamine stress echocardiography during chemotherapy and one study that observed changes in cardiac function post peak exercise in breast cancer survivors [23], [151]. Although there was heterogeneity in the types and doses of chemotherapy used, all participants received anthracyclines as a part of their chemotherapy treatment and five studies reported the use of trastuzumab following chemotherapy [152], [158], [159], [162], [163].

3.3.2: Changes in Cardiac Measures During Therapy

Systolic Parameters: Echocardiographic measures of strain (deformation) and strain rate (rate of deformation) with tissue Doppler imaging (TDI) or 2D-echocardiography were more sensitive than LVEF in the detection of change in systolic function. For example, two studies were able to detect a decrease in radial peak systolic strain, radial strain rate, and radial systolic strain [154], [161] or a decrease in longitudinal systolic strain rate and longitudinal systolic strain [154] without a change in LVEF. Moreover, decreases in peak global longitudinal and radial strain were able to predict the early onset of cardiotoxicity preceding a change in LVEF [162]. Further, change in longitudinal strain, circumferential strain or radial strain accompanied by a decline in LVEF were also evident despite the development of cardiotoxicity [155], [158], [159]. It is important to note, that changes in myocardial deformation imaging were detected at doses lower than those identified to be cardiotoxic doxorubicin (cumulative dose of $240 \text{ mg}\cdot\text{m}^{-2}$) or epirubicin (cumulative dose of $300 \text{ mg}\cdot\text{m}^{-2}$) [158], [159], while two of the studies reported chemotherapy regimens including PL-DOX $30 \text{ mg}\cdot\text{m}^{-2}$ and cyclophosphamide $500 \text{ mg}\cdot\text{m}^{-2}$ every three weeks for six

cycles, which is believed to be less cardiotoxic than other anthracycline regimens [154], [161]. Further, one study identified a change in LV contractile reserve through the use of dobutamine stress echocardiography in patients who developed cardiotoxicity during therapy [151]. In the same investigation, those who did not develop cardiotoxicity during therapy did not demonstrate a change in LV contractile reserve during therapy.

Diastolic Parameters: Changes in measures of diastolic function were evident in eight studies with a total of 428 participants over the time course of adjuvant therapy [44], [132], [150], [151], [153], [156], [157], [160]. A reduction in diastolic function was evident in four of the eight studies, with no change in any measure of LV function [44], [150], [151], [157]. A decline in diastolic function was evident through the use of 2D-echocardiography measures with a decrease in E/A ratio [150]–[152], [156], [157], [160] due to a decrease peak E filling velocity (indicating a decrease in early LV filling) [151], [156]. However, the E/A ratio remained within normal clinical limits between 1.0-2.0 throughout the course of therapy in many of these studies [150], [151], [156], [160]. Conversely, two of these study demonstrated normal diastolic function with E/A ratio between 1.0-2.0 with a subsequent drop below 1.0 during the course of chemotherapy [152], [157]. In this case, the change in the E/A ratio was due to an increase in the A wave accompanied by a decrease in the E wave [157] suggesting an reversal of the E/A ratio resulting in an increase in late LV filling, a marker of diastolic impairment. Importantly, two studies compared echocardiographic measures between women who developed cardiotoxicity versus women who did not, and each of these investigations demonstrated that those who experience a decrease in LVEF (>50%) had concurrent changes in measures of diastolic function [151], [160]. Lastly, only one study included a control group of healthy age matched controls and measured differences

between groups at each time point throughout chemotherapy [157]. This group identified that 1 to 2 yr following chemotherapy, women with a history of breast cancer had a significant decrease in Ea/Aa ratio due to a decrease in Ea diastolic velocity and significant increase in Aa diastolic velocity compared to apparently healthy age matched controls. Although these data suggest that reduced diastolic function might be plausible during chemotherapy, discrepancies between changes in measures of diastolic function are apparent. Because changes in diastolic function measured by 2D-echocardiography are load-dependent, changes in EDV (preload) may influence changes in diastolic outcomes, which may explain some discrepancies found in these studies. However, it is plausible that alterations in diastolic function might not be alleviated 1 to 2 yr following therapy, therefore further research is warrant.

Table 3.1: Systematic Review Outcomes During Treatment

Study	Country	Time of Assessment	Group(s)	Age (yr)	Treatment	Main Outcomes
Appel et al, 2011 ¹⁵⁰ (n = 80)	Denmark	Before and after 3 cycles of epirubicin (47-113 days)	Single group	52 ± 9	Epirubicin (mean cumulative dose, 273.7 ± 46.6 mg·m ⁻²)	↓ E/A ratio. A reduction in E/A ratio to abnormal values was only apparent in 2 women
Civelli et al, 2006 ¹⁵¹ (n = 49)	Italy	Before and after 3 cycles of chemotherapy. Follow up at 1,4,7 and 12-18 after chemotherapy	Group A-Cardiotoxicity (LVEF <50%) Group B- No Cardiotoxicity	42 ± 7	Taxotere (85 mg·mq), Epirubicin (200 mg·mq), and cyclophosphamide (4 g·mq) for three cycles every four weeks.	Group A: ↓ PWTs, ↓ peak E, ↓ E/A ratio, ↑ ESV, ↓ LVCR, ↓ resting and peak LVEF Group B: ↓ peak E and ↓ E/A ratio
Di Lisi et al, 2011 ¹⁵² (n = 72)	Italy	Before chemotherapy, following 3 and 6 months of chemotherapy. All groups were assessed together for all outcomes	Group A- 6 cycles of FEC Group B- 3 cycles FEC and Trastuzumab Group C- 6 cycles of Trastuzumab Group D- 3 cycles FEC and Taxotere Group E- 3 cycles FEC, Trastuzumab and Taxotere	57 ± 12	Fluorouracil (800 mg·m ⁻²), cyclophosphamide (800 mg·m ⁻²), trastuzumab (420 mg·m ⁻²), taxanes (150 mg·m ⁻²) and epirubicin (cumulative dose 840 mg·m ⁻²) (group A) or epirubicin (cumulative dose 420 mg·m ⁻²) (group B,C,D,E)	Data represents all groups: ↓ in E/A ratio, ↓ Em/Am ratio, ↓ Sm, ↓ TEI index and ↑ in IVRT. A total of 94.5% of participants demonstrated a change in measure of diastolic function
Erdim et al, 2009 ¹⁵³ (n = 41)	Turkey	Baseline and 1 year following chemotherapy	Single group	48 ± 8	4 cycles of cyclophosphamide and epirubicin (90 mg·m ⁻²) per cycle or 6 cycles of cyclophosphamide and epirubicin (75mg/m ²) per cycle	↓ E/A ratio in patients with an increase in cardiac troponin T, ↓ LVEF
Jurcut et al, 2008 ¹⁵⁴ (n = 16)	Belgium	Baseline, and following the 3 rd and 6 th cycle of chemotherapy	Single group	69. 8 ± 3.1	6 cycles of PL-DOX (30 mg·m ⁻²) and cyclophosphamide (500 mg·m ⁻²) every 3 weeks	↓ longitudinal E velocity, ↓ longitudinal systolic strain rate (1/s), ↓ longitudinal systolic strain (%), ↓ radial systolic strain rate (1/s) and ↓ radial systolic strain (%)

Study	Country	Time of Assessment	Group(s)	Age (yr)	Treatment	Main Outcomes
Stoodley et al, 2011 ¹⁵⁵ (n = 52)	Australia	Baseline, and following anthracycline treatment	Single group	49 ±9	four or six cycles of anthracyclines including doxorubicin (mean dose 236 ± 33 mg·m ⁻²), or epirubicin (mean dose 408 ± 110 mg·m ⁻²)	↓ LVEF, ↓ global longitudinal strain (%) (regional longitudinal strain: basal septum, mid-septum, apical septum, mid-lateral basal-lateral) ↓ global radial strain (%) (regional radial strain: anterior septum, inferior and septal)
Lotrionte et al, 2013 ¹⁵⁶ (n = 39)	Italy	Baseline, following treatment, 2 mo, 6 mo and 12-24 mo following completion of chemotherapy	Single group	Median: 49	Anthracyclines including doxorubicin (50 mg·m ⁻²), or epirubicin (90 mg·m ⁻²), every 3 wk in combination with cyclophosphamide (500 mg·m ⁻²), docetaxel (75 mg·m ⁻²), and 5-fluorouracil (500 mg·m ⁻²).	↓ LVEF, ↓ E wave, ↓ lateral Em wave, ↓ septal Em wave, ↓ mean E wave, ↓ E/A ratio, ↓ septal Em/Am, ↓ E/lateral wall Em, ↓ E/mean Em (data represents a change in parameters at one time point)
Mizia-Stec et al, 2013 ⁴⁴ (n = 35)	Italy	Baseline and 6 mo following anthracycline treatment	Single group	Range: 35-68	Various regimens including: 5- fluorouracil (500 mg·m ⁻²), epirubicin (75 mg·m ⁻²) cyclophosphamide (500 mg·m ⁻²); epirubicin (90 mg·m ⁻²) cyclophosphamide (600 mg·m ⁻²); 5-fluorouracil (500 mg·m ⁻²), doxorubicin (50 mg·m ⁻²) cyclophosphamide (500 mg·m ⁻²); or docetaxel (75 mg·m ⁻²), doxorubicin (50 mg·m ⁻²) cyclophosphamide (500 mg·m ⁻²)	↑ EDD, ↓ IVS, ↓ PW, ↑ EDV, ↑ SV, ↑ Tei Index, ↓ S wave, ↓ E wave, ↓ A wave, ↑ E/E' and ↓ in flow propagation velocity.

Study	Country	Time of Assessment	Group(s)	Age (yr)	Treatment	Main Outcomes
Nagy et al, 2008 ¹⁵⁷ (n = 60)	Poland	Baseline, 3 mo following the second dose of chemotherapy, immediately following chemotherapy, 1 and 2 yr following chemotherapy. Data assessed as differences between groups at each time point.	Group A: Breast cancer Group B: Healthy controls Data assessed as differences between groups at each time point.	49.2 ± 10.1 50 ± 9	Epirubicin (cumulative dose of 360 mg·m ⁻²) or doxorubicin (cumulative dose of 240 mg·m ⁻²) and cyclophosphamide	↓ Ea/Aa ratio, ↓ Ea diastolic velocity, and ↑ Aa diastolic velocity. These measures were significantly different between each group in the septal, posterior, septal, lateral, inferior, and anterior segments. Additionally, there was a ↓ in S velocity in the septal and posterior segments. Diastolic dysfunction was apparent in 97.5% of patients
Sawaya et al, 2011 ¹⁵⁸ (n = 43)	USA	Baseline (with the exception of 10 patients who completed anthracycline treatment), 3 and 6 mo of treatment	Single group	47 ± 11	Epirubicin (cumulative dose of 300 mg·m ⁻²) or doxorubicin (cumulative dose of 240 mg·m ⁻²) taxanes and Trastuzumab	↓ LVEF, ↑ ESV, ↓ longitudinal strain (%), ↓ radial strain (%), ↓ circumferential strain (%), ↓ peak early diastolic mitral annular velocity
Sawaya et al, 2012 ¹⁵⁹ (n = 81)	Australia	Baseline, at the end of anthracycline therapy and every 3 mo during trastuzumab therapy (total of 15 mo)	Single group	49 ± 10	Doxorubicin (cumulative dose of 240 mg·m ⁻²) or epirubicin (cumulative dose of 300 mg·m ⁻²) for 3 mo, weekly paclitaxel (80 mg·m ⁻²) and trastuzumab (2 mg·kg) for 3 mo, and (6 mg·kg) every 3 wk for 9 mo	↓ LVEF and ↓ longitudinal strain (%)

Study	Country	Time of Assessment	Group(s)	Age (yr)	Treatment	Main Outcomes
Stoodley et al, 2013 ¹⁶⁰ (n = 52)	Australia	Baseline and following anthracycline therapy (before radiation therapy and/or trastuzumab)	Single group	49 ± 9	Doxorubicin (cumulative dose of 236 mg·m ⁻²) or epirubicin (cumulative dose of 408 mg·m ⁻²) for four or six cycles	↑ Peak A velocity, ↓ E/A ratio, ↓ PV diastolic VTI, ↑ PV atrial fraction, ↑ PV systolic fraction, ↓ early strain rate. Participants who developed cardiotoxicity (LVEF<50%) had additional changes in indices of diastolic function (↓ E' velocities, ↓ atrial reversal duration) and systolic function (↓ bi-plane longitudinal systolic strain (%)).
Wildiers et al, 2008 ¹⁶¹ (n = 16)	Belgium	Baseline, before the fourth cycle, within 7–14 days after the sixth cycle and before radiation therapy and/or trastuzumab therapy when appropriate	Single group	Median: 69	PL-DOX (30 mg·m ⁻²) cyclophosphamide (500 mg·m ⁻²) every 3 weeks for 6 cycles	↓ radial peak systolic strain (%) and ↓ radial strain rate (1/s) with no change in LVEF
FallahRad et al, 2011 ¹⁶² (n = 42)	Canada	Baseline, before the initiation of trastuzumab therapy, weeks after the final cycle of chemotherapy, 6, 9, 12 mo after the initiation of trastuzumab	Group A: No cardiotoxicity Group B: Cardiotoxicity	46 ± 8 47 ± 10	Six cycles of 5-fluorouracil, epirubicin (cumulative dose 600 mg·m ⁻²) and cyclophosphamide or four cycles of doxorubicin (cumulative dose of 240 mg·m ⁻²) cyclophosphamide and trastuzumab	Mean S', ↓ peak global radial strain, ↓ peak global longitudinal strain and ↓ LVEF following a change in strain measures

STE-speckle tracking imaging; TDI- tissue Doppler imaging; Echo-echocardiography; PL-DOX-pegylated liposomal doxorubicin; LVEF- left ventricular ejection fraction; ESV-end systolic volume; EDV-end diastolic volume; PWTs-posterior wall thickness systole; EDD-end diastolic diameter; LVCR-left ventricular contractile reserve; IVS- interventricular septum; PV-pulmonary venous; VTI-velocity time integral

3.3.3: Changes in Cardiac Measures Following Therapy

Systolic Parameters: Only two studies have assessed changes in systolic function following therapy [23], [163]. In the absence of a reduction in LVEF, 2D- speckle tracking imaging (STE) including a reduction in global longitudinal strain was able to detect a change in systolic function following the completion of adjuvant therapy (average time since completion was 50 ± 22 mo following the last anthracycline treatment) in women treated with anthracyclines or anthracyclines and trastuzumab (cumulative dose of anthracyclines $402 \text{ mg}\cdot\text{m}^{-2}$). Moreover, Khouri and colleagues (2014) [23] suggested that changes in LVEF were not apparent with 2D echocardiography, whereas 3D echocardiography was able to detect a change in LVEF with subsequent change in post peak exercise SV, Q index and the mean change in Q index from baseline to post peak exercise when compared to healthy controls. The reduction in SV, Q and Q index provide some insight that either systolic and/or diastolic function might be impaired in breast cancer survivors [165]. Perhaps these data suggest that asymptomatic breast cancer survivors present with systolic impairment, while myocardial deformation imaging or exercise measures of cardiac function may play a pivotal role in the oncology setting for early detection of systolic dysfunction compared to conventional resting measures of LVEF.

Diastolic Parameters: Two studies investigated the use of echocardiography to measure the effects of adjuvant chemotherapy on diastolic function in breast cancer survivors [145], [163]. Radulescu et al, (2008) [145] reported that measures of diastolic function including peak E filling velocities, pressure half time of the E wave, and isovolumetric relaxation time (IVRT) were all reduced while peak A filling velocities were increased in breast cancer patients who completed chemotherapy. Moreover, despite normal resting LVEF

values, asymptomatic women with a history of breast cancer present with reduced measures of diastolic function including reversal of the E/A ratio, reduced global E' and reduced global Sm in comparison with women with no history of breast cancer [163]. Although information is limited, these data suggest that adjuvant therapy may play a role in reduced diastolic function in breast cancer survivors, while an increased resistance to filling of the ventricles might be seemingly acceptable.

Table 3.2: Systematic Review Outcomes Following Treatment

Study	Country	Time of Assessment	Group(s)	Age (yr)	Treatment	Main Outcomes
Khoury et al, 2014 ²³ (n = 77)	USA	Mean 26 ± 22 mo post-doxorubicin containing chemotherapy	Group A: Doxorubicin treated patients Group B: Controls	51 ± 10 57 ± 7	Doxorubicin containing chemotherapy (cumulative dose of 240 mg·m ⁻²) and tamoxifen in the absence of trastuzumab therapy	↓ LVEF by 3DE but not 2DE at rest. Post peak stress echo demonstrated a ↓ change in Q index, ↓ SV and ↓ Q.
Ho et al, 2010 ¹⁶³ (n = 130)	Dublin	Average 50 ± 22 mo following the last anthracycline dose	Group A: Anthracyclines and no Trastuzumab Group B: Anthracyclines and Trastuzumab Group C: Controls	53 ± 8 56 ± 8 52 ± 5	Anthracyclines (cumulative dose of 402 mg·m ⁻²), cyclophosphamide (cumulative dose of 425 mg·m ⁻²) and paclitaxel (cumulative dose of 861 mg·m ⁻²) and trastuzumab when appropriate	Whole chemotherapy group compared to controls: ↓ global Sm, ↓ transmitral Doppler E velocities, ↓ E/A, ↓ global E velocities, ↓ global longitudinal strain and a regional ↓ in longitudinal strain (posterior wall). The trastuzumab naïve group had a regional ↓ in longitudinal strain (septal, lateral, anterior) compared to controls.
Radulescu et al, 2008 ¹⁶⁴ (n = 68)	Romania	Measures were taken following treatment with epirubicin in the study group and before commencement of chemotherapy in the control group	Group A: Patients treated with Epirubicin (study group) Group B: Women with breast cancer who have not started chemotherapy (controls)	53.9 ± 10.01 46.3 ± 14.4	Epirubicin (50 mg·m ⁻²), cyclophosphamide (500 mg·m ⁻²) and 5-fluorouracil (500 mg·m ⁻²) every 3 weeks for 6 six cycles	↓ Emax, ↑ Amax, ↓ pressure half time of E wave, and ↓ IVRT compared to controls

IVRT- isovolumetric relaxation time; SV-stroke volume, Q-cardiac output; STE-speckle tracking imaging; TDI- tissue Doppler imaging; Echo-echocardiography.

3.3.4: Prevalence of Cardiovascular Disease Risk Factors in Breast Cancer

There are seven studies completed during treatment that reported the prevalence of cardiovascular disease or cardiovascular disease risk factors among the patient groups [132], [153], [154], [156], [162], [164]. The reported outcomes included the incidence of ischemic heart disease (6%) [155], [160], hypercholesterolemia (19% - 39%) [155], [156], [160], dyslipidemia (25.5%) [153], hyperlipidemia (36%) [162], smoking (5.9% - 17%) [153], [155], [160], [162], hypertension (18% - 68 %) [153], [155], [156], [160], [162], diabetes mellitus (4% - 15%) [153], [155], [156], [160], [162] and family history of cardiovascular disease (19.6% - 33%) [153], [156], [162]. In two studies, patients were treated with beta-blockers, ACE inhibitors, statins, diuretics or calcium channel blockers in 41% and 50% of the total population [153], [154]. One study reported individual data for various cardiac medications including treatment with ACE inhibitors (23.5%), beta-blockers (3.9%), statins (25.5%), aspirin (11.8%), and warfarin (2.5%) [153]. None of the studies conducted in survivors included women a history of cardiovascular disease or known risk factors for cardiovascular disease. The prevalence of cardiovascular risk factors or the use of cardiac medications during therapy may influence the change in echocardiography measures throughout therapy. For instance, the use of statins, angiotensin antagonists and beta-blockers have been suggested to be cardioprotective against cardiac injury during therapy [166], however patients with hypertension may be at increased risk of developing cardiotoxicity as a result of treatment ameliorating results of the investigation(s). Moreover, data that excludes women with known cardiovascular disease or cardiac risk factors, may underestimate the

degree of cardiac dysfunction that occurs due to the prevalence of co-morbid conditions in the breast cancer population.

Table 3.3: Cardiovascular Disease Risk Factors in Breast Cancer

Study	Population	Prevalence of Cardiovascular Disease or Cardiovascular Disease Risk Factors
Khoury et al, 2014 (n = 77)	Survivors	Patients with cardiovascular disease or with contraindications to cardiopulmonary testing were excluded.
Stoodley et al, 2013 (n = 52)	During Chemotherapy	History of ischemic heart disease (6%), hypercholesterolemia (22%), smokers (12%), hypertension (26%), diabetes mellitus (4%).
Lotrionte et al, 2013 (n = 39)	During Chemotherapy	Family history of cardiac disease (33%), hypertension (>140/90mmHg) (18%), hypercholesterolemia (39%) and diabetes mellitus (15%).
Mizia-Stec et al, 2013 (n = 35)	During Chemotherapy	Clinical or echocardiography evidence of heart failure, acute cardiotoxicity symptoms, severe or uncontrolled hypertension, diabetes, coronary artery disease, left chest wall radiation, active smoking, abnormalities in ECG, autoimmune or endocrine disease or infections were all excluded from this investigation.
Sawaya et al, 2012 (n = 81)	During Chemotherapy	Authors did not report the prevalence of cardiovascular disease risk factors. Patients with LVEF < 50 at baseline were excluded.
Sawaya et al, 2011 (n = 43)	During Chemotherapy	Authors did not report the prevalence of cardiovascular disease risk factors. Patients with LVEF < 50 at baseline were excluded.
Fallah-Rad, 2011 (n = 42)	During Chemotherapy	Smoking (17%), diabetes (14%), hyperlipidemia (36%), hypertension (12%), family history (29%).
Appel et al, 2011 (n = 80)	During Chemotherapy	Patients with heart rhythm other than sinus rhythm or prior cardiovascular disease were excluded from this investigation.
Di Lisi et al, 2011 (n = 72)	During Chemotherapy	Patients treated with beta-blockers, ACE inhibitors or statins (41%), diabetes mellitus (15%) and dyslipidemia (19%).
Stoodley et al, 2011 (n = 52)	During Chemotherapy	Ischemic heart disease (3%), Hypercholesterolaemia (21%), smoking (25%), hypertension (25%), diabetes mellitus (4%).
Ho et al, 2010 (n = 130)	Survivors	Patients with uncontrolled hypertension, significant valvular disease, widened QRS complex or surface ECG, heart rhythm other sinus rhythm or previous history of heart failure or coronary artery disease were excluded from this investigation.
Jurcut et al, 2008 (n = 16)	During Chemotherapy	10 (63%) patients had controlled hypertension, 2 (13%) had diabetes and 50% of patients received antihypertensive therapy including ACE inhibitors, diuretics, calcium channel blockers or beta-blockers.

Study	Population	Prevalence of Cardiovascular Disease or Cardiovascular Disease Risk Factors
Erdim et al, 2009 (n = 41)	During Chemotherapy	Hypertension (35.3%), dyslipidemia (25.5%), family history of cardiovascular disease (19.6%), diabetes (11.8%), and smoking (5.9%). Treatment with ACE inhibitors (23.5%), beta-blockers (3.9%), statins (25.5%), aspirin (11.8%), and warfarin (2.5%) was also reported.
Wildiers et al, 2008 (n = 16)	During Chemotherapy	Patients with a history of heart failure, unstable angina pectoris, previous history of myocardial infarction, uncontrolled hypertension or arrhythmias were all excluded.
Radulescu et al, 2008 (n = 68)	Survivors	Patients with a history of ischemic heart disease, valvular disease, heart hypertension or diabetes mellitus were excluded.
Nagy et al, 2008 (n = 60)	During Chemotherapy	Patients with hypertension (severe or mild), anemia, type 2 diabetes, coronary heart disease, left ventricular hypertrophy, severe aortic stenosis, mitral valve disease, or cardiomyopathy were excluded.
Civelli et al, 2006 (n = 49)	During Chemotherapy	Patients with a history of cardiovascular disease or use of anti-diabetic or cardiac related medications were excluded.

Reported number of patients with cardiovascular disease or cardiovascular disease risk factors in the included articles in this review.
Patients taking medications for cardiovascular disease were also reported.

3.3.5: Quality and Level of Evidence

There is level two and level four evidence to support that systemic adjuvant therapy for breast cancer leads to a change in systolic and diastolic function, with or without a subsequent decline in LVEF, which can be successfully measured by echocardiography. Many of these investigations are generally single arm cohort studies with small sample sizes which included baseline and follow up measurements. Moreover, level five evidence supports that systemic adjuvant therapy leads to a decline in diastolic and systolic function in asymptomatic women with a history of breast cancer. To date, only observational studies with a cross-sectional cohort study design are available. Although there are numerous large prospective clinical trials that have demonstrated that treatment for breast cancer is associated with cardiac toxic effects when defined by a decline in LVEF, more investigations are needed utilizing more sensitive echocardiography measures to assess the risk of cardiac dysfunction in those with a history of breast cancer.

The quality of evidence to support changes in cardiac function that occur as a result of systemic adjuvant therapy is seemingly low. This is in part, due to the study design of current investigations including lack of randomized control trials, lack of studies including control groups of healthy subjects or women with breast cancer who do not receive systemic adjuvant treatment (i.e. women treated with surgery and/or radiation only). Without such information it is difficult to conclude if the change in cardiac function is due to a result of treatment, or whether women with breast cancer have altered cardiac function upon commencement of therapy. Moreover, the heterogeneity of these studies and lack to control for variables including type and dose of treatment, prevalence of cardiac risk factors and type

of breast cancer diagnosis make it difficult to determine the risk of cardiac dysfunction associated with treatment for breast cancer. Lastly, many of these studies only report statistical difference between groups or changes in cardiac echocardiography parameters over time, therefore the number of women who experience a decline in cardiac function among the breast cancer population cannot be fully elucidated from currently available evidence.

3.4: DISCUSSION

There are significant key messages that are highlighted in this review. First, utilization of 2D and 3D echocardiography or more sophisticated measures using TDI and STE are useful for the measurement of subclinical changes in myocardial function during and following therapy for breast cancer. Measures of systolic and diastolic function seem to be altered despite a change in LVEF [44], [149], [150], [154], [161], [155], the currently accepted measure of cardiac toxicity in breast cancer [5]. Secondly, it is important to differentiate these changes between women currently undergoing therapy and breast cancer survivors. The information highlighted in this review demonstrates that women who undergo systemic adjuvant therapy experience impaired systolic and diastolic function, which is not alleviated during survivorship. Further, it seems that alterations in subclinical measures of myocardial function occur at doses that are generally considered non-cardiac toxic and it is difficult to conclude if alterations are due to specific drug combinations. Lastly, it is imperative to highlight the prevalence of cardiovascular disease risk factors identified in these investigations. As such, management of cardiovascular risk factors might be clinically significant in breast cancer. This information is important in the management of breast cancer in order to design effective treatment strategies to alleviate the risk of cardiac related morbidity and mortality during survivorship.

3.4.1: Cardiac Complications During Therapy

The importance of cardiac evaluation and monitoring of patients receiving breast cancer therapies is highlighted. Here, in this review, we emphasize important changes that occur in myocardial function as a result of treatment for breast cancer, and suggest considerations for the evaluation of cardiac dysfunction in the management of breast cancer. Currently, the incidence of overt heart failure varies depending on treatment regimens ranging from 4.3% and 20.4% within 5 yr following therapy [3]. However, the measurement of LVEF has important limitations and may under estimate the cardiac toxicity associated with treatment for breast cancer. As such, new investigations including the use of subclinical measures of cardiac dysfunction demonstrate that the incidence of cardiac toxicity may have been underestimated in previous clinical trials due to the limitations of monitoring LVEF.

Measures of strain and strain rate using TDI and STE have been employed to detect early myocardial changes in patients receiving chemotherapy for breast cancer with or without a change in LVEF [154], [159], [161]. Evidence suggest that radial and longitudinal strain rate are consistently reduced during therapy in breast cancer patients, while measures of longitudinal strain $<19\%$ may predict the development of symptoms of heart failure following therapy [159]. Consequently, myocardial strain imaging is successful in demonstrating changes in myocardial function during therapy, and can also predict the onset of cardiotoxicity as a result of treatment [146]. However, the use of myocardial strain imaging in predicting the late onset of cardiac toxicity or cardiac related prognosis during survivorship in breast cancer remains elusive. In other patients with systolic dysfunction, longitudinal deformation imaging is shown to be superior then the measurement of LVEF in predicting the onset of major averse cardiac events [167]. As such, future investigation is

needed to determine the use of strain imaging in the breast cancer population. Moreover, it is important to highlight that changes in myocardial strain imaging occur before a decline in LVEF and despite low doses or favorable drug regimens (PL-DOX) [154]. As expected, these alterations in strain rate imaging also occur in the elderly populations (69.8 ± 3.1 yr) despite a normal dose of anthracyclines [154], however Nagy and colleagues suggest that these measures are also reduced in younger women with no history of risk for cardiovascular disease [157]. It is possible that strain imaging by TDI and 2D-STE might be adequate measures to predict the onset of latent cardiac related morbidity in breast cancer patients who are considered low risk of developing cardiotoxicity, although future investigation is needed. Stress echocardiography (exercise or pharmacological stress) is well established for providing prognosis information for patients with coronary artery disease in predicting future cardiac events [168]. However, the use of stress echocardiography in the assessment of the hemodynamic consequences of breast cancer treatment and subsequent cardiac events is limited. The use of stress echocardiography may reveal latent or subclinical LV dysfunction in patients in who deterioration of LV function is not detected at rest. Briefly, this is due to the inability to increase the ejection fraction (EF) or reduce ESV with stress indicating impaired myocardial contractility [169]. One study identified in this review investigated the use of dobutamine stress echocardiography detected the onset of cardiotoxicity. Civelli and colleagues (2006) [151] demonstrated that the measurement of LV contractile reserve allowed for the identification of patients at risk of developing cardiac dysfunction in the short-term follow up. Moreover, one study assessed a change in LVEF with dobutamine radionuclide ventriculography demonstrating its ability to detect those at high or moderate risk of developing cardiotoxicity; however it was not included here in this

review due to differences in modalities used to assess cardiac function [170]. Together, these studies demonstrate the usefulness of the measurement of LV contractile reserve in the subclinical identification of cardiac dysfunction during therapy; however its role in evaluating cardiac related prognosis in long-term follow up is unknown.

Diastolic function has also been explored as a marker of early cardiac toxicity preceding a change in LVEF in several studies. Diastolic dysfunction is generally accepted as a prolongation or incomplete ability of myocardium to lose its ability to generate force and shorten awhile it returns to an unstressed length [171]. However, conflicting evidence makes it difficult to determine the extent of diastolic impairment that occurs during therapy, and which measures are most sensitive to changes in diastolic function. For instance, some investigations demonstrate that women with breast cancer experience a decline in diastolic function due to augmentation of the E/A ratio as result of an increase in the mitral inflow E velocities with no change in mitral inflow A velocities [151], [156], while other studies demonstrate a reversal of the E/A ratio due to a decline in the mitral inflow A velocities [154]. Accordingly, it is plausible that women undergoing therapy will experience an decrease in early ventricular filling due to an increase in filling pressure, an increase in late LV filling, or both, all indicating a change in diastolic function. This is also supported by changes in TDI measures signifying impairment in diastolic function, although changes in echocardiographic measures indicating a change in diastolic function are not consistent. Noteworthy, mitral inflow patterns are highly sensitive to changes in preload by 2D-echocardiography while TDI measures are perceived to be less load dependent [172]. Changes in LV volumes and diameter as a result of treatment may therefore pose a limitation to the value of mitral valve inflow patterns in the assessment of diastolic function with 2D-

echocardiography.

The use of TDI and 2D-STE imaging demonstrates that women experience a decline in diastolic function also experience a change in subclinical measures systolic dysfunction (strain and strain rate) [160]. Thus, it is likely that although women undergoing cytotoxic treatment may experience a decline in diastolic function, it is likely on a continuum with a reduction in systolic function as a result of increased LV stiffness and an increase in LV filling pressures. It is also noteworthy, that although treatment with anthracyclines is associated with a change diastolic function, these measures often stayed within normal clinical limits [152], [157]. As such, the extent of diastolic dysfunction that occurs due to treatment for breast cancer is unclear, and evidence to support whether changes in diastolic parameters lead to detriment in diastolic function during survivorship is limited.

3.4.2: Cardiac Complications Following Therapy

Cardiovascular complications of treatment remain an important limitation to the treatment of breast cancer. Here, in this review we identified only three studies that have assessed the use of echocardiography to detect subclinical changes in cardiac function in breast cancer survivors [23], [145], [163]. A reduction in 2D-STE-derived global and regional longitudinal strain in asymptomatic individuals previously treated with anthracyclines suggests that cardiac toxicity might persist event beyond the acute phase following treatment. Importantly, a subset of patients treated with anthracyclines and trastuzumab in this investigation did not show changes in global longitudinal strain compared to controls. As such, there is some evidence that trastuzumab-induced cardiac toxicity may differ from that induced by anthracyclines, with trastuzumab-induced cardiomyopathy being

less severe [163]. However, interpretation of these results is cautioned due to the low sample size ($n = 17$) of this investigation.

Exercise ('stress') echocardiography has also shown that LV contractile reserve impairment is evident in breast cancer survivors treated with anthracyclines despite normal LVEF. This was indicated by a significant difference in post-peak Q index, SV and change in Q index compared to healthy controls [23]. The ability to increase SV and Q ($Q=SV \times HR$) throughout exercise depends on the amount of blood returning to the heart (preload), which in turn may be influenced by the ability of the myocardium to relax during diastole. Moreover the ability to eject blood from the heart per beat depends on the inotropic state of the heart and the ability to eject blood from the heart during systole. As such, a reduction in either of these parameters might suggest impairment in diastolic function or systolic function but it most likely not isolated but rather a combination of both. Lastly, one investigation suggests that breast cancer survivors may present with diastolic impairment due to poor LV compliance [145], however further investigation is needed. Thus, with lack of guidelines for follow up and monitoring during survivorship, women at risk of developing cardiac toxicity may go untreated until reversal or medical management is too late.

3.4.3: Methodological Considerations

It is plausible that with the use of 2D-echocardiography may not be reliable to detect small changes in LVEF, including a decline of 5% with accompanying signs and symptoms of heart failure. For example, Thavendiranathan et al (2013) [173], found that the difference in temporal variability of LVEF by 3D or 2D-echocardiography was 6-10%, which therefore a change in LVEF by 5% could be underestimated by 2D-echocardiography. Moreover, 2D-echocardiography assess load dependent changes in LV cavity size and may underestimate

LV volumes and LVEF [174]. TDI is believed to be less dependent on loading conditions than conventional 2D-echocardiography Doppler imaging in the assessment in systolic and diastolic function, and might be more clinically useful in the detection of changes in cardiac function [172], [174]. Moreover, 2D-STE strain rate imaging is also more sensitive to subtle changes in LV wall deformation allowing discrimination between active and passive myocardial tissue movement [175]. The use of advanced measures of echocardiography including 3D-echocardiography, TDI and 2D-STE should be considered in standard of care if possible. Although these newer techniques might be more sensitive to the detection of cardiac toxicity, it is also important to consider their limitations due to high costs and the skills required to acquire these images and interoperate results.

3.4.4: Cardiovascular Disease Risk Factors

Although it is widely accepted that women with breast cancer might be at increased risk of cardiac related morbidity and mortality due to treatment, the prevalence of cardiac risk factors among the breast cancer population is not well defined. Current practice guidelines recommend that patients undergoing anticancer therapy should be encouraged follow standard guidelines for reducing cardiovascular disease risk, such as blood pressure control, lipid level reduction, smoking cessation and lifestyle modifications [118]. Thus, identification of cardiac risk factors and their prevalence among breast cancer patients may assist in the implementation of screening for cardiovascular risk factors and provide focus for lifestyle management in breast cancer survivors. Moreover, the development of cardiovascular risk factors may increase the likelihood of developing cardiac toxicity, while early medical management may decrease the likelihood of developing heart failure in breast cancer during survival years [159]. Indeed, identification of cardiovascular disease risk

factors in breast cancer is of central importance, in order to design effective treatment strategies with potential to alleviate co-morbidities during survivorship.

3.5: CONCLUSION

The information regarding the role of echocardiography in cardiac monitoring and management in breast cancer remains limited. It is difficult to distinguish which cardiac parameters are most sensitive to change in cardiac function in breast cancer, and subsequently, which measures provide meaningful information regarding cardiac prognosis. The earlier clinical trials regarding outcomes to treatment for breast cancer also only focused on the development of overt heart failure and a decline in LVEF as a result of treatment. Newer investigations suggest that cardiovascular disease is accepted as a competing outcome of mortality in breast cancer [15]. Indeed, it is unknown if subclinical changes in cardiac function lead to adverse cardiac events or the development of cardiovascular diseases other than heart failure, therefore the incidence of cardiac toxicity may be underestimated in the literature. Finally, it is evident that echocardiography is successful at identifying subclinical changes in cardiac function in breast cancer, although large clinical trials are needed to determine the importance of changes and whether these changes translate into the development of cardiac related morbidity and mortality.

Chapter 4: Thesis Investigation: Cardiovascular Function in HER2-positive Breast Cancer Survivors

The purpose of this chapter is to provide a rationale for the thesis investigation, a detailed description of the methods and results of our findings. This chapter also contains discussions of our findings. A version of this chapter will be submitted for peer review. This research was conducted under Human Ethics approval certificate number: H13-02916.

4.1: INTRODUCTION

Cardiac injury as a result of treatment for breast cancer (chemotherapy, radiation, ligand targeted therapies) is shown to lead to alterations in subclinical measures of cardiac function and overt heart failure [176]. However, alterations in subclinical measures of cardiac function in early-stage breast cancer survivors and the prognosis associated with these changes are not well characterized in the literature. In a recent investigation from Riihimäki et al (2012) [143], they recognized outcomes of mortality in breast cancer compared to age matched apparently healthy women, and identified that breast cancer survivors carry a 29% increased risk of dying of heart failure. Moreover, they also identified diseases due to coronary artery disease to carry a 9% increased risk in the breast cancer population. As such, it is pertinent to understand changes in cardiovascular function that occur in breast cancer survivors in order to effectively manage breast cancer survivors while reducing the risk of cardiac related mortality. Moreover, reliable and valid measures that provide prognostic information regarding long-term risk of adverse cardiac events is also warrant.

Trastuzumab (Herceptin™), a new recombinant DNA-derived humanized monoclonal antibody against the proto-oncogene, HER2/neu gene product is an important addition for the treatment of breast cancer. Trastuzumab has lead to improved rates of disease

free and overall survival in the breast cancer population [176]. However, trastuzumab treatment is limited by the development of cardiac injury leading to a reduction in LVEF during and following treatment. In fact, in breast cancer survivors the incidence of heart failure was 12.1% for trastuzumab, 4.3% for anthracyclines, and 20.1% for anthracyclines and trastuzumab treated patients at 5 yr follow up [3]. As such, women who are treated with trastuzumab may be at increased risk of dying due to cardiac toxicity associated with treatment, due to an increase in survival years and treatment related cardiac damage. With this information, it seems apparent then women treated with trastuzumab (especially in combination with anthracyclines) may have a greater risk of developing cardiotoxicity as a result of treatment. However, reports from Ho and colleagues (2010) [163] demonstrated that trastuzumab and anthracycline treated breast cancer survivors differed from those treated with anthracyclines alone, and unexpectedly did not demonstrate a reduction in global longitudinal strain rate within 6 yr follow up. Indeed, the extent and magnitude of treatment related cardiac toxicity associated with trastuzumab treatment required further investigation. With an increasing number of women surviving breast cancer, it is imperative to understand the long-term cardiac consequences of treatment in order to alleviate co-morbidities and improve survival among the breast cancer population.

In order to determine if subclinical cardiovascular dysfunction is evident in asymptomatic breast cancer survivors treated with trastuzumab, we aimed to use an integrated approach of measuring resting and exercise (“stress”) echocardiography and vascular function. In order to accomplish our goals we conducted resting measures of vascular function including PWV, arterial compliance and BRS. Further, we assessed echocardiography parameters (LV volumes) to calculate ventricular-vascular coupling at rest

and during exercise to assess the reserve capacity of the heart. We also included measures of cardiac haemodynamics (SV and Q) and heart rate response to exercise. Resting echocardiography measures were also collected to determine if stress echocardiography was able to uncover cardiac impairment in breast cancer survivors, which was not evident using conventional resting 2D-echocardiography. We hypothesized that resting measures of cardiac function would be similar between groups; however exercise would unveil a change in cardiac function in breast cancer survivors compared to age-matched apparently healthy controls. Moreover, differences in vascular function at rest would be apparent between breast cancer survivors and control subjects.

4.2: PROCEDURES AND METHODS

4.2.1: Study Design

A cross-sectional study design was used to assess asymptomatic breast cancer survivors and age-matched apparently healthy controls. All women with breast cancer had histologically confirmed HER2-positive breast cancer, who completed primary therapy (radiation, surgery and/or chemotherapy) and adjuvant therapy with trastuzumab. The participants underwent two days of testing to evaluate resting and exercise measure of cardiac and vascular function in the Cardiovascular Physiology and Rehabilitation Laboratory at the University of British Columbia. This study was approved through University of British Columbia human ethics board H13-02916.

4.2.2: Recruitment and Participants

Recruitment: Women were recruited between February 2014 and July 2014 from local community centers and support groups for breast cancer survivors. Posters were placed in community buildings or electronically sent by support group leaders and all women

interested were asked to contact the investigators. All women were approached by the co-investigator (Alis Bonsignore) and provided with a letter of initial contact (Appendix A). All participants were asked to commit to two days of testing with 7-14 days between each day of testing. All participants were required to be free of symptoms of disease and contraindications to exercise testing. This was confirmed through pre-screening with administration of the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) by a qualified exercise professional [177] (Appendix A). An additional health-history questionnaire was also provided (Appendix A). All participants were asked to provide informed written consent (Appendix A) prior to the initiation of testing. Participants also completed the Healthy Physical Activity Questionnaire and the Godin-Shephard Leisure-Time Physical Activity Questionnaire by Shephard and colleagues [178], [179](Appendix A).

Participants: Inclusion criteria included women diagnosed with HER2-positive breast cancer 1 yr prior to testing. Women treated with or without radiation, surgery and hormone therapies if appropriate were also included. Additionally, age-matched apparently healthy women were also included as a control group. Individuals who were not cleared for unrestricted physical activity, those with chronic conditions including diabetes, cardiovascular disease, or metastatic breast cancers, received prior treatment for anthracyclines, or were not treated with trastuzumab therapy were not included. Individuals taking medication for cardiovascular disease risk factors or diabetes (for example, lipid-lowering, antihypertensive or hypoglycemic medications) were also not included.

4.2.3: Procedures

Assessment Day 1: Questionnaires and Familiarization Phase

Participants were asked to volunteer for two days of testing with a total time

commitment of 3 hr (60 min day one; 120 min day two) in the Cardiovascular Physiology and Rehabilitation Laboratory. On each testing day participants were asked to refrain from exhaustive exercise for 24 hr, caffeine/alcohol for 12 hr, smoking and food for 2 hr prior to testing. Each participant completed questionnaires to determine clearance for physical activity, informed consent, individual characteristics, individual health history, and physical activity behavior. All participants were then evaluated for standing height and weight. Resting blood pressure and heart rate was taken in duality and averaged together following 5 min of rest using an automated blood pressure cuff. The participants underwent an incremental exercise test on an electronically based cycle ergometer (Ergometrics er800s; Ergoline Blitz, Germany) in order to assess VO_2peak . Breath-by-breath gas samples and ventilatory parameters were collected and averaged over a 15-second period using a calibrated metabolic cart (Medisoft Ergocard, Belgium). Heart rate (Polar, Lachine, Canada), rating of perceived exertion (Borg scale 6-20) and blood pressure was taken at the end of each stage.

Assessment Day Two: Resting and Exercise Measures of Cardiovascular Function

Upon arrival on the second testing day, the participants standing height and weight was recorded. Each participant then rested for 5 min and an automated blood pressure cuff was used to record blood pressure and heart rate in duality and averaged over the two readings. The participants then underwent a series of resting vascular measures following 5 min of supine rest while they were asked to lie on their backs on the treatment table. Each of the participants underwent assessment of resting vascular measures including baroreceptor sensitivity, PWV and arterial compliance. Following this, a certified sonographer collected all resting 2D echocardiography measures. The participant then performed a standard

incremental exercise test on a cycle ergometer following the same protocol as day one.

Throughout the test, breath-by-breath gas samples and ventilatory parameters were collected and averaged over a 15-second period using a calibrated metabolic cart (Medisoft Ergocard, Belgium). Heart rate (Polar, Lachine, Canada), rating of perceived exertion (Borg scale 6-20), and blood pressure was recorded at the end of each stage. Each participant also underwent a cardiac ultrasound assessment during the last minute of each exercise stage.

4.2.4: Methods

Echocardiography: A highly trained board certified (American Registry of Diagnostic Medical Sonography) clinical sonographer performed all echocardiographic assessments with a portable ultrasound unit (Vivid i, GE Healthcare), a 2.0-MHz transducer (GE Medical Systems Wauwatosa, WI USA) and simultaneous ECG recordings. 2D transthoracic images were acquired from the apical two- and four-chamber views for the assessment LV function and EDV and ESV using Simpson's bi-plane method for which three consecutive beats were measured and analyzed. Primary measurements of mitral inflow including the peak early filling (E-wave) and late diastolic filling (A-wave) velocities, the E/A ratio, and deceleration time of early filling velocity, were also obtained by placing the cursor in the LV outflow tract to simultaneously displaying the end of aortic ejection and the onset of mitral inflow. Measures of LV cavity size and wall thickness were acquired in the long axis parasternal view and recorded at the end of systole (maximal opening of the aortic valve) and diastole (complete closing of the aortic and mitral valve). The opening and closing of each valve was confirmed with the ECG tracings to determine the beginning and end of each cardiac cycle. All measures were computed in accordance with the recommendations of the American Society of Echocardiography[180]. All resting echocardiographic images were

collected with participants positioned in the left lateral decubitus position and all exercise images were collected during the last minute of each exercise stage on an upright cycle ergometer. All cardiac images were stored with patient identification numbers for later offline analysis.

Arterial Compliance: Arterial compliance was measured noninvasively using Applanation tonometry with the HDI CR-2000 (Hypertension Diagnostics, Eagan, MN) for diastolic pulse contour analysis following 5 min of supine rest. This method is considered optimal for measuring systemic compliance estimation of large (capacitive) artery and small (oscillatory) artery compliance using waveform analysis. To acquire these measures, the subject's wrist was stabilized in order to obtain the maximizing signal strength, and an automated sphygmomanometer acquired the blood pressure reading on the opposite arm.

Baroreceptor Sensitivity: Each participant was secured with a three-lead ECG and finger plethysmography worn on the middle finger, while measures of a beat-by-beat blood (Finapres; Ohmeda Inc, Englewood, CO) were obtained following 5 min of supine rest. Automated blood pressure measurements were also obtained in duplicate every 3 min for later calibration. All readings were continuously recorded and sent to a data acquisition system for later analysis (PowerLab/16SP ML 795, ADInstruments, Colorado Springs, CO), while beat-by-beat blood pressure and ECG recordings were displayed during collection (version 7.0, ADInstruments, Colorado Springs, CO).

Pulse Wave Velocity: PWV was represented mathematically by determination of pulse transit time and the distance traveled between two recording sites (common carotid artery and femoral artery). Pressure waveforms were collected using two infrared photoelectric sensors (MLT1020PPG Plethysmograph, ADInstruments), one placed on the

base of the neck for the common carotid artery and over the right femoral artery where simultaneous waveforms were recorded for calculation of PWV. A three lead ECG was also secured to each participant and all recordings were sent to a data acquisition system for later analysis (PowerLab/16SP ML 795, ADInstruments, Colorado Springs, CO). Each waveform and ECG reading was visually available and inspected during data collection (version 7.0, ADInstruments, Colorado Springs, CO). The distance between the sites of the pressure waveforms were measured to the nearest 0.5 cm using a standard measuring tape.

VO₂peak Assessment: The participant underwent an incremental exercise test on an electronically based cycle ergometer (Ergometrics er800s; Ergoline Blitz, Germany) in order to assess VO₂peak. Prior to each test, the metabolic cart was calibrated with ten samples from a 3 L calibration syringe. The gas analyzers were also calibrated before each test to room air and medically certified calibration gases (12.09% O₂ and 4.94% CO₂, respectively). The patient was instructed to maintain a minimum rate of 60 revolutions per minute on the cycle ergometer at a pace that was comfortable to the individual. The test began at workload of 25W and increased by 25W every 3 min thereafter. The workload increased until volitional fatigue (participants could not maintain 60 rpm). Breath-by-breath gas samples and ventilatory parameters were collected and averaged over a 15-second period using a calibrated metabolic cart (Medisoft Ergocard, Belgium). Heart rate (Polar, Lachine, Canada), and rating of perceived exertion (Borg scale 6-20) was recorded at the end of each stage. The same experienced technician also recorded brachial blood pressure at the end of each stage via a manual sphygmomanometer. The same experience exercise physiologist completed all VO₂peak assessments.

4.2.5: Data Analysis

Vascular assessments and cardiac parameters were analyzed by the same investigator (A.B). Data that was acquired through echocardiography including LV volumes, diameters and function were analyzed offline (EchoPAC, GE Healthcare, v. 110.1.1) by the same qualified sonographer who was blinded to the participant groups. EDV, ESV and LVEF were computed using Simpson's bi-plane method for which three consecutive beats were measured and analyzed. SV was calculated as $EDV - ESV$ and averaged between the two and four chamber views. Q was calculated as $SV \times HR$. LVEF was calculated as $EF = (EDV - ESV) / EDV \times 100$ [181]. E_a was calculated as $E_a = ESP / SV$ where $ESP = 0.9 \times$ systolic blood pressure. E_{LV} was calculated via the formula $E_{LV} = ESP / ESV$. Ventricular-vascular-coupling was then computed as the ratio of E_a / E_{LV} [127]. Mean arterial pressure (MAP) was calculated at rest and during exercise as $[(2 \times DBP) + SBP] / 3$ and $\frac{1}{2} (SBP - DBP) + DBP$ respectfully where DBP= diastolic blood pressure and SBP= systolic blood pressure [182]. All baseline echocardiography measures were indexed to body surface area (BSA), which was calculated as $BSA (m^2) = [Height (cm) \times Weight (kg) / 3600]^{1/2}$ [183].

PWV was collected over a minimum of 30 consecutive cardiac cycles, which were chosen based on visual interpretation of the clearest waveform signals. The cardiac cycles obtained from the ECG readings were averaged to calculate the foot-to-foot pulse transit time between the carotid artery and femoral artery (two-sites). The segmental distances were divided by the corresponding pulse transit time in order to calculate PWV between the carotid artery and femoral artery. The shortest distances between the sites of the carotid and femoral artery were measured to the nearest 0.5 cm using a standard measuring tape. An adjustment factor of multiplying PWV measures by 0.8 was applied. While analyzing PWV

files, the investigator was blinded to the subject ID and study group.

Quantification of BRS was assessed using power spectral analysis and sequencing methods. Files were analyzed by selecting a 5 min segments of the chart file with a minimum of 180 s of continuous cardiac cycles evaluated in the determination of heart rate variability (HRV), blood pressure variability (BPV) and BRS (average beats used 295.6 ± 90.9) which was analyzed using available software (Nevrokard NKFP 8.7.0 and BRS 5.7.0, Nevrokard, Izola, Slovenia). For the sequence method, the sensitivity of the baroreflex was computed as the average of the slope of the regression lines relating changes in systolic pressure to changes in RR interval (RRI) [140]. Criteria for inclusion for the sequence method included a change in the RRI greater than 5 ms with a corresponding change in blood pressure greater than 0.5 mmHg over a minimum sequence duration of three beats with a correlation coefficient greater than 0.85. The power spectral method used the fast Fourier transformation of the BPV and the RRI. The alpha coefficients of the high frequency (HF) and low frequency (LF) bands represented the baroreceptor sensitivity spectral method [184]. The spectral gain of oscillations was set to fixed signal bandwidths of low frequency (0.04 – 0.15 Hz, LF) and high frequency (0.15 – 0.45 Hz, HF) according to current recommendations [140]. Data acquisition of the spectral method included the alpha LF, HF (n.u) and LF (n.u) of blood pressure variability and the RRI in addition to the RRI HF/LF. The sequence method baroreceptor sensitivity measures were obtained as an averaging of the slopes of all sequences evaluated, as well as examining up and down direction slopes individually. In addition, measures of mean normal-to-normal (NN) interval of SBP and DBP and RRI were obtained. The standard deviation of the NN intervals (SDNN), the standard deviations of differences between adjacent NN intervals (SDSD), the root mean square successive

difference of intervals that were extracted from heart rate signals (RMSSD) were also provided.

Measures of SV and Q were collected at the end of the completed stage of the exercise test or at peak exercise in the event that the stage was not completed. The highest VO_2 peak recorded in the final exercise stage was identified and averaged over the last 30 seconds of the stage and normalized for body mass (reported in $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Blood pressure and heart rate were also recorded as the maximal value obtained during the final stage of the exercise test. Maximal power output was defined as the maximal power output obtained in the final stage. In the event that a participant was able to complete 50% (90 s) of a stage, this was determined as the maximal aerobic power achieved on the exercise test. Anaerobic and aerobic thresholds were defined by a combination of changes in ventilatory parameters (VE , FeCO_2 , FeO_2) and the respiratory exchange ratio (RER) obtained from the print outs from the metabolic cart (15 second averages). Detailed ventilator graphs also confirmed the gas analysis results.

4.2.6: Statistical Analysis and Power Calculation

Data were analyzed using SPSS Version 20.0 software (SPSS Inc, Chicago, IL). Descriptive data including means and standard deviations were calculated for all variables of interest. The Leveners Test was used to determine the homogeneity of variance. The Leveners test was used to determine if the assumption of homogeneity of variance was met. Normal distribution of data was explored using descriptive data, plots and the Shapiro Wilk normality test. For non-normally distributed data, log transformations were applied if appropriate. If data violated the homogeneity of variance assumption, the Welsh F statistic was reported. Baseline differences between groups including participant characteristics (height, weight, age, body mass

index), resting haemodynamic profiles, and cardiopulmonary exercise test outcomes were evaluated using a one way Analysis of Variance (ANOVA). Resting vascular function and echocardiography measures were also assessed using one-way ANOVAs. Mixed between-within subjects ANOVA was used to evaluate temporal changes in cardiovascular parameters during exercise conditions and the Wilks Lambda statistic was reported. Equality of covariance and the Lenveres test was also assessed to determine if assumptions were met. The Greenhouse-geissers correction was reported if assumptions were violated. The level of significance was set *a priori* at $p < 0.05$.

Our study was powered based on the primary outcome variable (i.e., the difference in the temporal response of vascular-vascular coupling during exercise between the breast cancer and control groups). Based on the mean difference observed in prior studies of ventricular-vascular coupling in breast cancer at submaximal intensities we estimated the magnitude of this difference would be $3.51 \text{ mmHg}\cdot\text{mL}^{-1}$. Twelve participants were required per group ($p = 0.05$) in order to obtain strong power (0.80), a *priori* sample size calculation from published means of ventricular-ventricular coupling between groups.

4.3: RESULTS

A total of eight women with a history of HER2-positive breast cancer with a mean age of 53.0 ± 8.2 yr (range 32 – 65 yr) were included in this investigation. Baseline participant characteristics are outlined in table 4.1. Briefly, the mean time since completion of trastuzumab therapy was 3.5 ± 2.72 yr (range 1-8 yr). A total of 67.5% ($n = 5$) were treated for right sided and 37.5% ($n = 3$) were treated for left sided breast cancer. All patients were treated in the adjuvant setting and 75% ($n = 6$) received ACT followed by trastuzumab for 1 yr and 25% ($n = 2$) received FEC followed by trastuzumab for 1 yr. Only 67.5% ($n = 5$) received radiation, and

67.5% (n = 5) were treated with tamoxifen. A total of eight age matched apparently healthy control subjects, with a mean age of 54.6 ± 9.6 yr (range 32 – 63 yr) were also included.

Baseline characteristics including height (1.62 ± 0.04 vs. 1.64 ± 0.09 m, $p = 0.58$), weight (64.5 ± 12.0 vs. 65.5 ± 6.3 kg, $p = 0.83$), and body mass index (24.4 ± 4.2 vs. 24.0 ± 2.1 kg·m⁻², $p = 0.81$) were not significantly different between the breast cancer group and control groups respectively.

Table 4.1: Participant Characteristics

Variable	Breast Cancer (n = 8)	Controls (n = 8)	p-value
Age (yr)	53.0 ± 8.2	54.6 ± 9.6	0.72
Height (m)	1.62 ± 0.04	1.64 ± 0.09	0.58
Weight (kg)	64.46 ± 12.01	65.49 ± 6.31	0.83
Body Mass Index (kg·m ⁻²)	24.43 ± 4.23	23.97 ± 2.12	0.81
Treatments for Breast Cancer, n (%)			
ACTT	6 (75)	-	-
FEC	2 (25)	-	-
Tamoxifen	5 (62.5)	-	-
Aromatase Inhibitor	9 (25)	-	-
Radiation	5 (67.5)	-	-
Lumpectomy	2 (25)	-	-
Mastectomy	1 (12.5)	-	-
Partial Mastectomy	2 (25)	-	-
Bilateral Mastectomy	3 (37.5)	-	-
Disease Characteristics, n (%)			
HER2+	8 (100)	-	-
ER+	5 (62.5)	-	-
PR+	2 (25)	-	-
Stage I	1 (12.5)	-	-
Stage II	4 (50)	-	-
Stage III	3 (37.5)	-	-
Right Side	5 (62.5)	-	-
Left Side	3 (37.5)	-	-

Data are presented as the mean \pm standard deviation unless otherwise indicated; FEC- fluorouracil, epirubicin, cyclophosphamide; ACTT-anthracyclines, cyclophosphamide, taxanes, trastuzumab; ER- estrogen receptor; PR- progesterone receptor; $p < 0.05$

4.3.1: Resting Haemodynamic Profiles and VO_{2peak}

Table 4.2 displays the differences in resting haemodynamic profiles and outcomes to the graded cardiopulmonary exercise test. Resting heart rate (78.4 ± 12.9 vs. 66.9 ± 4.2 beats

per min, $p = 0.04$) was significantly different between the breast cancer and control groups respectfully. VO_2peak was not statistically significant between the breast cancer group and healthy controls (27.0 ± 3.18 vs. $31.9 \pm 11.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectfully, $p = 0.28$). There was no significant between-group differences in any of the other resting haemodynamic outcomes or VO_2peak variables obtained from the cardiopulmonary exercise test (all $p > 0.05$).

Table 4.2: Haemodynamic Profiles

Variable	Breast Cancer (n = 8)	Controls (n = 8)	p-value
Resting Haemodynamics			
Heart Rate (beats per min)	78.4 ± 12.9	66.9 ± 4.2	0.04
Systolic Blood Pressure (mmHg)	117.9 ± 15.8	115.3 ± 15.1	0.73
Diastolic Blood Pressure (mmHg)	75.4 ± 10.3	74.6 ± 6.6	0.87
Mean Arterial Pressure (mmHg)	89.5 ± 11.6	88.2 ± 9.2	0.77
Q index ($\text{L}\cdot\text{min}^{-2}$)	2.29 ± 0.47	1.92 ± 0.10	0.47
SV index ($\text{mL}\cdot\text{beat}\cdot\text{m}^{-2}$)	29.33 ± 3.28	28.01 ± 2.49	0.38
Maximal Aerobic Outcomes			
VO_2peak ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	27.0 ± 3.18	31.9 ± 11.3	0.28
Maximal Power Output (Watts)	118.8 ± 29.1	156.3 ± 54.7	0.11
Aerobic Threshold (Watts)	40.6 ± 18.6	65.6 ± 46.2	0.17
Anaerobic Threshold, (Watts)	93.8 ± 22.2	115.6 ± 42.1	0.22
Aerobic Threshold (%)	33.1 ± 8.7	38.6 ± 14.1	0.36
Anaerobic Threshold (%)	79.8 ± 9.8	74.1 ± 7.6	0.21
Mean Arterial Pressure (mmHg)	124.4 ± 14.9	129.8 ± 13.18	0.46
Systolic Blood Pressure (mmHg)	171.8 ± 20.6	182.3 ± 21.26	0.96
Diastolic Blood Pressure (mmHg)	77.0 ± 10.3	77.3 ± 7.6	0.33
AVO_2 Difference ($\text{mL}\cdot 100 \text{ mL}^{-1}$)	14.4 ± 3.5	17.9 ± 7.0	0.22

Data are presented as the mean \pm standard deviation; $p < 0.05$, Q-cardiac output; SV-stroke volume

4.3.2: Resting Vascular Function

Differences in BRS and HRV function were observed between the breast cancer patients and the controls, as outlined in table 4.3. In the breast cancer patients, the slope of the +RR/+SBP sequence (9.2 ± 3 vs. $22.6 \pm 12.6 \text{ mmHg}\cdot\text{ms}^{-1}$, $p = 0.05$) and the +RR/+SBP sequence (6.7 ± 3 vs. $24.1 \pm 12.6 \text{ mmHg}\cdot\text{ms}^{-1}$, $p = 0.02$) were lower compared to controls. The average BRS (8.0 ± 3.5 vs. $23.4 \pm 12.6 \text{ mmHg}\cdot\text{ms}^{-1}$, $p = 0.03$) was also significantly lower in breast cancer group compared to controls. The RRI LF measurement of HRV was significantly

reduced in breast cancer group compared to controls ($p = 0.05$). All other measures of BPV, HRV and BRS were similar between the two groups (all $p > 0.05$). No differences in resting arterial distensibility (cPWV) or arterial compliance were observed (all $p < 0.05$).

Table 4.3: Resting Vascular Measures

Variable	Breast Cancer (n = 8)	Controls (n = 8)	p-value
Arterial Stiffness			
Large Artery Compliance (mmHg x 10)	12.3 ± 4.3	13.3 ± 7.6	0.77
Small Artery Compliance (mmHg x 100)	5.6 ± 3.3	6.3 ± 4.6	0.71
cPWV	6.7 ± 2.6	5.1 ± 2.8	0.28
Baroreceptor Sensitivity			
BRS+/- (mmHg·ms ⁻¹)	9.2 ± 3.3	22.6 ± 12.6	0.05
BRS -/- (mmHg·ms ⁻¹)	6.7 ± 3.5	24.1 ± 12.6	0.02
BRS _{average} (mmHg·ms ⁻¹)	8.0 ± 3.5	23.4 ± 12.6	0.03
BRS-αLF (Hz)	4.6 ± 4.9	19.3 ± 14.6	0.07
BPV Variability			
Systolic Mean NN (mmHg)	108.1 ± 9.8	107.3 ± 7.5	0.12
Diastolic Mean NN (mmHg)	60.5 ± 3.6	68.7 ± 9.1	0.50
SDNN (mmHg)	6.1 ± 3.2	2.9 ± 2.5	0.18
RMSD (mmHg)	5.6 ± 2.9	2.9 ± 2.6	0.16
SBP LF n.u	102.7 ± 30.2	66.4 ± 26.2	0.32
SBP HF n.u	18.8 ± 5.4	30.1 ± 23.4	0.08
HR Variability			
Mean RRI	1058.5 ± 375.9	1031.1 ± 77.4	0.88
RMSD	44.3 ± 22.6	35.1 ± 13.5	0.46
SDNN	44.2 ± 22.8	35.2 ± 13.5	0.47
RRI HF n.u	51.9 ± 16.3	35.0 ± 19.8	0.18
RRI LF n.u	38.2 ± 13.2	59.8 ± 16.5	0.05
HF/LF	0.8 ± 0.4	2.4 ± 1.6	0.07

Data are presented as the mean ± standard deviation; cPWV-central pulse wave velocity; BRS-baroreceptor sensitivity; RRI- RR interval; HF-high frequency, LF-low frequency; NN- mean normal-to-normal; SDNN-standard deviation of the NN intervals; SDNN- standard deviations of differences between adjacent NN intervals RMSSD- the root mean square successive difference of intervals that were extracted from heart rate signals; $p < 0.05$

4.3.3: Resting Echocardiography

There were no between group differences in LVEF in the breast cancer group (61.7 ± 2.25 vs. 59.8 ± 4.53 , $p = 0.30$) compared to the control group with no participants presenting with a LVEF < 55%. Differences in IVSd were observed in the breast cancer group when compared to control subjects (0.95 ± 0.11 vs. 1.07 ± 0.09 cm respectfully, $p = 0.03$). PWTd was

also significantly lower in the breast cancer group when matched to the control group (0.9 ± 0.12 vs. 1.0 ± 0.09 cm respectfully, $p = 0.30$). However, when adjusting for BSA, there were no differences between groups (all $p > 0.05$). No other resting echocardiography measures were different in breast cancer compared to the control group (all $p > 0.05$) (see Table 4.4).

Table 4.4: Resting Echocardiographic Measures

Variable	Breast Cancer (n = 8)	Controls (n = 8)	p-value
LVEF	61.7 ± 2.25	59.8 ± 4.53	0.30
IVSd (cm)	0.95 ± 0.11	1.07 ± 0.09	0.03
IVSd BSA ($\text{cm} \cdot \text{m}^{-2}$)	0.57 ± 0.11	0.62 ± 0.07	0.24
LVd diameter (cm)	3.8 ± 0.41	3.6 ± 0.40	0.38
LVd BSA ($\text{cm} \cdot \text{m}^{-2}$)	1.53 ± 0.27	1.45 ± 0.24	0.52
LVs diameter (cm)	2.6 ± 0.43	2.5 ± 0.52	0.73
LVs BSA ($\text{cm} \cdot \text{m}^{-2}$)	2.2 ± 0.27	2.1 ± 0.24	0.25
PwTd (cm)	0.9 ± 0.12	1.0 ± 0.09	0.04
Pwd BSA ($\text{cm} \cdot \text{m}^{-2}$)	0.53 ± 0.11	0.59 ± 0.06	0.24
E	0.77 ± 0.12	0.81 ± 0.22	0.58
A	0.59 ± 0.30	0.71 ± 0.30	0.44
E/A	1.03 ± 0.52	1.33 ± 0.61	0.32
MV Deceleration Time (s)	197 ± 29.22	212 ± 33.27	0.32
ESV (mL)	30.62 ± 2.47	32.31 ± 2.61	0.21
ESV BSA ($\text{mL} \cdot \text{m}^{-2}$)	11.62 ± 0.91	12.1 ± 2.11	0.60
EDV (mL)	80.0 ± 2.90	80.75 ± 4.04	0.68
EDV BSA ($\text{mL} \cdot \text{m}^{-2}$)	47.5 ± 5.1	46.8 ± 2.72	0.74

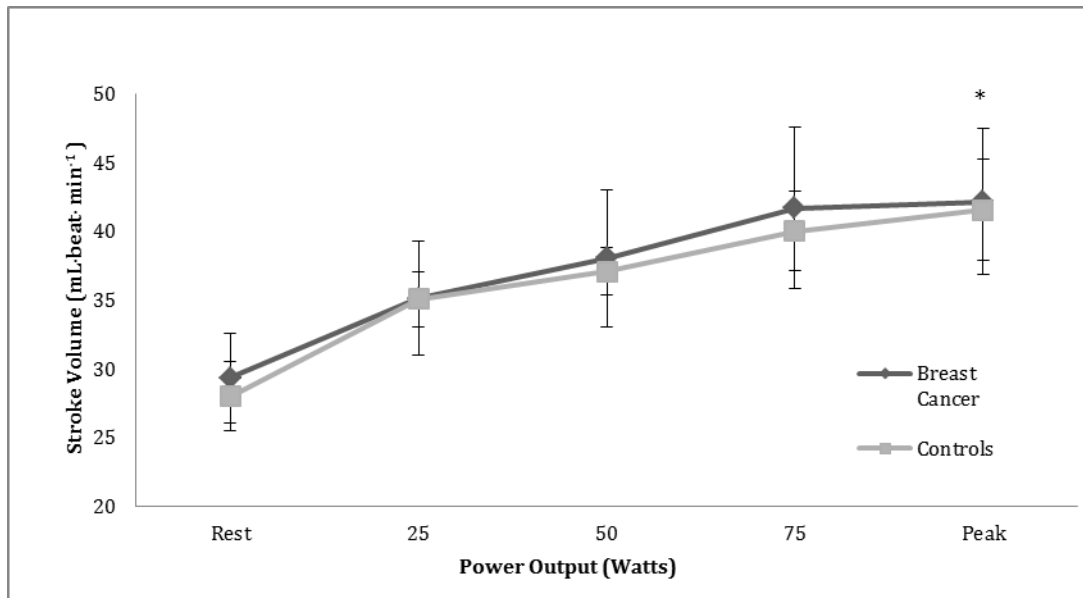
Data are presented as the mean \pm standard deviation; IVSd- interventricular septum in diastole; IVSs- interventricular septum in systole; LVd-left ventricular diameter in diastole; LVs-left ventricular diameter in systole; PwTd-posterior wall thickness in diastole; MV-mitral valve; ESV- end systolic volume; EDV-end-diastolic volume; LVEF- left ventricular ejection fraction BSA- body surface area $p < 0.05$

4.3.4: Exercise Haemodynamics

A mixed between-within subjects ANOVA was applied to assess changes in SV index in response to exercise and to determine potential differences between groups. There was no significant interaction effect $F(4, 11) = 1.265$, $p = 0.34$, partial eta squared = 0.315 for SV index and time between the breast cancer and control groups from baseline to peak exercise. There was a substantial main effect for time, $F(1, 14) = 60.034$, $p < 0.001$, partial eta

squared = 0.956, with both groups showing an increase in SV index in response to exercise (see figure 4.1). Importantly, three of the patients and one of the controls did not reach their maximal SV during maximal exercise (reflecting a continual increase in SV with increasing work intensity) (see Figure 4.2 and 4.3). There was also no significant interaction effect $F(4, 11) = 1.023, p = 0.437$, partial eta squared = 0.271 between the breast cancer and control groups for changes in Q index with an increase in exercise intensity. As expected, Q index did increase during exercise $F(4, 11) = 97.528, p < 0.001$, partial eta squared = 0.973 in both groups (see figure 4.4). There was also no significant interaction effect $F(1, 14) = 2.081, p = 0.171$, partial eta squared = 0.129 for the heart rate response to exercise between each group. Also, there was a main effect for time and heart rate, $F(1, 14) = 500.503, p < 0.001$, partial eta squared = 0.973, from rest to peak exercise (see figure 4.7).

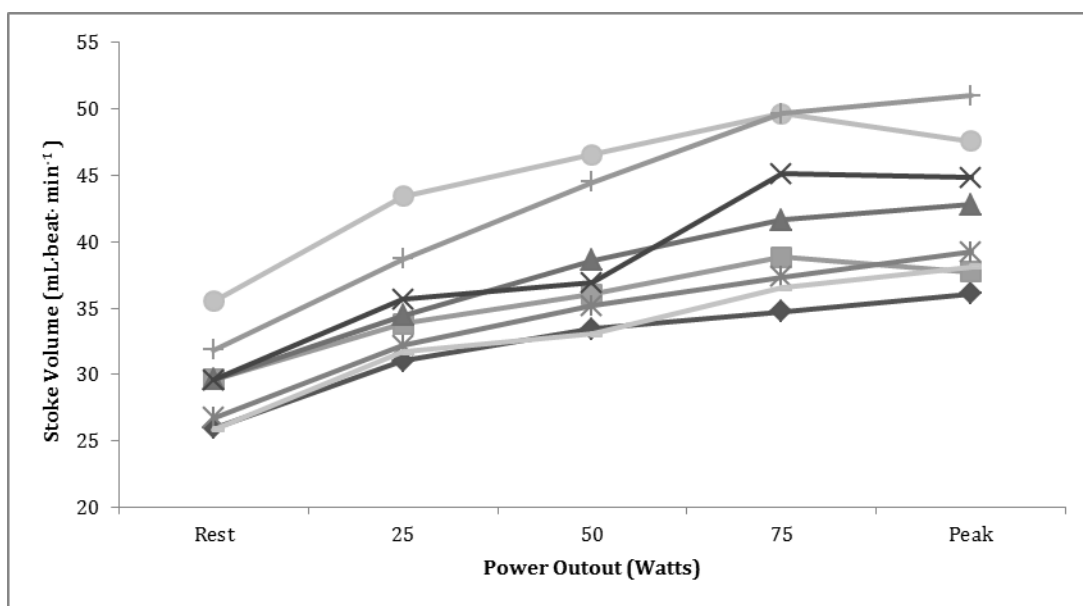
Figure 4.1: Stroke Volume Response During Incremental Exercise Test



Data represents mean values for the repeated measures ANOVA for stroke volume response indexed to body surface area during each stage of the incremental exercise test for the breast cancer and control group.

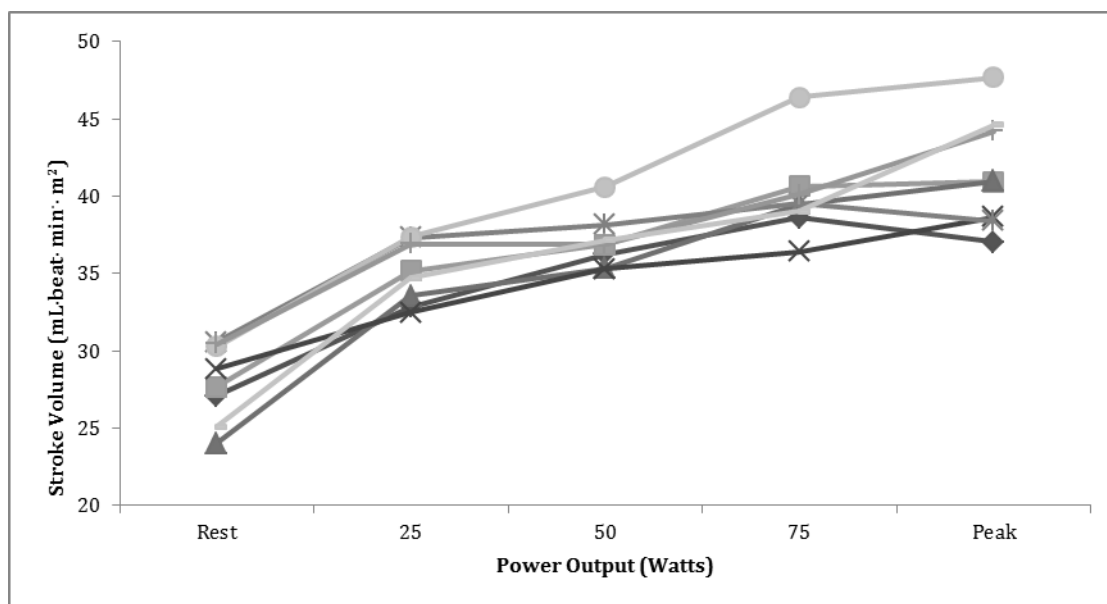
* Indicates a main effect for time for each group

Figure 4.2: Stroke Volume Response During Incremental Exercise Test (Breast Cancer)



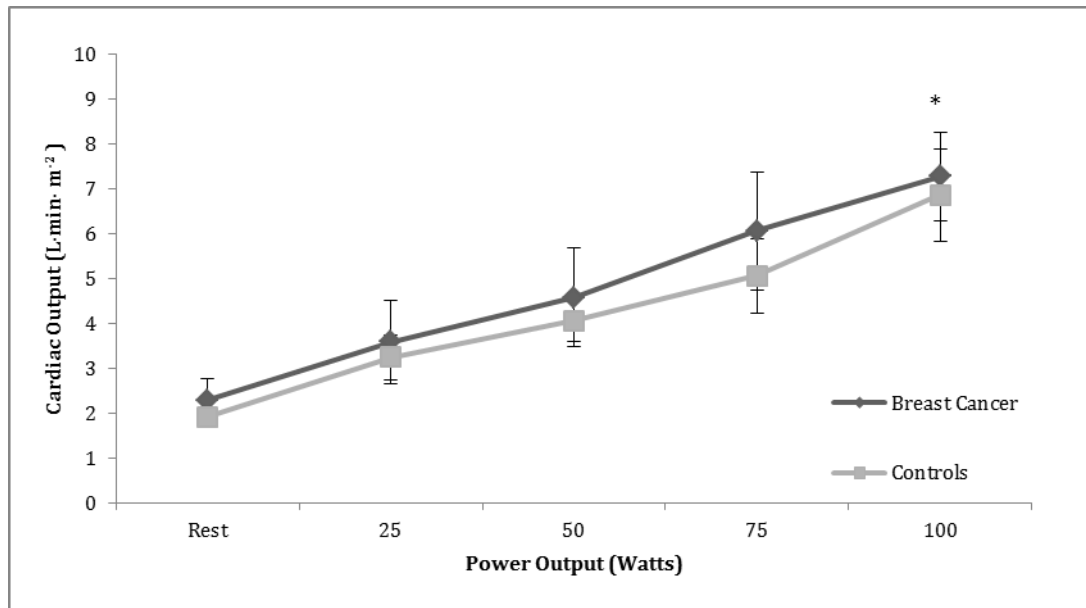
Represents individual data for the stroke volume response indexed to body surface area during each stage of the incremental exercise test for the breast cancer group.

Figure 4.3: Stroke Volume Response During Incremental Exercise Test (Control Group)



Represents individual data for the stroke volume response indexed to body surface area during each stage of the incremental exercise test for the control group.

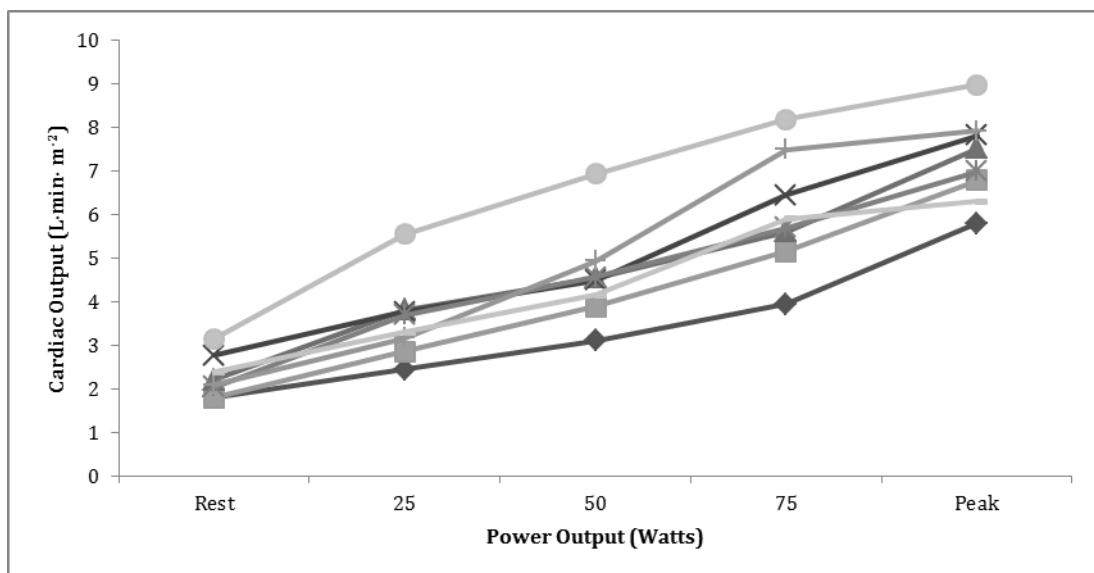
Figure 4.4: Cardiac Output Response During Incremental Exercise Test



Data represents mean values for the repeated measures ANOVA for stroke volume response indexed to body surface area during the incremental exercise test for the breast cancer and control group.

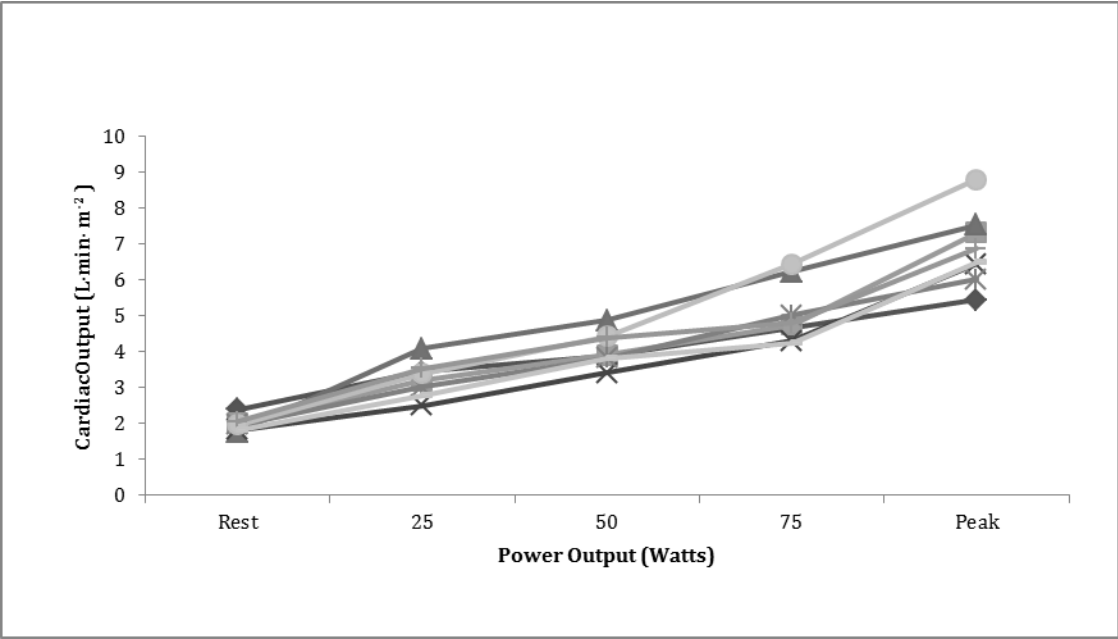
* Indicates a main effect for time for each group

Figure 4.5: Cardiac Output Response During Incremental Exercise Test (Breast Cancer)



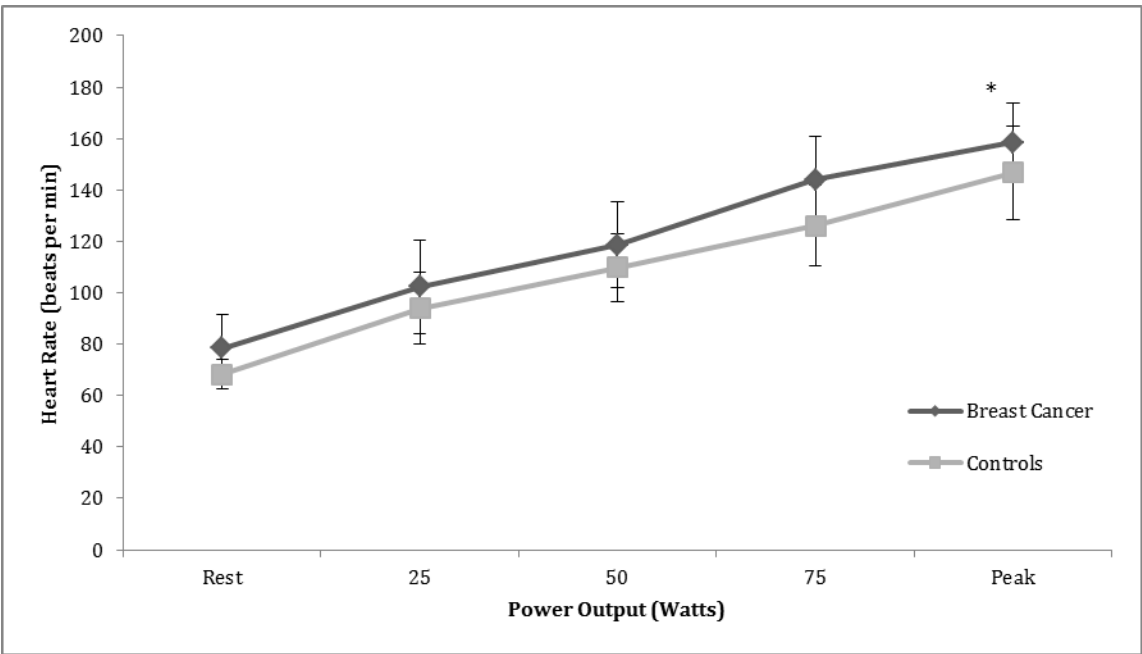
Represents individual data for the cardiac output response indexed to body surface area during each stage of the incremental exercise test for the breast cancer group.

Figure 4.6: Cardiac Output Response During Incremental Exercise Test (Control Group)



Represents individual data for the cardiac output response indexed to body surface area during each stage of the incremental exercise test for the control group.

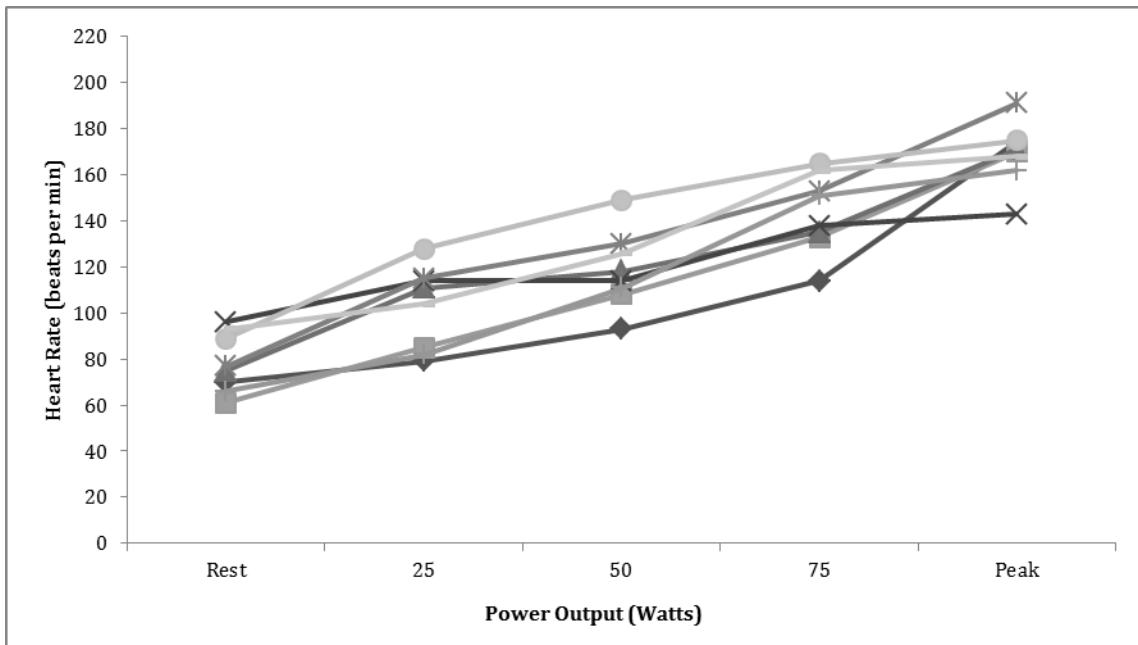
Figure 4.7: Heart Rate Response During Incremental Exercise Test



Represents mean data for the average heart rate during each stage of the incremental exercise test for the breast cancer and control group.

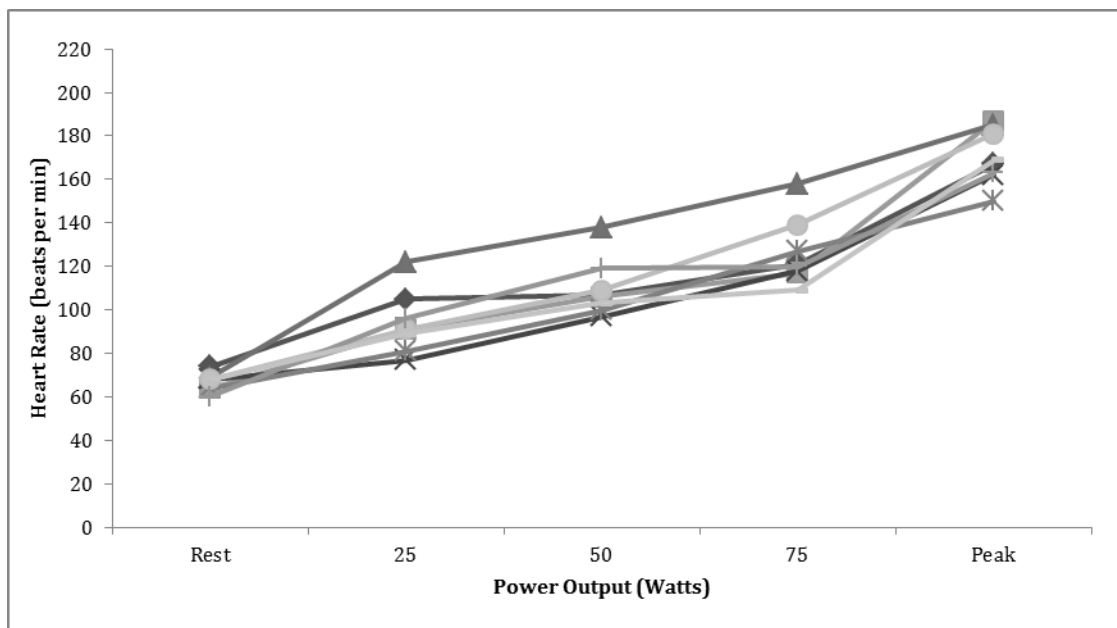
* Indicates a main effect for time for each group

Figure 4.8: Heart Rate Response During Incremental Exercise Test (Breast Cancer)



Represents individual data for the average heart rate during each stage of the incremental exercise test for the breast cancer group.

Figure 4.9: Heart Rate Response During Incremental Exercise Test (Controls)

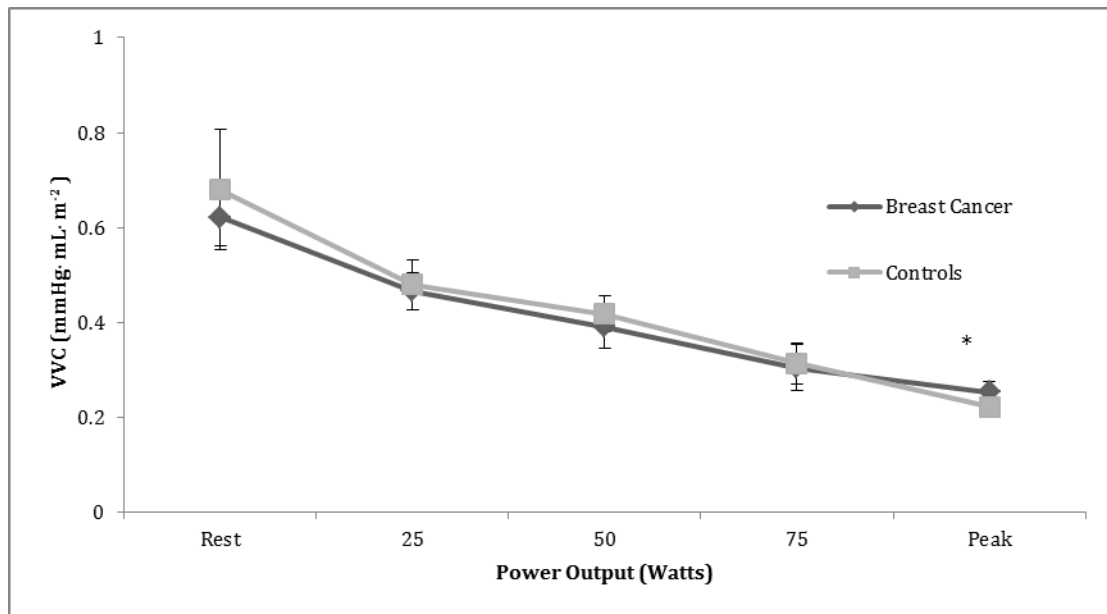


Represents individual data for the average heart rate during each stage of the incremental exercise test for the control group.

4.3.5: Ventricular-Vascular- Coupling

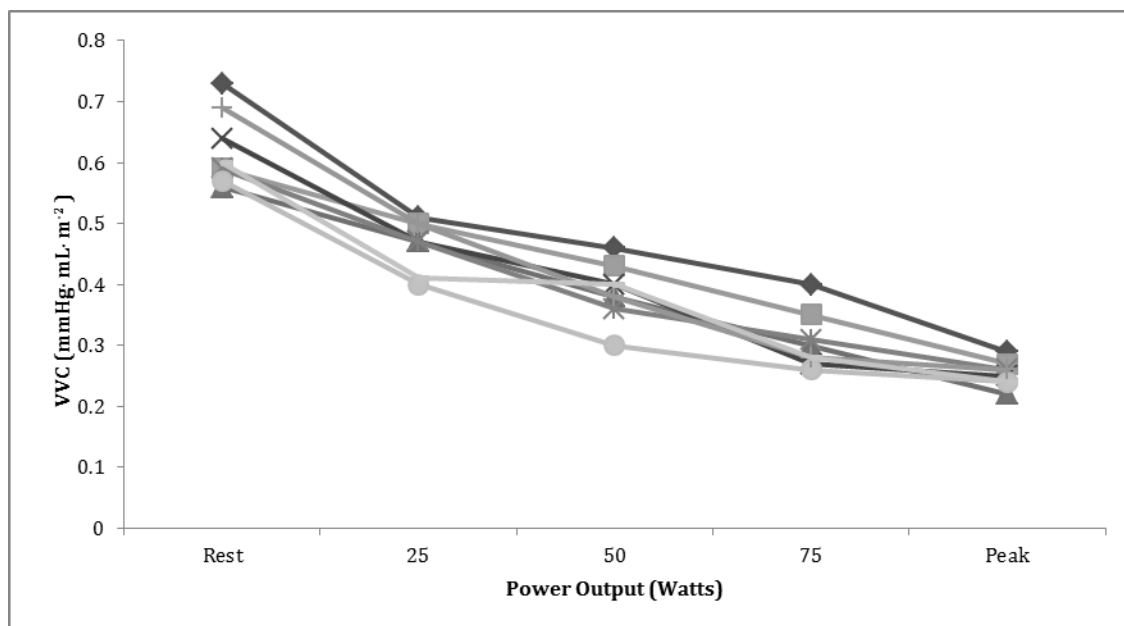
A mixed between-within subjects ANOVA was conducted to assess the change in ventricular-vascular coupling index from resting to peak exercise. There was no significant interaction effect $F(1, 14) = 2.264, p = 0.135$, partial eta squared = 0.139 between the breast cancer and control groups from baseline to peak exercise. There was a substantial main effect for time, $F(1, 14) = 223.107, p < 0.001$, partial eta squared = 0.94, with both groups showing a decrease in ventricular-vascular coupling index with an increase in exercise intensity (see figure 4.10). The effect of an increase in exercise intensity on Ea index was also assessed. There was no significant interaction effect $F(3, 12) = 0.534, p = 0.64$, partial eta squared = 0.04 between the breast cancer and control groups from baseline to peak exercise suggesting that both groups had similar Ea index responses to an increase in exercise intensity (see figure 4.13). There was a main effect for time, $F(1, 14) = 7.633, p < 0.001$, partial eta squared = 0.353 suggesting a change in Ea index with an increase in exercise intensity in each group. The breast cancer group demonstrated a blunted response to increase E_{LV} index compared to controls with a significant interaction effect $F(1, 14) = 7.427, p = 0.005$, partial eta squared = 0.347 between groups from baseline to peak exercise. There was also a substantial main effect for time, $F(4, 11) = 199.952, p < 0.001$, partial eta squared = 0.935, from baseline to peak exercise (see figure 4.16). The interaction effect occurred above 75 watts.

Figure 4.10: Ventricular-Vascular Coupling Response During Incremental Exercise Test



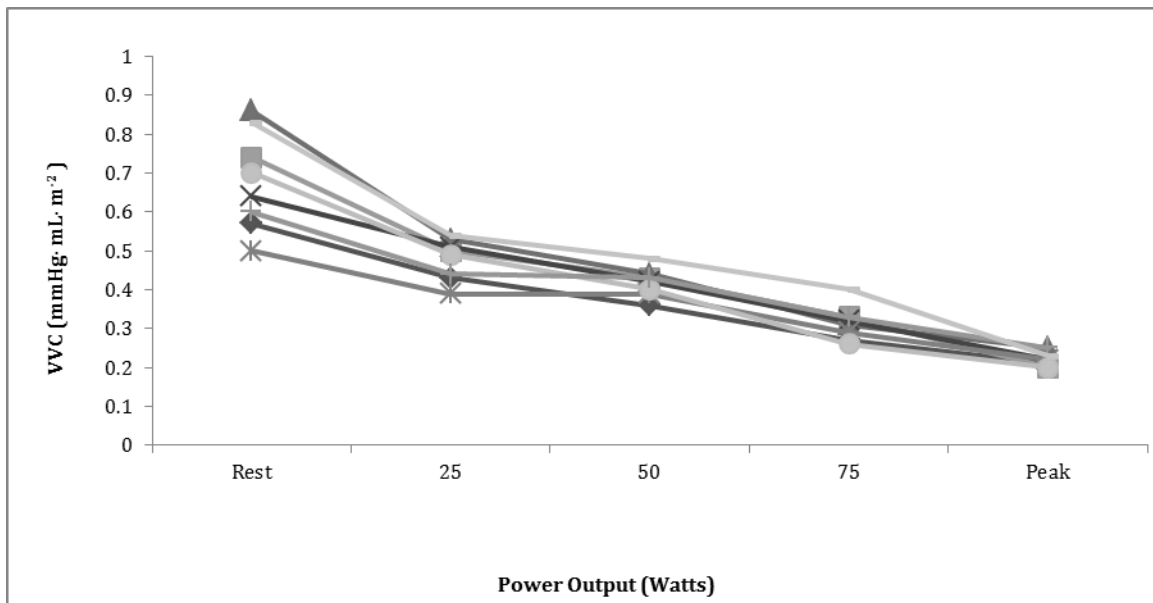
Data represents mean values for the repeated measures ANOVA for ventricular-vascular coupling indexed to body surface area during each stage during the incremental exercise test for the breast cancer and control group.

Figure 4.11: Ventricular-Vascular Coupling Response During Incremental Exercise Test (Breast Cancer)



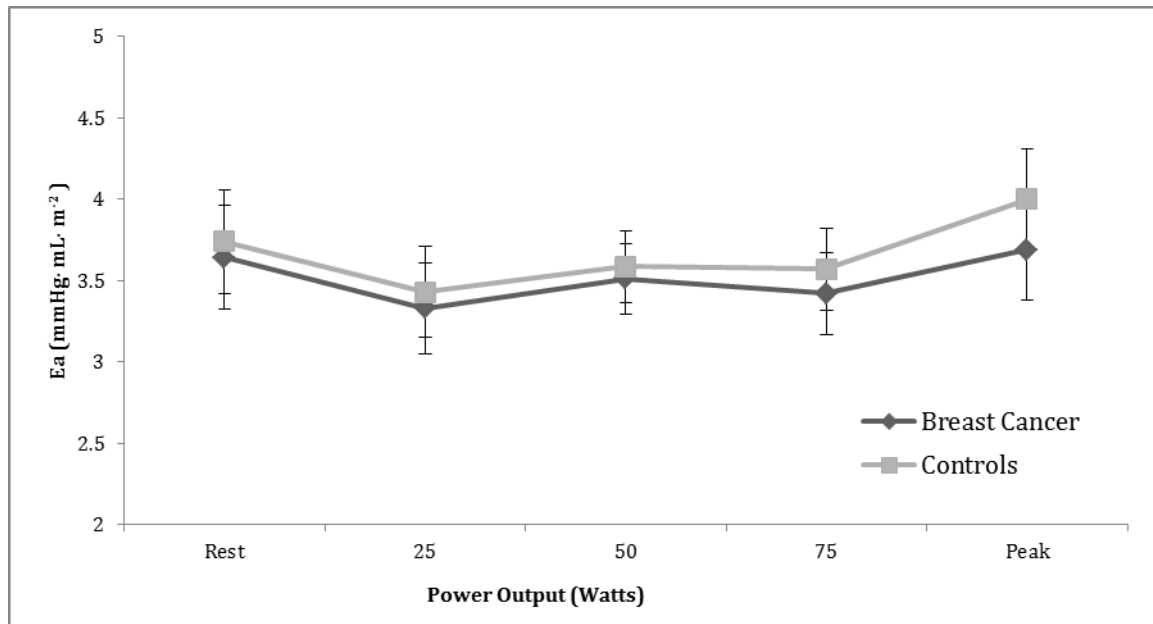
Represents individual data for ventricular- vascular coupling indexed to body surface area during each stage of the incremental exercise test for the breast cancer group.
VVC- ventricular-vascular coupling

Figure 4.12: Ventricular-Vascular Coupling Response During Incremental Exercise Test (Controls)



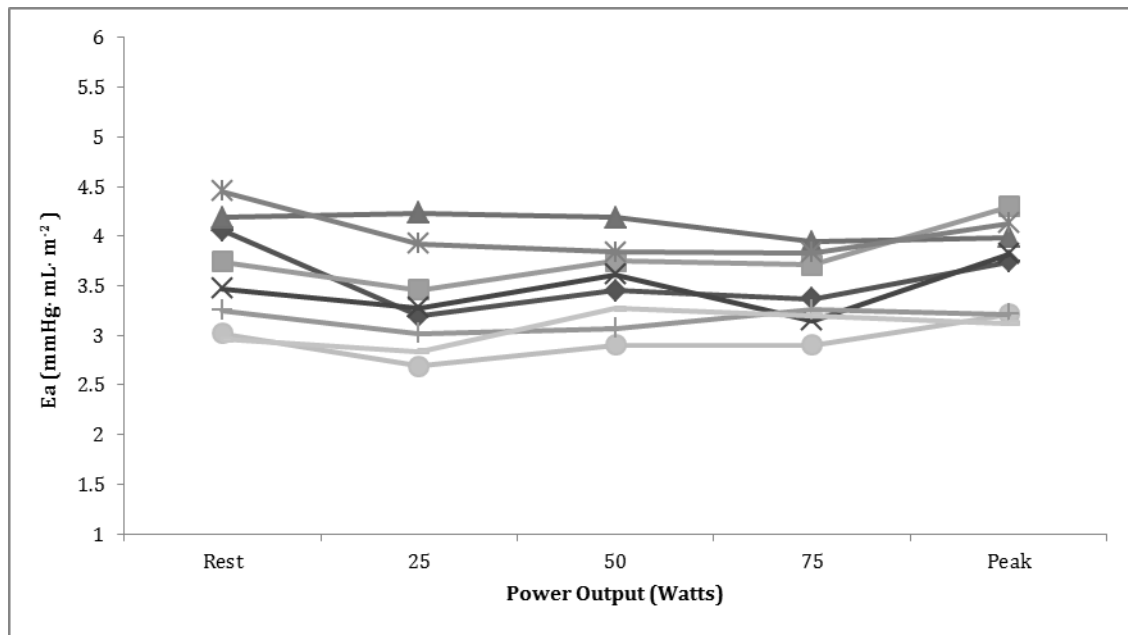
Represents individual data for ventricular-vascular coupling indexed to body surface area during each stage of the incremental exercise test for the control group.
VVC- ventricular-vascular coupling

Figure 4.13: Arterial Elastance Response During Incremental Exercise Test



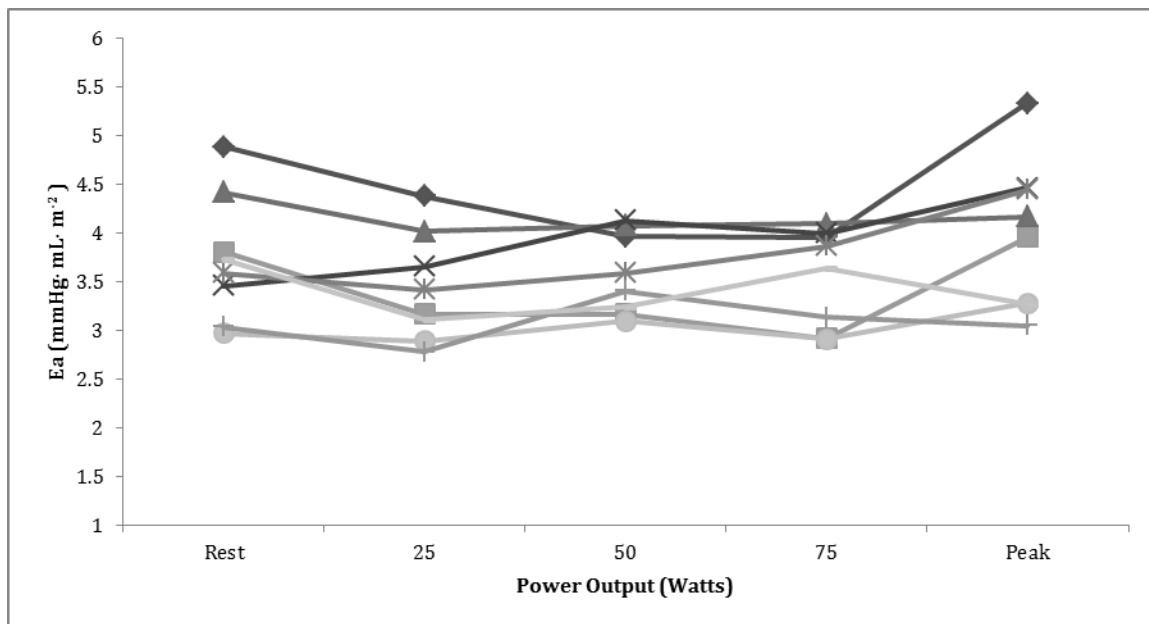
Data represents mean values for the repeated measures ANOVA for arterial elastance indexed to body surface area at each stage during the incremental exercise test for the breast cancer and control group
* Indicates a main effect for time for each group.
Ea- arterial elastance

Figure 4.14: Arterial Elastance Response During Incremental Exercise Test (Breast Cancer)



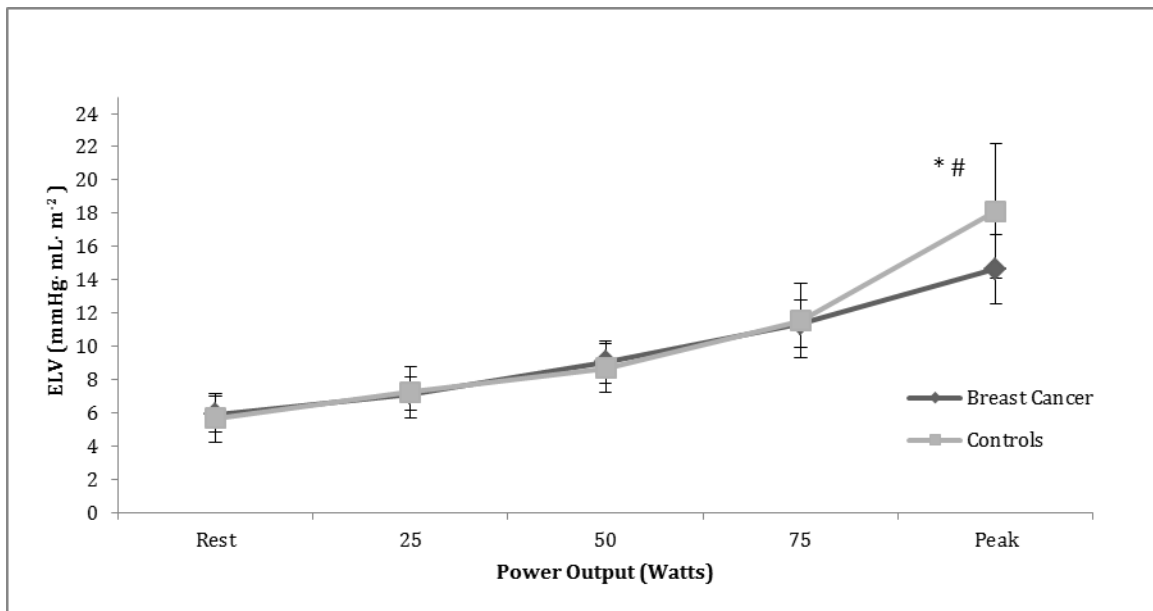
Represents individual data for arterial elastance indexed to body surface area during each stage of the incremental exercise test for the breast cancer group.
Ea-arterial elastance

Figure 4.15: Arterial Elastance Response During Incremental Exercise Test (Controls)



Represents individual data for arterial elastance indexed to body surface area during each stage of the incremental exercise test for the control group.
Ea- arterial elastance

Figure 4.16: Left Ventricular Elastance Response During Incremental Exercise Test



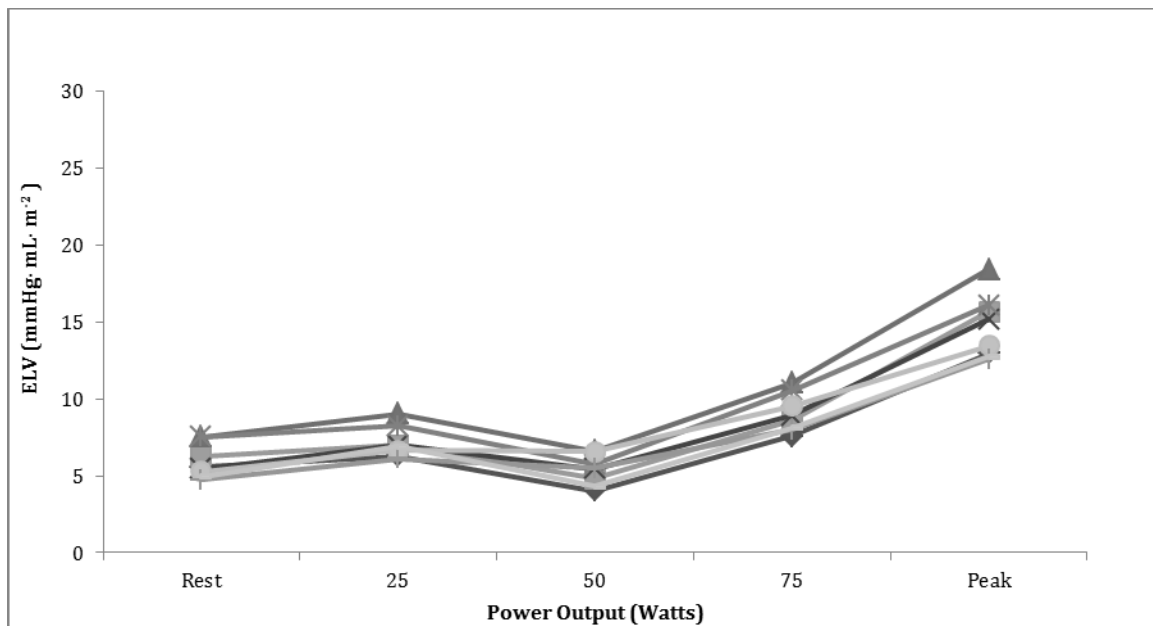
Data represents mean values for the repeated measures ANOVA for left ventricular elastance indexed to body surface area during each stage of the incremental exercise test for the breast cancer and control group.

* Indicates a main effect for time for each group

Indicates an interaction effect between the breast cancer and control groups

ELV- left ventricular elastance

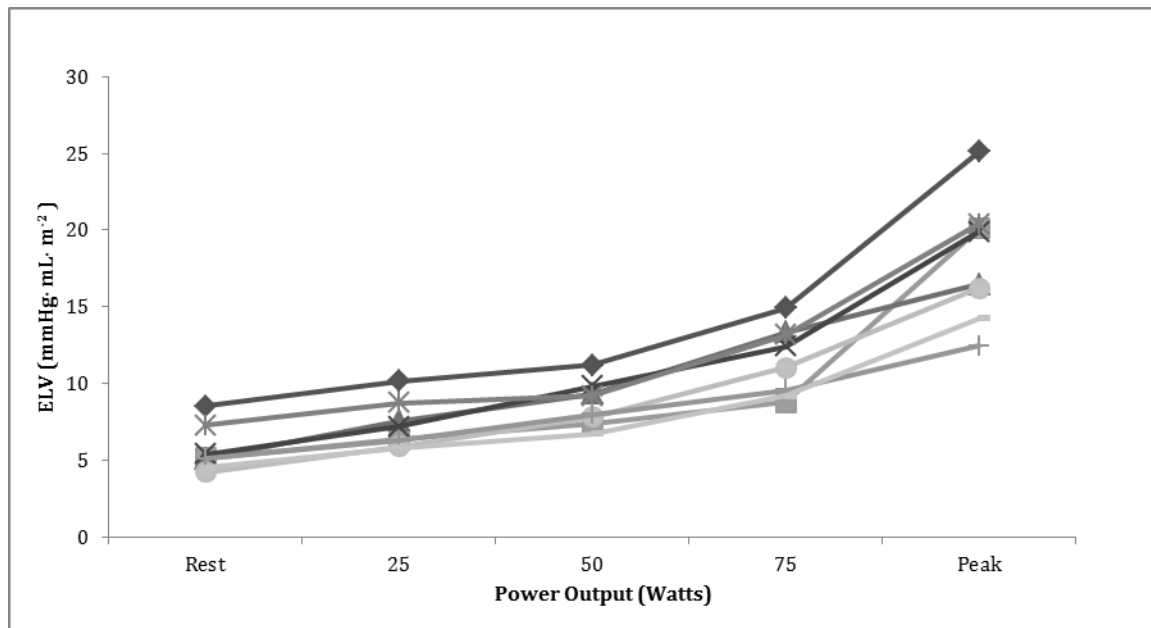
Figure 4.17: Left Ventricular Elastance Response During Incremental Exercise Test (Breast Cancer)



Represents individual data for left ventricular elastance indexed to body surface area during each stage of the incremental exercise test for the breast cancer group.

ELV- left ventricular elastance

Figure 4.18: Left Ventricular Elastance Response During Incremental Exercise Test (Controls)



Represents individual data for left ventricular elastance indexed to body surface area during each stage of the incremental exercise test for the control group.

ELV- left ventricular elastance

Table 4.5: Ventricular-Vascular Coupling Reserve

Variable	Pre-Exercise (n = 8)	Post Exercise (n = 8)	Difference	p-value
Ea Index				
<i>Breast Cancer</i>	3.64 ± 0.56	3.69 ± 0.46	0.05	
<i>Controls</i>	3.73 ± 0.65	4.00 ± 0.78	0.26	0.33
Elv Index				
<i>Breast Cancer</i>	5.91 ± 1.10	14.63 ± 2.06	9.72	
<i>Controls</i>	5.67 ± 1.47	18.11 ± 4.04	12.45	0.009
Ea/Elv Index				
<i>Breast Cancer</i>	0.62 ± 0.06	0.25 ± 0.02	-0.37	
<i>Controls</i>	0.68 ± 0.13	0.22 ± 0.20	-0.46	0.09

Data represented as mean ± standard deviation; Ea-arterial elastance; E_{LV}- left ventricular elastance; $p < 0.05$

4.4: DISCUSSION

To our knowledge, this was the first study to examine resting measures of vascular function and the role of stress echocardiography to determine changes in cardiac and vascular

performance in HER2-positive breast cancer survivors. Resting cardiac performance in HER2-positive female breast cancer survivors has been assessed in one investigation [163] and one investigation has explored endothelial function in HER2-positive breast cancer using FMD [96] demonstrating no change in either cardiac or vascular performance compared to healthy controls. One study has also assessed the role of resting ventricular-vascular-coupling in breast cancer patients treated with anthracyclines demonstrating augmentation of the ventricular-vascular coupling ratio can predict the onset of cardiotoxicity in women undergoing chemotherapy [185]. To the best of our knowledge, no other study has investigated the role of ventricular-vascular coupling during exercise conditions in HER2-positive breast cancer, and its role in predicting cardiac and vascular impairment that is not apparent under resting conditions. Moreover, no other study has assessed vascular stiffness (compliance and distensibility) or BRS in HER2-positive breast cancer survivors. This investigation therefore provides unique insight into resting indices of cardiac and vascular function and the role of measurement of LV contractile reserve in HER2-positive breast cancer. Results of this investigation demonstrate innovative findings: 1) Resting indices of cardiac and vascular performance are apparently normal in HER2-positive breast cancer survivors and age-matched apparently healthy controls 2) The SV, Q and heart rate response to an increase in exercise intensity did not differ between each group 3) Autonomic function is altered in HER2-positive breast cancer survivors indicating an increase in sympathetic drive compared to controls 4) Although the ventricular-vascular-coupling ratio was not altered in breast cancer, there was a disproportionate increase in E_{LV} during exercise, indicating a reduced ability to increase LV contraction during exercise conditions compared to apparently healthy counterparts.

4.4.1: Cardiovascular Function at Rest

Although it is accepted that trastuzumab treatment is associated with the development of cardiotoxicity, whether asymptomatic women with a history of breast cancer carry a long-term risk for the development of latent cardiac events is not well characterized. In our investigation, we demonstrate that asymptomatic breast cancer survivors did not present with alterations in ventricular-vascular-coupling at rest compared to their healthy counterparts suggesting that LV function might not be altered in HER2-positive breast cancer survivors who do not experience a decline in LVEF as a result of treatment. However, resting measures of ventricular-vascular-coupling are often altered (due to an increase in E_a or reduced E_{LV}) in patients with progressive disease [186], and therefore alterations in ventricular-vascular-coupling at rest might not be sufficient in detecting subtle alterations in LV function in breast cancer survivors. Moreover, SV and Q were not altered in breast cancer survivors compared to controls. This is expected, as even in patients with LV dysfunction, Q is maintained until later stages of disease when Q is unable to meet metabolic demands of the body. As such, a reduction in SV or Q might not be apparent in breast cancer survivors at rest, especially those with only mild cardiac impairment.

Outcomes of this investigation demonstrate that a reduction vascular compliance or an increase in PWV (indicating an increase in vascular stiffness) in HER2-positive breast cancer survivors was not apparent. However, many researchers have demonstrated that treatment with anthracyclines leads to an increase vascular stiffness or reduced endothelial function [44], [187]. Although acute changes in vascular function are apparent, it is possible that these changes do not persist following treatment. Indeed, changes in vascular stiffness

have been demonstrated in other investigations, however, it is important to note, that these measures are still within normal limits (compared to normative age and gender data) and are not associated with increased cardiovascular disease risk [188]. As such, augmentation of vascular stiffness may not play an important role in asymptomatic changes in LV function in breast cancer, rather it might play an instrumental role in the development of cardiotoxicity [185]. Further, our investigation was the first to demonstrate that BRS is altered in HER2-positive breast cancer survivors indicating an increase in sympathetic activity or a reduction in parasympathetic activity. These data are consistent with a recent investigation from Lenneman and colleagues (2014) [189] demonstrating that HER2 antagonism administration caused an increase in norepinephrine with a corresponding increase in systolic and diastolic blood pressure within 3 mo of administration. As such, it appears that autonomic regulation of cardiovascular performance is altered in breast cancer (as indicated by reduced BRS sensitivity), leading to increase in heart rate, however these changes seem to be isolated from a change in blood pressure or an increase in vascular stiffness in this investigation. Further research is needed to determine the role of autonomic function in breast cancer as an early marker of cardiovascular dysfunction.

4.4.2: Cardiovascular Function During Exercise

The haemodynamic response to incremental exercise in our investigation fails to demonstrate impaired LV function in breast cancer. Typically, in the presence of LV dysfunction Q fails to rise normally during exercise. Failure to increase Q is due to abnormal systolic function causing a defect in the ejection of blood and abnormal diastolic function causing to a defect in ventricular filling [120]. Moreover, the ability to increase heart rate is also blunted in the presence of LV dysfunction. Our data does not support the presence of LV

dysfunction in the assessment of exercise haemodynamics as heart rate, Q and SV were similar in the breast cancer population; and changes in diastolic function measured by deceleration time during exercise was also not altered (data not shown). Importantly, many of our patients and controls were able to increase their SV throughout incremental exercise reflecting the absence of marked pericardial constraint and the ability of the human myocardium to enhance diastolic filling during strenuous exercise conditions [190]. However, these findings provide insightful information regarding exercise intolerance in breast cancer. Despite Q and SV during exercise being similar between groups, the breast cancer group had a reduction in total time to exhaustion and reduced $\text{VO}_{2\text{peak}}$ by 12.9% compared to the age matched controls. Thus, although the breast cancer group is able to increase delivery of oxygen to the working muscle during exercise (indicated by a matched Q and SV response to exercise), they might be limited in their ability to extract and utilize oxygen suggesting peripheral limitations to exercise tolerance. This is also supported by the reduced AVO_2 difference observed in the breast cancer group compared to controls. Peripheral limitations to exercise tolerance is plausible, as it is suggested that impaired mitochondrial activity as a result of increased ROS production leads to the development of heart failure following anthracycline treatment [191]. Similar alterations may also occur in other tissue, including muscle leading to reduced metabolic activity and inability to extract and utilize oxygen despite efficient delivery. Such information is important in order to design effective exercise treatment strategies to reduce the possibility of a trajectory towards progressive exercise intolerance and perhaps functional limitations during survivorship in breast cancer.

Assessment of the end-systolic pressure volume relationship via exercise changes in ventricular-vascular coupling and its components provides a load-independent measure of

contractile function [127]. In our investigation, a blunted response to increase E_{LV} was apparent suggesting that LV contractile function might be impaired during exercise in breast cancer, despite normal resting indices of cardiac function. It should be noted as well, that the blunted response to increase E_{LV} during exercise despite a relatively small increase in E_a suggests that impaired LV function might be isolated to the contractile state of the LV rather than due to an increase in arterial loading conditions imposed on the LV (indicated by reduced augmentation of E_a during exercise). Importantly, exercise induced changes in E_{LV} (E_{LV} reserve) have been shown to predict the onset of late cardiac events, where changes in E_a (E_a reserve) did not in other patients populations [186]. In our investigation, E_{LV} reserve was significantly different between groups, suggesting that although ventricular-vascular-coupling was not altered during exercise conditions, stress echocardiography might play an instrumental role in identifying patients at risk of cardiac dysfunction in HER2-positive breast cancer survivors, despite being asymptomatic at rest.

These data opposes the findings that that cardiac performance (via evaluated Q and SV) was normal with the assessment of Q and SV throughout exercise in our investigation. Importantly, SV and therefore Q are critically dependent on the amount of blood returned to the heart during diastole (preload). In our investigation, SV was similar between each group, however ESV was significantly higher in the breast cancer group at peak exercise subsequently leading to a reduced measure of E_{LV} . As such, the breast cancer group was able to increase preload in order to maintain SV and Q. In patients with asymptomatic systolic dysfunction, recruitment of preload during exercise is responsible for maintaining SV and Q response. In patients with symptomatic heart failure, the ability to increase preload is diminished and the Q response decreases or is solely maintained by heart rate [192]. Thus,

exercise measures of SV and E_{LV} in this investigation reflects clearly the capacity for diastolic filling in the human heart to compensate for alterations in LV systolic function while the significant elevation in ESV may represent impairment in LV systolic function.

4.4.3: Study Limitations and Methodological Considerations

Stress echocardiography is accepted as a valuable assessment to determine cardiovascular dysfunction in asymptomatic patients. However, the use of stress echocardiography is particularly difficult to perform during exercise conditions [193]. We were fortunate to be able to hire a clinical sonographer with internationally recognized expertise in using 2D-echocardiography during exercise conditions. Unlike many other investigations in the field, we were able to utilize images throughout incremental to peak exercise. In order to increase the reliability and validity of our measures, the same skilled sonographer analyzed all LV volumes in an offline and blind analysis. Despite these stringent controls, we acknowledge that the LV EDV assessed during this trial are likely to be underestimated owing to the inherent difficulty of obtaining end-diastolic images during exercise conditions. These issues are known to affect EDV measures more than ESV. However, these limitations were consistent across groups.

Whether changes in vascular function and BRS are associated with adverse cardiac events has not been validated in the breast cancer population. As such, whether changes in BRS observed in this investigation are associated with increased risk of cardiac events in breast cancer survivors is unknown. Moreover, the small sample size in our investigation may have made it difficult to detect differences between groups due to inadequate power. As such, larger clinical trials are needed to confirm the results obtained in this investigation.

5.0: CONCLUSION

This investigation was the first to report changes in cardiovascular performance in HER2-positive breast cancer survivors using exercise-induced indices of cardiovascular function. Moreover, for the first time, the effects of HER2 directed therapies on vascular stiffness and baroreceptor function have been elucidated. This information provides insight to the possible mechanisms associated with the development of heart failure or adverse cardiac events in breast cancer survivors. Further, our data suggests that LV systolic function might be impaired in breast cancer; however, this difference does not appear to be as greatly influenced by arterial function as we had postulated. These observations provide insight regarding utilization of stress echocardiography to identify women at risk of latent cardiac events following therapy and improve our knowledge concerning limitations to exercise tolerance in the breast cancer population. Such information is important to clinicians, patients and researchers alike, in order to create effectively treatment strategies for breast cancer patients during survivorship.

Chapter 5: Discussion, Future Directions and Conclusion

This final chapter provides a discussion of our findings from each chapter of the thesis. Also we provide suggestions for future areas of research and concluding remarks.

5.1: GENERAL DISCUSSION

Recent investigations using subclinical markers of cardiac dysfunction in persons with a history of breast cancer or those undergoing treatment have led to an improved understanding regarding the extent and magnitude of cardiac dysfunction that occurs in the breast cancer population. Moreover, these investigations have demonstrated that subclinical markers of cardiac dysfunction including strain and strain rate imaging are efficient at predicting early onset of cardiotoxicity [146]. Despite these advancements, the guidelines regarding monitoring for cardiotoxicity in breast cancer are still limited, and the optimal screening methods for cardiotoxicity in breast cancer is unknown. Accordingly, this thesis investigation highlights important findings regarding subclinical markers of cardiac dysfunction in the breast cancer population. First, as outlined in chapter two, the development of cardiac dysfunction in HER2-positive breast cancer is multifactorial involving interplay between direct and indirect effects of treatment consequently increasing the risk of cardiac related morbidity and mortality in breast cancer. Moreover, chapter three provides a comprehensive summary of subclinical changes in cardiac echocardiographic measures that occur in the breast cancer population, which may precede the development of cardiotoxicity. Thus, it is apparent that direct consequences of treatment might lead to alterations in cardiac function beyond resting LVEF, which may lead to long-term cardiac dysfunction with poor prognosis. These changes are also accompanied by a high prevalence of cardiac risk factors in the breast cancer population, which should be addressed in the management of breast cancer survivors. Lastly, chapter four provides evidence

that although breast cancer survivors are asymptomatic at rest; exercise measures of cardiac function suggest that LV contractile function is impaired which may carry a long-term risk of cardiac events.

Recent chemotherapeutic agents and HER2 directed therapies are limited in use due to cardiotoxic effects, with the most prevalent being the development of heart failure and cardiomyopathy. As previously discussed, the detection of cardiotoxicity currently relies on a reduction in LVEF, which has important limitations. Chapter three highlights important limitations to current standards for monitoring of cardiotoxicity in breast cancer, suggesting that a plethora of alterations in cardiac function might occur in breast cancer, despite a reduction in LVEF. Although few studies have suggested that alterations in strain or strain rate imaging, or exercise measures of cardiac function may predict the onset of cardiotoxicity [151], [162], longitudinal studies are needed to determine the clinical significance of alterations in subclinical measures of cardiac function in predicting latent cardiac effects of treatment (1 to 10 yr) in breast cancer survivors. Lastly, evidence highlighted in Chapter three suggests that alterations in subclinical measures of cardiac function may occur at lower doses than previously believed to be cardiotoxic, in those who do not carry cardiac risk factors, and those > 60 yr of age. Thus, some patients might go undetected due to lack of recommendations for cardiotoxic screening in breast cancer, and therefore may only be identified when cardiac function is impaired and non-reversible.

Contrary to our hypothesis, outcomes of chapter four did not demonstrate that women with a history of HER-positive breast cancer present with changes in vascular compliance and PWV under resting conditions. However, these findings were consistent with the exercise measures indicating that Ea was not markedly different from controls, resulting in a decrease in

ventricular-vascular coupling with exercise in each group. Thus it seems that in our investigation net arterial load on the LV does not impede function of the LV under resting or exercise conditions. As expected, BRS was altered in our study group, providing some explanation for an increase in heart rate observed in HER2-positive breast cancer survivors when compared to healthy controls [96]. In acceptance with our hypothesis, LV contractile function was impaired upon stress echocardiography in our study group, despite normal indices of systolic and diastolic function at rest. These findings are important, and suggest that stress echocardiography is useful in identifying LV systolic impairment in asymptomatic breast cancer survivors. Moreover, these data suggest that cardiac dysfunction may persist following treatment with trastuzumab; however the long-term implications of this are unknown. It is also important to highlight that exercise haemodynamics were not altered in our study group compared to age-matched healthy controls; therefore the use of ventricular-vascular-coupling and its components might be superior for the identification of cardiac dysfunction in asymptomatic breast cancer survivors.

5.2: LIMITATIONS

The current investigation presents with many limitations. First, the small sample size makes it's difficult to determine if non-significant findings were due to a lack of statistical power to detect changes. We did however have sufficient statistical power to detect differences in myocardial contractility during exercise conditions, a very important contribution to the literature. Due to lack to access to hospital charts, we were limited in our ability to fully describe the disease characteristics and treatments (grade of disease, number of lymph nodes involved and doses of anthracycline treatments), which are important indicators of risk of cardiotoxicity following a breast cancer diagnosis. However, it is important to highlight that all breast cancer participants provided detailed medical records of

their treatments supplied by their oncologists, therefore characteristics including stage of disease, lymph node involvement, combination of chemotherapy and trastuzumab regimens and a diagnosis of HER2-positive breast cancer was plausible. Further investigations are needed in statistically powered investigations to confirm results obtained in our investigation. However owing to the limited research regarding the use of subclinical measures of cardiovascular dysfunction in HER2-positive breast cancer survivors, these results provide a framework for future research.

5.3: FUTURE RESEARCH

Future research in this field should focus on the clinical utility of LV contractile reserve in predicting long-term cardiac events in the breast cancer population. Given that HER2-positive breast cancer survivors are living longer, it is imperative to identify if changes in cardiac function during treatment carry a long-term cardiac risk in this breast cancer population. It should also be noted that the effects of HER2-directed therapies on vascular function is not well understood. Future investigations should focus on the effects of trastuzumab on vascular function, and if these effects are different then those seen with other cytotoxic agents including anthracyclines which results in impaired vascular function, at least, in the acute phase during and following treatment. Lastly, longitudinal studies assessing changes in subclinical measures during and following treatment are needed to determine if changes in these parameters, when reduced during therapy, are still apparent in long-term follow up. This is important, because although alterations in echocardiographic parameters are evident during and following treatment, there is lack of evidence investigating changes in cardiac function in long-term survivors who might be at the greatest risk of developing cardiotoxicity (5-10 yr following therapy).

5.4: CONCLUSION

This thesis investigation has numerous important findings. First, outcomes of our systematic review provide evidence for important early markers of cardiac dysfunction in breast cancer. These findings are sufficient, suggesting that early markers of systolic and diastolic dysfunction are apparent in breast cancer, preceding changes in LVEF, and therefore may pose a long-term risk for cardiac related morbidity in breast cancer. However, many of these women may go undetected due to lack of follow up or insufficient evidence to suggest which markers of cardiac function are important in predicting long-term cardiac events. Moreover, our first hypothesis stated that measures of vascular function would be evident between subjects and controls with an increase in vascular stiffness leading to reduction in BRS. However, this was not the case. It seems that direct effects of treatment may impair BRS despite changes in measure of vascular stiffness. Next we hypothesized that differences in exercise haemodynamics including reduced SV would be evident in breast cancer survivors. However, our investigation does not support this hypothesis. Further investigation is warranted to determine factors that may preserve SV and Q in this patient population. Lastly, we hypothesized that differences in ventricular-vascular coupling would be apparent in HER2-positive breast cancer survivors due to reduced LV function coupled with impaired arterial function. Outcomes of this investigation support this hypothesis that impaired LV systolic function is evident during exercise in asymptomatic HER2-positive breast cancer survivors; however variances in ventricular-vascular coupling were not clear between groups at rest or under exercise conditions.

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Appendix A

Letter of Initial Contact

Title of Project: Cardiovascular Function in Breast Cancer Survivors

Investigators: D. Warburton, PhD- School of Human Kinetics
A. Bonsignore (Masters student)- Faculty of Experimental Medicine

Institution: Faculty of Experimental Medicine, the University of British Columbia

Institution: School of Human Kinetics, The University of British Columbia
Contact Person: Dr. Darren Warburton's Office: [REDACTED]

To the participant,

We are looking for female breast cancer survivors, who have completed primary therapy (chemotherapy, surgery, radiation) with HER2+ breast cancer treated with Herceptin. We are evaluating the impact of trastuzumab on cardiovascular function. Specifically, we would like to measure the cardiovascular function at rest and during exercise conditions. The results of our study will provide important information regarding the changes that occur to heart and vascular function following treatment with trastuzumab. The procedures are outlined in detail in the Consent Form, which is attached.

Your total time commitment will be approximately 60 minutes on one day of testing and 90 minutes of testing on the second day at the University of British Columbia.

Physical measures taken will include: (1) blood pressure and heart rate, (2) 3 lead electrocardiogram (ECG) tracings, which will measure the electrical activity of your heart, and allow the investigators to safely monitor your heart function, (3) echocardiographic measures (images) of your heart (4) measures of vascular function.

If, after reading the study description carefully, you would like to participate in this study, please sign the consent form. Should you have any questions about this study, please contact Dr. Darren Warburton ([REDACTED]) at UBC. Thank you for your interest in this investigation.

Sincerely,
Dr. Darren Warburton, Ph.D.
Principal Investigator
Telephone: ([REDACTED])

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	<input type="checkbox"/>	<input type="checkbox"/>
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Are you currently taking prescribed medications for a chronic medical condition?	<input type="checkbox"/>	<input type="checkbox"/>
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	<input type="checkbox"/>	<input type="checkbox"/>
7.	Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

If you answered NO to all of the questions above, you are cleared for physical activity.



Go to Section 3 to sign the form. You do not need to complete Section 2.

- › Start becoming much more physically active – start slowly and build up gradually.
- › Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- › You may take part in a health and fitness appraisal.
- › If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist* (CSEP-CEP) or CSEP Certified Personal Trainer* (CSEP-CPT).
- › If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the questions above, please GO TO SECTION 2.



Delay becoming more active if:

- › You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- › You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- › Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?	<input type="checkbox"/> If yes, answer questions 1a-1c	<input type="checkbox"/> If no, go to question 2
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	<input type="checkbox"/>	<input type="checkbox"/>
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you have Cancer of any kind?	<input type="checkbox"/> If yes, answer questions 2a-2b	<input type="checkbox"/> If no, go to question 3
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	<input type="checkbox"/>	<input type="checkbox"/>
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm	<input type="checkbox"/> If yes, answer questions 3a-3e	<input type="checkbox"/> If no, go to question 4
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	<input type="checkbox"/>	<input type="checkbox"/>
3c.	Do you have chronic heart failure?	<input type="checkbox"/>	<input type="checkbox"/>
3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	<input type="checkbox"/>	<input type="checkbox"/>
3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	<input type="checkbox"/>	<input type="checkbox"/>
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes	<input type="checkbox"/> If yes, answer questions 4a-4c	<input type="checkbox"/> If no, go to question 5
4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	<input type="checkbox"/>	<input type="checkbox"/>
4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	<input type="checkbox"/>	<input type="checkbox"/>
4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)	<input type="checkbox"/> If yes, answer questions 5a-5b	<input type="checkbox"/> If no, go to question 6
5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
5b.	Do you also have back problems affecting nerves or muscles?	<input type="checkbox"/>	<input type="checkbox"/>

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure	<input type="checkbox"/> If yes, answer questions 6a-6d	<input type="checkbox"/> If no, go to question 7
	6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
	6b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	<input type="checkbox"/>	<input type="checkbox"/>
	6c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	<input type="checkbox"/>	<input type="checkbox"/>
	6d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	<input type="checkbox"/>	<input type="checkbox"/>
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia	<input type="checkbox"/> If yes, answer questions 7a-7c	<input type="checkbox"/> If no, go to question 8
	7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
	7b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	<input type="checkbox"/>	<input type="checkbox"/>
	7c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	<input type="checkbox"/>	<input type="checkbox"/>
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event	<input type="checkbox"/> If yes, answer questions 8a-c	<input type="checkbox"/> If no, go to question 9
	8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
	8b. Do you have any impairment in walking or mobility?	<input type="checkbox"/>	<input type="checkbox"/>
	8c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	<input type="checkbox"/>	<input type="checkbox"/>
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?	<input type="checkbox"/> If yes, answer questions 9a-c	<input type="checkbox"/> If no, read the advice on page 4
	9a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	<input type="checkbox"/>	<input type="checkbox"/>
	9b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	<input type="checkbox"/>	<input type="checkbox"/>
	9c. Do you currently live with two chronic conditions?	<input type="checkbox"/>	<input type="checkbox"/>

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+



If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.



Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider or other designator) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES

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The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.



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CSEP approved Sept 12 2011 version

ID _____

Date: _____

Health History Questionnaire

Please take the time to answer the following questions about your ethnicity and health history. This questionnaire is voluntary and you are free to leave any questions unanswered. Please be assured that all information will be coded and will remain strictly confidential and only be available to the researchers.

REGARDING YOU:

- 1.0 What is your current age? _____ yr
- 1.1 What is your current height and weight?
Height: _____ feet _____ in Weight: _____ pounds
- 1.2 When did you last:
- Engage in exhaustive exercise? _____ hours ago
 - Consume Alcohol? _____ hours ago
 - Smoke? _____ hours ago
 - Drink caffeine? _____ hours ago
 - Eat? _____ hours ago
- 1.3 What is the highest level of education that you completed? **Please check only one.**
- | | |
|--|--|
| <input type="checkbox"/> 8 th grade or less | <input type="checkbox"/> Vocational school or some college |
| <input type="checkbox"/> Some high school | <input type="checkbox"/> College degree |
| <input type="checkbox"/> High school diploma | <input type="checkbox"/> Professional or graduate degree |
- 1.4 What is your current marital status? **Please check only one.**
- | | | |
|--|-----------------------------------|--|
| <input type="checkbox"/> Single, Never married | <input type="checkbox"/> Married | <input type="checkbox"/> Living Common-law |
| <input type="checkbox"/> Separated | <input type="checkbox"/> Divorced | <input type="checkbox"/> Widowed |
- 1.5 How about your job situation? **Please check one that fits you best.**
- | | |
|--|--|
| <input type="checkbox"/> Homemaker | <input type="checkbox"/> Retired |
| <input type="checkbox"/> Paid full-time employment | <input type="checkbox"/> Paid part-time employment |
| <input type="checkbox"/> Temporarily unemployed | |
- 1.6 Type of job (Please fill-in): _____
- 1.7 Are you currently a student?
_____ Yes _____ No
- 1.8 If you are a student, are you:
_____ Full-time _____ Part-time

1.9 What is your annual personal income? **Please check only one**

- | | | |
|---|---|---|
| <input type="checkbox"/> \$5000 or less | <input type="checkbox"/> \$5001 to \$10,000 | <input type="checkbox"/> \$10,001 to \$20,000 |
| <input type="checkbox"/> \$20,001 to \$40,000 | <input type="checkbox"/> \$40,001 to \$75,000 | <input type="checkbox"/> More than \$75,000 |

2.0 Medical History and Status:

2.1 Are you currently taking any medications?

_____ Yes _____ No

If yes, what medication(s) are you taking? _____

What are these medication(s) for? _____

2.2 Do you have heart disease, such as angina (chest pain), a heart attack, coronary bypass surgery, stroke, etc.?

_____ Yes _____ No

2.3 Is there a history of heart disease in your family?

_____ Yes _____ No

2.4 If yes, who was affected?

_____ Mother	_____ Father
_____ Grandmother	_____ Grandfather
_____ Sister	_____ Brother
_____ Daughter	_____ Son

2.5 If yes, how old were they?

Mother	_____ yr	Father	_____ yr
Grandmother	_____ yr	Grandfather	_____ yr
Sister	_____ yr	Brother	_____ yr
Daughter	_____ yr	Son	_____ yr

2.6 Do you have diabetes?

_____ Yes _____ No

2.7 Have you had gestational diabetes (women only)?

_____ Yes _____ No

2.8 Has your doctor told you your blood pressure is too high?

_____ Yes _____ No

2.9 Has your doctor told you your cholesterol is too high?

_____ Yes _____ No

2.10 What is your menopausal status (women only)?

- ☐ Have not yet started menopause (still get regular periods)
- ☐ Am pregnant
- ☐ Started menopause less than 10 years ago (stopped having regular periods less than 10 years ago)
- ☐ Started menopause 10+ years ago (stopped having regular periods 10 or more years ago)

2.11 What is your smoking status? (Please check all that apply)

- | | |
|--|---|
| <input type="checkbox"/> Never Smoked | <input type="checkbox"/> Smoke 10 or less cigarettes a day |
| <input type="checkbox"/> Quit MORE than 2 years ago | <input type="checkbox"/> Smoke 11 to 20 cigarettes a day |
| <input type="checkbox"/> Quit LESS than 2 years ago | <input type="checkbox"/> Smoke 20 or more cigarettes a day |
| <input type="checkbox"/> Am exposed to 2nd hand smoke | <input type="checkbox"/> Use chew tobacco |
| <input type="checkbox"/> Only smoke with others | <input type="checkbox"/> Smoke a pipe or cigar |

2.12 If you have smoked in the past 30 days, do you smoke mainly:

- ☐ When you are with other people
- ☐ Mainly when you are alone
- ☐ As often by yourself as with others

2.13 During the past 12 months, have you had a drink of beer, wine, liquor or any other alcoholic beverage?

_____ Yes _____ No

2.14 During the past 12 months, how often did you drink alcoholic beverages?

- ☐ Less than once a month
- ☐ Once a month
- ☐ 2 to 3 times a month
- ☐ Once a week
- ☐ 2 to 3 times a week
- ☐ 4 to 6 times a week
- ☐ Every day

2.15 How often in the past 12 months have you had 5 or more alcoholic drinks on one occasion?

- ☐ Never
- ☐ Less than once a month
- ☐ Once a month
- ☐ 2 to 3 times a month
- ☐ Once a week
- ☐ More than once a week

2.16 If you have had alcohol in the past 30 days, do you drink mainly:

- ☐ When you are with other people
- ☐ Mainly when you are alone
- ☐ As often by yourself as with others

2.17 What was your breast cancer diagnosis?

2.18 Do you have estrogen positive breast cancer?

_____ Yes _____ No

2.19 What types of medications and doses were you prescribed during chemotherapy?

2.20 Are you currently on any medications for breast cancer and what is the dose?

2.21 Did you undergo radiation therapy?

_____ Yes _____ No

2.22 What type of surgery did you have?

THANK YOU FOR YOUR PARTICIPATION!

PHYSICAL ACTIVITY QUESTIONNAIRE

1. Frequency

Over a typical seven-day period (one week), how many times do you engage in physical activity that is sufficiently prolonged and intense to cause sweating and a rapid heart beat?

- ☐ Five or more times
- ☐ Three to four times
- ☐ Normally once or twice
- ☐ Rarely or never

2. Intensity

When you engage in physical activity, do you have the impression that you:

- ☐ Make an intense effort
- ☐ Make a moderate effort
- ☐ Make a light effort

3. Perceived Fitness

In a general fashion, would you say that your current physical fitness is:

- ☐ Very Good
- ☐ Good
- ☐ Average
- ☐ Poor
- ☐ Very Poor

PHYSICAL ACTIVITY QUESTIONNAIRE (MONTHLY)

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number)

	Times/Week	Average
Duration		
a. STRENUOUS EXERCISE		
(HEART BEATS RAPIDLY, SWEATING)		
(e.g., running, jogging, hockey, soccer, football, squash, basketball, cross country skiing, judo, roller skating/roller blading, vigorous swimming, vigorous long distance bicycling, vigorous aerobic dance classes, heavy weight training)		
b. MODERATE EXERCISE		
(NOT EXHAUSTING, LIGHT PERSPIRATION)		
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)		
c. MILD EXERCISE		
(MINIMAL EFFORT, NO PERSPIRATION)		
(e.g., easy walking, yoga, bowling, archery, fishing, bowling, horseshoes, golf, snow-mobiling)		

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

- ☐ OFTEN
- ☐ SOMETIMES
- ☐ NEVER/RARELY

Participant Information and Consent Form

Title of Project: Cardiovascular Function in Breast Cancer Survivors

Principal Investigator: D. Warburton, Ph.D.- School of Human Kinetics, UBC
Co-Investigators: A. Bonsignore (Masters Student)– Experimental Medicine, UBC

Institutions: University of British Columbia

Contact Person: Dr Darren Warburton's Laboratory: [REDACTED]

We would like to invite you to participate in an investigation evaluating the vascular and cardiac dynamics in women with a history of breast cancer who have undergone treatment with trastuzumab. Women breast cancer survivors who are 19 years or older who were treated for early-stage breast cancer and who have completed chemotherapy and trastuzumab treatment will be included. We are interested in assessing the cardiac and vascular health, structure, and function at rest and during exercise among women with a history of breast cancer compared to age matched controls. The results of our investigation will provide information regarding the potential cardiovascular risk associated with trastuzumab treatment. In this investigation we are seeking 23 individuals with breast cancer and apparently healthy age-matched controls.

You should not participate in this investigation if you: 1) are younger than 19 at the start of the investigation, 2) have uncontrolled cardiovascular disease or diabetes which has been diagnosed by your physician, 5) are taking any medications for cardiovascular disease or diabetes, 6) are unable to participate in physical activity or have not been cleared for unrestricted physical activity, or 6) are pregnant

Your Participation is Voluntary:

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. If you decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

Procedures:

Please review the following consent form, which will outline all aspects of the investigation. If you have any questions or are interested in participating in this investigation please contact the laboratory by phone ([REDACTED]). A researcher with the study will further discuss the investigation and schedule the first measurement sessions (see below) at the University of British Columbia. The consent form will be reviewed at the first session and your written consent will be sought should you decide to participate. This study will require 1 day of testing, requiring a time commitment of approximately 2 hours.

The measurement sessions

The health assessments will be conducted and will consist of measurements of vascular health (arterial compliance, blood pressure variability, heart rate variability and pulse wave velocity), ultrasound images of the heart, and the artery in the neck. Vascular and ultrasound measures will be obtained before during and after an exercise session.

Prior to each day of testing, **you will be asked to refrain from exhaustive exercise for 24 hours, caffeine/alcohol for 12 hours, and smoking and food for 2 hours.** Both of these testing sessions will be conducted at the Cardiovascular Physiology and Rehabilitation Laboratory at the University of British Columbia. For each testing session, you will be asked to wear or bring clothing and footwear suitable for exercise.

Questionnaires: You will be asked to complete 2 questionnaires upon arrival at the laboratory. The first aims to assess general physical activity and the second is a health history questionnaire that asks about aspects of your medical history relevant to this study, your educational background, your approximate income and your ethnic and cultural background. You will not have to answer any questions that you are not comfortable with on any of the questionnaires. These questionnaires should take about 10 minutes or less to complete.

Body Composition/Anthropometric and Grip Strength Measurements: First we will ask you to remove your shoes to measure your height and weight. Then we will ask you to lift up the bottom of your shirt slightly to expose your waist to measure your waist circumference. To measure your grip strength, you will be asked to squeeze a grip strength dynamometer as hard as you can, twice with each hand. These are all non-invasive and painless. These measures should take about 5 minutes to complete.

Vascular health: Both before and after the exercise session, we will non-invasively measure the compliance of your arteries while you are lying on a bed. We will ask you to rest, lying down, for 10 min. Then we will assess blood pressure wave forms (similar to a standard blood pressure measurement) using a specially designed pressure device placed on the right arm in combination with an automatic measurement of blood pressure on the left arm. This test is completely painless and non-invasive. You will remain lying down for the next assessment, which is heart rate and blood pressure variability, and pulse wave velocity. For this you will wear an ECG (electrocardiogram) to record heart rate and a small blood pressure cuff that goes on your finger. You will also wear sensors to measure your pulse at 3 places on your body: at the neck, hip and toe. These sensors, and the 3 sensors on your chest for the ECG will consist of a small sticker-like pad on your skin with an electrode attached to the pad. This is painless and non-invasive. These measures should take about 15 minutes to complete.

Vascular ultrasound of the neck: Prior to and following exercise, we will non-invasively measure the artery in your neck while you are lying on a bed. We will take pictures and short videos (3 to 5 heart beats long) of the artery in your neck with an ultrasound machine. During these measurements you will wear an ECG (electrocardiogram) to record heart rate. This procedure uses sound waves (ultrasound) to produce an image of the internal structures of the artery. In order to produce an image, gel will be applied to your neck and a transducer (a wand-like apparatus) is moved over your neck. There is absolutely no pain or discomfort with this procedure and will take approximately 15 minutes.

Cardiac function (Echocardiography): A trained sonographer (someone who is trained in taking ultrasound images) will image your heart before, during and after each exercise. The scanning will take place with you lying on your left side. This procedure uses sound waves (ultrasound) to produce an image of the internal structures of your heart. In order to produce an image of the heart muscle, gel will be applied to your chest area and a transducer (a wand-like apparatus) is moved over your chest. There is absolutely no pain or discomfort with this procedure and this procedure will take approximately 15 minutes for each assessment.

Cardiovascular fitness: Your cardiovascular fitness will be assessed using a maximal, graded (increasing in resistance, or the work you pedal against), leg cycle test, or VO_{2max} (the maximum amount of oxygen your muscles can use in one minute per kilogram of body weight). This will be determined by cycling on an exercise bike against increasing resistance (described more fully below). You will be instructed to cycle at 60-80 revolutions per minute (RPM), with an initial workload of 75 watts (W) (which is the resistance you will pedal against). Every 3 minutes, the resistance will increase by 25 W, but the rate of pedaling will remain at 60-80 rpm. You will be asked to exercise until you are too tired to continue, unless you experience symptoms that would cause you or the research team to terminate the test early. This exercise test should take approximately 12 to 20 minutes. During this test you will be asked to wear a mask that covers your mouth and nose while cycling. This will allow us to collect the gases you expire during the activity. This apparatus is safe, painless, and is used regularly in exercise tests with individuals of all ages. The tests will be stopped early if you experience abnormal symptoms (e.g. lightheadedness, and muscle cramping). During this task you will also be asked to wear a non-invasive hemodynamic monitor. This device will provide continuous, accurate, reproducible and sensitive measurements cardiovascular hemodynamics during exercise. There is absolutely no pain or discomfort with this procedure.

A university-trained clinical exercise physiologist will be conducting the exercise sessions. During the exercise test, your heart rate will be recorded using an ECG. Your heart rate (via a heart rate monitor and an ECG) and blood pressure (using a blood pressure cuff and stethoscope) will be measured non-invasively throughout the test. You will also be asked to rate your level of effort during this test. Your heart rate and breathing rate will increase; you may sweat and feel some fatigue, which may cause some temporary discomfort. You may ask to terminate the test at any time.

Disclosure of Race/Ethnicity

Studies involving humans now routinely collect information on race and ethnic origin as well as other characteristics of individuals because these characteristics may influence how people respond to different medications and experience different chronic conditions. Providing information on your race or ethnic group is voluntary.

Risks:

The tests of physical fitness may cause you to become tired and short of breath for a short period of time. There are no known permanent adverse side effects that have resulted from these exercise sessions. Data from individuals with or without heart disease indicates that the likelihood of having a heart attack or dying during an exercise stress test is 1 in 10,000 tests. All exercise testing and body composition measures will be performed under the supervision of a Certified Exercise Physiologist. These individuals have received the most advanced exercise training in Canada and are certified in first aid, CPR and the use of an automated external defibrillator (AED). The laboratory is equipped with

an AED and is located less than 1km from the hospital on the University of British Columbia campus (response time approximately 3 minute).

Since there is a variable response from individuals during exercise, unanticipated complications may occur that would require treatment. Few problems have been associated with exercise testing, and the shortness of breath and muscular soreness usually clear quickly with little or no treatment. Every effort will be made to conduct the test in such a way as to minimize discomfort and risk. However, there exists the small possibility of potential risks from maximal exercise such as vomiting (5%), abnormal blood pressure, such as low blood pressure or hypotension following exercise (less than 1%), disorders of heartbeat, or arrhythmia (0.1%), and very rare instances of heart attack (less than 0.001%). The percentage of risk of heart attack based on males and females (age 18-35) that have been given these tests is very small (less than 0.0001%). Having Exercise Physiology trained professionals implement the tests will further reduce the risks associated with exercise testing. There is the risk of discomfort, bruising, pain, tingling or numbness during the cuff occlusion of the vascular function assessment. As a participant, you may not benefit from the study directly. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

Benefits:

You will receive complete individual and group summaries of body composition and cardiovascular health measure results from this investigation. You will also get an assessment of your risk for cardiovascular disease. If an abnormality in any measurement is observed (at any time period) that indicates an elevated risk a member of our research team will contact you directly regarding the findings.

Reimbursement

You will receive no financial reimbursement for your participation in the study

Rights and Welfare of the Individual:

You have the right to refuse to participate in this study. It is understood that you are free to withdraw from any or all parts of the study at any time without penalty. Your identity will remain confidential as all individual records and results will be analyzed and referred to by number code only and kept in a locked cabinet in the Cardiovascular Physiology and Rehabilitation Laboratory at the University of British Columbia. This lab will remain locked and only those directly involved in the study (namely the principal investigators) will have access to your records and results. You will not be referred to by name in any study reports or research papers. Your individual results will remain confidential as they will not be discussed with anyone outside the research team.

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the investigator or his or designate by representatives of Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the investigators offices.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected

about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Please be assured that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. Should you have any concerns about this study or wish further information, please contact Dr. Darren Warburton [REDACTED] at the University of British Columbia. If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics [REDACTED]

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected. Further details about these laws are available on request to your study investigator

INFORMED CONSENT FORM

Participant Consent:

I, _____
(Please Print Your Name)

I have read and understood the subject information and consent form. I have had sufficient time to consider the information provided and to ask for advice if necessary. I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study. I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives. I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or withdraw from this study at any time without changing in any way the quality of care I receive. I understand that I am not waiving any legal rights as a result of signing this consent form. .

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

Signature of Participant

Printed name

Date

Signature of Investigator

Printed name

Date