Pathophysiology of Exercise Intolerance in Breast Cancer Survivors Treated with Anthracycline Chemotherapy

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Abstract

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Anthracyclines emerged as frontline breast cancer adjuvant therapy in the late 1960's. Within half a decade of their clinical adoption, dose-limiting cardiotoxicity was recognized and cumulative dose limits were established to acceptably balance anti-tumor and cardiotoxic properties. Despite these longstanding dose limits, anthracycline treated breast cancer (BC) survivors have advanced biological aging, as evidenced by a marked impairment in peak oxygen uptake ($VO_{2\ peak}$). Given that the pathophysiology of reduced $VO_{2\ peak}$ in BC is poorly understood, the aim of this dissertation was to: 1) develop a non-invasive imaging technique to investigate cardiac function under exercise stress; 2) investigate the cardiac limits to exercise in BC; and 3) investigate muscle blood flow, oxygen use, and bioenergetics as components of exercise intolerance in BC.

In study 1 (Chapter 2) an exercise cardiac magnetic resonance imaging (cMRI) technique was developed using commercially available hardware and in-product image sequences. Rest and exercising cardiac volumes were measured in eight young healthy subjects (mean age: 25 years) and a meta-analysis was performed to demonstrate consistency of results, thus demonstrating feasibility and establishing the normative cardiac response to supine exercise cMRI. From rest to exercise, cardiac output increased by an increase in heart rate and stroke volume, through preserved end-diastolic volume and reduced end-systolic

volume. In study 2 (Chapter 3), using exercise cMRI and cardiopulmonary exercise testing, cardiac function and $VO_{2\ peak}$ were measured in 29 BC patients (mean age: 48 years) prior to receiving anthracycline based chemotherapy and in 10 age- and sex-matched healthy controls. On average, BC patients had 20% lower $VO_{2\ peak}$ (l/min or ml/kg/min) than healthy controls, which was related to lower peak exercise cardiac output. Importantly, decrements in peak cardiac output were apparent prior to anthracycline administration. In study 3 (Chapter 4), using femoral artery Doppler ultrasound, leg blood flow was measured during single-leg knee extension (SLKE) in 14 BC survivors (mean age: 61 years, mean time post anthracycline therapy: 12 years) and 9 age- and sex-matched controls. Peak SLKE power output, heart rate and blood pressure were comparable between groups. Leg (femoral artery) blood flow was measured during 25, 50 and 75% of peak SLKE, and was not significantly different between groups at rest or during submaximal exercise. However, estimated quadriceps muscle mass was reduced in BC survivors, despite normal leg blood flow and conductance. In study 4 (Chapter 5), using MRI, lower leg (superficial femoral vein) blood flow, VO_2 and bioenergetics were measured during plantar flexion exercise in 16 BC survivors (mean age: 56 years, mean time post anthracycline therapy: 13 months) and 16 age-, sex- and body mass index matched controls. Muscle oxidative capacity was not impaired, nor was muscle blood flow or oxygen extraction. However, BC survivors tended to have abnormal leg composition (increased fat and reduced muscle) that contributed to reduced $VO_{2\ peak}$. Taken together, the data herein demonstrate a strong non-cardiac component to exercise intolerance in anthracycline treated BC survivors, similar to that found in sex- and age-matched controls. Future research building upon non-cardiac interventions for prevention of anthracycline related reductions in $VO_{2\ peak}$ are needed to reduce cardiovascular risk in BC survivors.

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Dedication

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Chapter 1

1.1 Pathophysiology of Exercise Intolerance in Breast Cancer: Review of the literature.

1.1 a) Introduction:

Breast cancer (BC) is the most frequently diagnosed malignancy in women, and the second leading cause of cancer mortality.[1] Approximately 1 out of every 8 women will be diagnosed with BC in their lifetime, amounting to nearly a quarter million new cases annually in the United States.[2] Over the past 4 decades, improvements in prevention, detection and treatment have resulted in a nearly 40% BC mortality reduction.[1] A direct implication of improved survival is the rapidly expanding population of survivors, for which competing causes of morbidity and mortality are growing concerns. Specifically, cardiovascular disease (CVD) is a leading cause of death in BC survivorship.[3] Most strikingly is the reported 35% increase (adjusted incidence rate ratio) in risk of cardiomyopathy/heart failure (HF).[4] This excess risk has traditionally been attributed to cardiotoxic BC therapies including mediastinal chest irradiation and chemotherapy; however, in 2007 this understanding evolved to the "Multiple Hit Hypothesis", explaining excess risk as a culmination of underlying CVD risk factors at diagnosis, direct cardiotoxic anti-cancer therapy, and indirect effects of anti-cancer therapy (poor nutrition, decline in physical activity).[5] Limited data were available to support the Multiple Hit Hypothesis when it was proposed; however, over the last decade a wealth of new data has emerged from the physiologic to epidemiologic level confirming and advancing the understanding of elevated CV mortality in BC survivorship. The present paradigm explaining excess CV risk posits a combination of pre-existing CVD risk factors, shared biologic pathways between CVD and BC, and treatment and lifestyle toxicity result in elevated CV mortality.[6]

Herein, the focus will be anthracycline related cardiotoxicity and HF in BC. Anthracycline class drugs were first introduced to oncology practice in the late 1960's.[7, 8] Their

robust antitumor efficacy resulted in widespread use and anthracycline based chemotherapy regimens quickly became frontline therapy. Within 5 years of their clinical induction, dose limiting cardiotoxicity was reported.[9] Today, anthracyclines remain a treatment mainstay; anti-tumor effects must carefully be balanced with risk of cardiotoxicity.

1.1 b) Anthracycline Cardiotoxicity: Evolution to the present state of knowledge:

In 1979 the first largescale study of anthracycline related HF was released.[10] This study consisted of retrospective analysis of 4018 anthracycline treated patients with histopathological examination of HF cases, as well as post-mortem non-HF cases when available.[10] Von Hoff *et al.* found overall incidence of doxorubicin (the clinical standard anthracycline at the time) induced HF was 2.2%; a conservative cumulative incidence given that many patients received low doses, and only clinically diagnosed HF cases were counted. Heart failure occurred on average 33 days after the last doxorubicin dose, and was lethal in 60% of patients. Remarkably, only 37% of HF cases demonstrated histopathological evidence of doxorubicin cardiotoxicity, and an additional 8% of a cohort subset (n=347) showed post-mortem evidence of subclinical damage. These investigators identified three factors associated with cardiotoxicity; first, they confirmed earlier reports of cumulative dose as the primary risk factor. Second, dosing schedule appeared to influence development of cardiotoxicity, with a trend for greater peak plasma concentration eliciting cardiotoxicity. Third, increasing age was associated with cardiotoxicity however it was unclear if the latter finding was related to age, or age associated declines in CV health (i.e. functional status, cardiovascular comorbidities).

This study identified two critical research areas[10]. First, research was needed to advance cardiotoxicity risk stratification, and improve early detection/monitoring and therapy for

cardiotoxicity. In the cohort, the leading cause of death was progressive malignant disease, establishing the need for higher cumulative doses of doxorubicin in patients at low risk of cardiotoxicity and HF. The authors recognized that their sample was drawn from clinical trials prone to selection bias through exclusion of "sick" patients, thus limiting application of their results. Underlying CV risk factors and low functional status were previously demonstrated as relevant risk factors for cardiotoxicity, but were not statistically significant in the selected retrospective analysis. As suggested by the authors, the use of developing methodologies (cardiac biomarkers, ECG, echocardiography) for monitoring cardiac function and detecting subclinical cardiotoxicity could aid in earlier and more effective intervention. More research was needed to identify effective therapies for reversal of cardiotoxicity.

Second, Von Hoff *et al.*[10] reported that 1) subclinical cardiotoxicity (post-mortem evidence of cardiac damage without HF) was present in 8% of non-HF cases, and 2) the vast majority of HF cases were not consistent with doxorubicin induced cardiac damage. This curious phenomenon marks a discord between anthracycline mediated cardiotoxicity and development HF, and raises the possibility of an indirect relationship between anthracyclines and HF.

The present state of knowledge of anthracycline cardiotoxicity is based on the seminal study by Cardinale *et al.* who demonstrated the feasibility of early detection and pharmaceutical intervention to reverse cardiotoxicity.[11] This prospective study was designed to elucidate the prevalence and pathophysiology of anthracycline cardiotoxicity through serial monitoring using state of the art echocardiography.[11]

At the time, the accepted model of anthracycline cardiotoxicity included 3 subtypes: 1) acute, occurring after a single dose/course with clinical manifestation in less than 2 weeks, 2)

early-onset chronic, developing within 1 year of therapy and characterized by cardiac hypokinesis, and 3) late-onset chronic, developing years to decades after therapy. Sparse data were available to support the model, as the vast majority of studies were retrospective, and only included patients who had developed clinically overt HF. As a result, it was unclear when cardiotoxicity occurred in relation to the onset of clinical HF. To address this, Cardinale *et al.*[11] prospectively enrolled 2625 consecutive patients scheduled for anthracycline based chemotherapy and monitored patient's quarterly using echocardiography. Cardiotoxicity was defined as a decline in left ventricular ejection fraction (LVEF) by 10% or to a value below 50% (biplane) and evidence based anti-HF medications were administered given evidence of cardiotoxicity. Notably, this definition of cardiotoxicity is not synonymous with clinical presentation of HF, but identifies subclinical cardiac dysfunction which may progress to clinical cardiac dysfunction and overt HF.

Cardinale *et al.*[11] reported the presence of cardiotoxicity in 9.7% of BC patients, similar to findings by Von Hoff *et al.* more than 3 decades earlier.[10] Ninety eight percent of cases occurred within the first year (median 3.5 months) of therapy cessation, and when treated with contemporary HF medication, 11% of patients had full recovery, 71% made partial recovery (defined as an increase in LVEF to >50% or an increase in LVEF by 5 percentage points), while 18% showed no improvement.[11] The authors argued for a singular pathophysiology of early onset cardiotoxicity, which could insidiously decline until symptomatic detection of HF years after incipient damage if left undetected and untreated. In spite of their compelling evidence that 98% of cases occurred within the first year, the median follow-up was only 5 years, with few cases available beyond 10 years. Importantly, as in the general population, age is the greatest risk factor for the development of CVD and HF with women diagnosed with BC who are >7

years having nearly 23-fold greater risk of developing HF relative to women diagnosed between 50 and 54 years of age.[12]

Detection of cardiotoxicity resulting in cardiac dysfunction was contingent upon changes in resting cardiac function, as measured by echocardiography.[11] Cardinale *et al.* provide promising results with this clinically relevant and cost effective strategy, however, there remains discord between resting cardiac measures and exercise intolerance- the hallmark feature of HF. Cardiac stress imaging and more robust non-invasive imaging strategies could provide earlier insight into functional declines and changes in cardiac tissue (i.e. fibrosis), opening the window for potentially earlier and more effective intervention.

1.1 c) Exercise Intolerance in Breast Cancer:

The hallmark feature of HF is exercise intolerance, measured objectively as decreased peak oxygen uptake ($VO_{2\ peak}$) during maximal exercise. $VO_{2\ peak}$ is the strongest independent predictor of CV, and all-cause mortality in healthy and clinical populations, and is related to survival in BC.[13-15] Across the survivorship continuum, $VO_{2\ peak}$ is approximately 20% lower relative to age and sex matched controls.[13, 16-18] This decrement in cardiorespiratory fitness likely contributes to elevated CVD risk as a 40 year-old BC survivor has been shown to have a $VO_{2\ peak}$ similar to that found in healthy sedentary women 20-30 years older.[13] Currently, the mechanisms underpinning impairments in $VO_{2\ peak}$ are poorly understood.

In accordance with the Fick principal (VO_2 = the product of cardiac output [Q] and arterial-venous oxygen difference [$Ca_{O2} - Cv_{O2}$]), the reduced in $VO_{2\ peak}$ may be due to central and/or peripheral, factors with the caveat that there is interdependency between flow and oxygen extraction equilibration.

1.1 c) i: Cardiac Determinants of impaired VO_{2peak}:

Cardiac output is the product of heart rate (HR) and stroke volume (SV), the difference between end-diastolic (EDV) and end-systolic volume (ESV). Given the known cardiotoxic nature of anthracycline chemotherapy, impaired peak Q may be an important contributor to exercise intolerance in anthracycline treated BC survivors. To date, 3 previous investigations have examined cardiac function in anthracycline treated BC survivors.

Jones *et al.* using impedance cardiography and expired gas analysis, studied 42 anthracycline treated BC survivors (34 months post therapy cessation) and 11 age- and sex-matched controls.[17] Breast cancer survivors had 21% lower VO_{2peak} that was due to a lower peak Q secondary to a lower peak SV with no significant difference found between groups for peak, mean arterial pressure, systemic vascular resistance or estimated $Ca_{O2} - Cv_{O2}$. Of note, resting SV was lower while HR was higher resulting in a blunted HR reserve (peak minus rest value). A limitation of impedance cardiography is that it is unable to evaluate the contribution of changes in left ventricular (LV) systolic and diastolic volumes and function in limiting exercise SV.

A later study by Khouri *et al.*, using echocardiography at rest and during post-exercise recovery, equal to 83% of maximal HR, measured LV volumes and Q in 57 middle-aged anthracycline treated BC survivors (51 years) and 20 age and sex-matched controls (57 years).[16] Compared to controls, VO_{2peak} was 20% lower in BC survivors with no significant difference between groups for maximal exercise HR or SBP. Post-exercise Q was lower in BC survivors secondary to a lower SV and EDV.[16] A limitation of this study was that assessment of cardiac function occurring in the recovery period.

Finally, Koelwyn *et al.*[19], using 2D echocardiography during sub-maximal cycle exercise, measured cardiac function in 30 anthracycline treated BC survivors and 30 age-, BMI- and activity- matched women. In contrast to the above mentioned studies[16, 17], VO_{2peak} was not significantly different between groups, and control subjects achieved greater peak systolic blood pressure.[19] Similar to prior studies[16, 17], the BC group a had higher resting HR and both groups achieved the same peak HR- suggesting reduced HR reserve in BC survivors. No differences were found for LV EDV, ESV, SV or LVEF at rest, however, at 25, 50 and 75% of maximal work rate, ESV was increased in BC survivors while LVEF reserve was blunted. These findings were explained by impaired ventricular-arterial coupling in BC mediated by an increase in non-invasively derived single-point end-systolic elastance (a surrogate for LV contractility). Importantly, the estimated increase in end-systolic elastance may have been due to increased myocardial stiffness due to fibrosis/extracellular matrix expansion, cardiomyocyte loss, or impaired cardiomyocyte contractile function independent of LV contractility.

In an effort to better understand perturbations in end-systolic elastance, myocardial tissue characteristics were characterized in a cross-sectional study of anthracycline treated cancer survivors (n=37), non-anthracycline treated cancer survivors (n=17), pre-therapy cancer patients (n=37), and cancer-free controls (n=236).[20] Specifically, magnetic resonance imaging (MRI) was used to non-invasively assess LV native T1 (an indicator of myocardial fibrosis) and T2 relaxation (a measure of myocardial edema). Previously, extracellular volume fraction (the ratio of extracellular matrix to cardiomyocytes) was linked to cardiac tissue movement and diastolic compliance.[21] Consistent with findings of impaired end-systolic elastance from Koelwyn *et al.*[19], anthracycline treated cancer survivors had increased myocardial fibrosis (measured as T1 and extracellular volume fraction with gadolinium) relative to cancer-free controls.[20] However, pre-

treatment cancer patients also had elevated native T1, suggesting that even prior to therapy, cancer patients may have increased LV chamber fibrosis and stiffness. T2 was within normal ranges for both pre-treatment cancer patients and cancer survivors.

In summary, the role anthracycline induced cardiac impairment in reduced VO_{2peak} is unclear. Studies performed to date consistently demonstrate that BC survivors do not have a reduced peak HR, however; resting HR is elevated and as a result, HR reserve is reduced.[16, 17, 19] Some studies have shown that peak Q is secondary to reduced SV[16, 17], however studies in which LV volumes were measured during exercise stress remain equivocal. Khouri *et al.*[16] reported normal LVEF, but were underpowered to detect subtle differences in LV volumes, while Koelwyn *et al.*[19] reported a blunted LVEF reserve secondary to impaired ventricular-arterial coupling during sub-maximal exercise. Further, the impaired ventricular-arterial coupling is consistent with studies reporting increased myocardial extracellular volume fraction and fibrosis following anthracycline therapy. However, there is evidence to suggest underlying differences in myocardial tissue characteristics prior to therapy.[20] There remains the possibility of reduced peak Q prior to anthracycline therapy, suggesting underlying structural and/or functional cardiac differences even in the absence of a cardiotoxic stimuli.

1.1 c) ii: Non-Cardiac Determinants of impaired VO_{2peak}:

Non-cardiac peripheral abnormalities, may result in decreased oxygen delivery to and or extraction by the active muscles during exercise. The volume of oxygen in a given volume of arterial blood is described by the equation:

$$CaO_2 = (SaO_2\%) \times [Hb\ g/dl] \times 1.34\ ml\ O_2/g\ Hb \times blood\ volume$$

Where SaO_2 is arterial oxygen saturation, Hb is hemoglobin, and 1.34 is a constant for the oxygen carrying capacity of hemoglobin.

During peak exercise, there is no evidence to indicate reduced SaO_2 in the absence of overt pulmonary disease in BC. Although reduced pulmonary function is reported in BC and linked to exercise capacity, reductions in pulmonary function do not contribute to reduced arterial oxygen content.[18]

Anemia is a well-known consequence of anthracycline therapy[22, 23], and can directly lower the oxygen carrying capacity of blood. Reductions in hemoglobin are related to the change in $VO_{2\ peak}$ over the course of chemotherapy.[24] In a 3-arm study comparing usual care (UC, n=82), aerobic exercise training (AET, n= 78) and resistance exercise training (RET, n=82), exercise did not attenuate declines in hemoglobin, but did preserve $VO_{2\ peak}$.[24] In another study, anemic cancer patients, including a subset of BC patients, were administered darbepoetin, a synthetic stimulator of red blood cell production, with (n=26) or without (n=29) concomitant AET. Peak oxygen uptake significantly improved in the AET group, and at study end, hemoglobin trended to increase greater in the darbepoetin alone group (123.4±12.8 vs 120.6±16.3 g/l, p=0.067).[25] Taken together, these data indicate a relationship between reduced oxygen carrying capacity of blood and VO_{2peak}, but do not support reduced oxygen carry capacity of blood as a limiting determinant of exercise capacity. Further complicating the role of anthracycline induced anemia are the findings by Kirshner *et al.* who reported that 31.3% of BC patients had anemia prior to receiving chemotherapy[22], a finding that suggests the possibility of impaired $VO_{2\ peak}$ in the absence of a cardiotoxic and anemic stimulus.

Given that CaO_2 and SaO_2 remain constant during exercise, a reduction in venous oxygen saturation (SvO_2%) drives increased oxygen extraction. SvO_2% is a composite term dictated by the Fick principle of diffusion:

$$\text{Rate of Diffusion} = k \times A(P_2 - P_1)/D$$

Where k is a diffusion constant dependent upon solubility of the gas and temperature, A is the surface area of gas exchange, $(P_2 - P_1)$ is the diffusion gradient (partial pressure in mmHg) and D is the diffusion distance.

Currently, no information is available directly linking perturbations involving factors governing oxygen diffusion and VO_{2peak} in anthracycline treated BC survivors. However, during exercise, the predominant source of increased oxygen demand is in the active skeletal muscle.[26] Accordingly, VO_2 is determined by the mass of skeletal muscle that is active, the ability to deliver oxygen to the muscle, and the ability of the muscle to utilize oxygen. In health, large muscle mass exercise (>5-6 kg) is limited by oxygen delivery[26], however, whether this is the case in BC remains unknown.

In a seminal study by Demark-Wahnefried *et al.*, BC patients receiving chemotherapy (n=36, age: 41 years) were shown to gain weight, with a concomitant drop in skeletal muscle mass from pre-to-post-therapy.[27] This finding was linked to a ~25-75 kcal/day reduction in physical activity energy expenditure, without a compensatory adjustment in caloric intake. Over the course of one year (study end), BC patients treated with chemotherapy increased their fat mass by 2.3 kg, and decreased their lean body mass by 0.5 kg, with lean body mass loss predominantly localized to the thigh. While this study did not measure VO_{2peak}, findings may be inferred; absolute VO_2 may decline as a result of decreased muscle mass, and relative VO_2 would

decline due to a two-fold hit- increased fat mass and decreased active skeletal muscle mass.[27] Indeed, therapy associated weight gain is a near ubiquitous finding, with a recent meta-analysis demonstrating a pooled mean gain of 2.7 kg (95% CI: 2.0-3.3 kg) across 34 studies and 2620 BC patients; increased adiposity is likely to be a contributing factor to reduced oxygen uptake in BC.[28]

Reding *et al.* provided the first evidence of a link between anthracycline related changes in body composition and declines in VO_{peak}.[29] In a cross sectional study, 14 cancer survivors (age: 54 years, 8 BC, >1 year post anthracycline therapy) and 14 sex- age- and BMI-matched controls underwent magnetic resonance imaging (MRI) to characterize intermuscular fat (IMF) and skeletal muscle (SM) in the paraspinal skeletal muscles at the level of the second lumbar vertebra while cardiopulmonary exercise testing was performed to determine $VO_{2\ peak}$. Survivors had 22% lower VO_{2peak}, and trended to have greater paraspinal IMF (BC 13.9 ± 5.6 vs HC 11.7 ± 4.3 cm^2, p=0.11) and IMF to SM ratio (BC 0.26 ± 0.10 vs HC 0.22 ± 0.10, p=0.13). In both groups combined, $VO_{2\ peak}$ was strongly and inversely related to the IMF:SM ratio (r -0.67, p<0.01). Correlations persisted after adjustment for subcutaneous and visceral fat depots, implicating a key role of IMF in reduced exercise capacity.[29] The authors speculate that IMF competes for blood flow and oxygen delivery to active muscle[29], as has been proposed as a mechanism of exercise intolerance in HF with preserved ejection fraction.[30] Despite the strong relationships reported by Reding *et al.*, caution must be warranted as the paraspinal muscles play a minimal role in VO_2 compared to major locomotor muscles during peak whole body exercise.[29]

Mijwel *et al.* extend findings of skeletal muscle loss from pre-to post-BC chemotherapy by measuring muscle fiber size and type, capillary to fiber ratio, and fiber oxidative capacity.[31] In a sample of 10 BC patients, muscle fiber cross-sectional area decreased, as well as the capillary

to fiber ratio and citrate synthase activity- the rate limiting enzyme in oxidative metabolism. Markers of muscle atrophy were not increased, while markers of mitophagy were reduced, a possible indicator of dysregulated quality control of mitochondria production. These changes may contribute to a decrease in surface area available for oxygen exchange, reduced oxygen use and therefore diffusion gradient, and potentially mixed effects on diffusion distance. Reduced fiber cross sectional area would reduce diffusion distance from the cell membrane to mitochondria, however, given fewer capillaries per muscle fiber, the diffusion distance from capillary to mitochondria may be increased. In the same study, 13 BC patients underwent exercise training which prevented a decrease in muscle fiber cross sectional area, capillary to fiber ratio, and citrate synthase activity.[31] How these findings translate to whole body VO_2 is unclear beyond skeletal muscle mass loss contributing to reduced $VO_{2\ peak}$ as previously discussed, however, reductions in muscle fiber oxidative capacity (citrate synthase activity) could contribute to reduced whole body $VO_{2\ peak}$ under the condition of adequate muscle oxygen supply at peak exercise.

Muscle oxygen supply is determined by ability to deliver Q to active skeletal muscle. Previously, vascular dysfunction has been hypothesized as a mechanism contributing to impaired $VO_{2\ peak}$ and elevated CV risk in BC, as vascular dysfunction is considered an incipient step in the pathogenesis of CVD.[32] An early study reported that over the course of anthracycline based chemotherapy, vascular function measured as brachial artery flow mediated dilation (FMD) in response to cuff ischemia did not decline (n=20, mean age=49).[33] Results were later confirmed in a larger sample (n=35), and in line with this finding, two, independent studies report no difference in FMD between BC survivors (n=47, 30) and healthy controls (n=11, 30).[17, 19, 34] Further, brachial artery function was not related to peak VO_2.[17] Taken together, evidence to date

suggests a minimal role of vascular (dys)function in limiting $VO_{2\ peak}$ in anthracycline treated BC survivors.

Vascular stiffening has also been proposed as a mechanism contributing to reduced oxygen delivery (through impaired peak Q) and impaired exercise tolerance. In a case-control study of cancer patients (BC n=19, lymphoma n=11, leukemia n=10) scheduled to receive anthracycline based chemotherapy and control participants (n=13), aortic distensibility (relative change in cross-sectional area of the artery normalized to pulse pressure) and pulse wave velocity (PWV, pressure propagation speed) were measured as indicators of aortic stiffness.[35] From pre to post therapy (4 months), PWV increased by 95%, with no corresponding change over the same duration in the control group. Aortic distensibility consistently mirrored PWV findings as distensibility dropped by more than 50% in cancer patients, and remained constant in controls.[35] In a follow-up study, Drafts *et al.* replicated PWV findings and provided time-course information in 53 cancer patients (BC n=22, lymphoma n=17, leukemia n=13, myelodysplastic syndrome n=1) undergoing anthracycline based chemotherapy.[36] Pulse wave velocity was measured prior to the start of therapy, and at 1, 3 and 6 months; PWV increased steadily from baseline (6.7±0.5) to 6 months (10.1±1 m/s). In contrast, a recent study of 35 BC patients found that over the course of anthracycline based therapy carotid-femoral, carotid-femoral PWV decreased (16.7±11.8 to 14.9±8.4 m/s, *n.s.*), while aortic compliance (assessed by ultrasound) did not change (0.061±0.04 to 0.046±0.03 mm/mmHg, *n.s.*).[34] Taken together, these findings indicate potential structural vascular involvement in the determination of $VO_{2\ peak}$. Despite mixed longitudinal findings, it should be noted that prior to anthracycline based chemotherapy, studies consistently report aberrant vascular stiffness, suggesting underlying structural vascular changes

independent of potentially vascular-toxic anthracycline therapy.[34-36] Whether increased vascular stiffness limits blood flow to working muscle and potentially impairs $VO_{2\ peak}$ was not addressed.

To date, only one study has investigated the ability to deliver blood to working muscle.[37] Small muscle mass exercise can be used as a paradigm to isolate ability to deliver blood to active muscle, as metabolic demands of small muscle mass exercise do not approach the limits of peak Q.[38] In a mixed cohort of cancer survivors (n=11, BC n=10) treated with adjuvant therapy (anthracycline based n=4), ultrasound was used to assess forearm blood flow in response rhythmic handgrip exercise at 20% of maximal voluntary contraction.[37] Cancer survivors achieved significantly lower forearm blood flow and mean arterial pressure during exercise, with no difference in forearm vascular conductance between groups. Despite using a paradigm designed to remove the heart as a limiting component of exercising blood flow, the authors attribute the finding of reduced forearm blood flow to an impaired blood pressure response, secondary to reduced LV ejection time (derived from the arterial pressure waveform, a measure of ventricular systolic performance).[37] The blunted forearm blood flow response could not be attributed to different relative or absolute workloads, raising the possibility that given the same absolute workload and oxygen demand for the given workload, lower forearm blood flow must be compensated for by increased oxygen extraction. In the context of whole body VO_2, the same effect would imply preserved VO_{2peak} despite any cardiac or vascular perturbations associated with BC.

1.1 d) Statement of the problem:

The pathophysiology of exercise intolerance in anthracycline treated BC is not well understood. Anthracyclines have clearly defined cardiotoxicity, however, only a small portion of anthracycline treated BC survivors have evidence of subclinical cardiac dysfunction at rest, and

the sparse literature available leaves the possibility of underlying cardiac impairment open.[11, 16, 17] Further, the paucity of studies that have examined isolated (cardiac, vascular and skeletal muscle function) components of the oxygen cascade have not examined components during maximal exercise.

The following studies aim to 1) develop a clinically viable MRI exercise stress imaging technique to assess cardiac function during exercise (Chapter 2); 2) examine exercising cardiac function in BC patients prior to anthracycline therapy as a determinant of $VO_{2\ peak}$ (Chapter 3); 3) examine peripheral hemodynamic responses during submaximal single leg knee extension (SLKE) exercise in BC survivors and controls (Chapter 4); and 4) examine muscle composition, lower leg blood flow, oxygen utilization and bioenergetics as potential factors contributing to exercise intolerance in BC survivors (Chapters 5). Taken together, these studies aim to elucidate pathophysiological mechanisms underpinning reduced cardiorespiratory fitness in anthracycline treated BC.

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Chapter 2

2.1 Exercise Cardiac Magnetic Resonance Imaging: A feasibility study and Meta-Analysis.

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2.1 a) Abstract

Cardiac stress testing improves detection and risk assessment of heart disease. Magnetic resonance imaging (MRI) is the clinical gold-standard for assessing cardiac morphology and function at rest; however, exercise MRI has not been widely adapted for cardiac assessment due to imaging and device limitations. Commercially available MR ergometers, together with improved imaging sequences, have overcome many previous limitations, making cardiac stress MRI more feasible. Here, we aimed to demonstrate clinical feasibility, and establish the normative, healthy response to supine exercise MRI. Eight young, healthy subjects, underwent rest and exercise cinematic imaging to measure left ventricular volumes and ejection fraction. To establish the normative, healthy response to exercise MRI we performed a comprehensive literature review and meta-analysis of existing exercise cardiac MRI studies. Results were pooled using a random effects model to define the left ventricular ejection fraction, end-diastolic, end-systolic, and stroke volume responses. Our proof-of-concept data showed a marked increase in cardiac index with exercise, secondary to an increase in both heart rate and stroke volume. The change in stroke volume was driven by a reduction in end-systolic volume, with no change in end-diastolic volume. These findings were entirely consistent with 17 previous exercise MRI studies (226 individual records), despite differences in imaging approach, ergometer, or exercise type. Taken together, the data herein demonstrate that exercise cardiac MRI is clinically feasible, using commercially available exercise equipment and vendor-provided product sequences, and establish the normative, healthy response to exercise MRI.

2.1 b) New & Noteworthy:

Cardiac stress testing improves detection and risk assessment of heart disease; however, exercise MRI has not been widely adapted for cardiac assessment due to imaging and device limitations. Here, we demonstrate clinical feasibility of cardiac stress MRI using a commercially available MRI compatible ergometer and vendor provided product sequences. Moreover, by performing a comprehensive literature review and meta-analysis of existing exercise cardiac MRI studies, we establish the normative, healthy response to supine exercise MRI.

2.1 c) Introduction

Exercise stress testing provides important prognostic information for assessing cardiovascular risk and detection of heart disease [39-45]. Combining exercise stress testing with cardiac imaging, such as echocardiography, nuclear imaging or positron emission tomography, significantly improves diagnostic sensitivity and specificity [46-50]. Accordingly, exercise stress imaging is commonly performed world-wide [51-55].

Cardiac magnetic resonance imaging (cMRI) is the clinical gold-standard for assessing cardiac morphology and global systolic function [56-58]. Despite its excellent spatial resolution, and unique potential for additional structural and functional quantification (e.g. fibrosis assessment and/or myocardial energetics), cMRI is not currently utilized for cardiac exercise stress imaging. While many factors may contribute to the underutilization of stress cMRI, the general lack of MR compatible exercise equipment and inadequate imaging sequences likely play a major role [59].

To overcome these limitations, several investigators have advocated exercising outside of the MRI followed by a brief transition to the scan table and subsequent imaging [60-68]. Under this paradigm, with much familiarization and the use of innovative body molds (to ensure anatomical image registration), image acquisition can be performed within 60 seconds of peak exercise [63]. Marked hemodynamic recovery during the transition from exercise to imaging [60-63, 67, 68], and the technical training and familiarization required of both the patient and imaging staff to achieve rapid and accurate body placement, has prevented wide spread adoption of this approach. Accordingly, several investigators have focused on MRI compatible cycle ergometers, which allow patients to exercise inside the bore of the magnet [69-78]. While several studies have

evaluated cardiac output dynamics using velocity encoding approaches [70, 79-85], only a few studies have attempted to evaluate ventricular morphology and function [69, 73, 75, 77, 78, 86-94]; the vast majority of which have utilized a real-time, ungated free-breathing pulse sequence [73, 87-90, 94]. While this latter approach increases overall feasibility by eliminating ECG-gating artifacts, patient breath-holds, and minimizes the impact of chest movement, post-processing is incredibly complex and time consuming, negating the possibility of "real-time" image reconstruction during the cardiac stress test (and thus real-time physician feedback). Such limitations have also prevented the wide-spread adoption of this approach.

With this background, and the goal of making exercise cMRI clinically translatable, the primary aim of this study was to test the feasibility of using a commercially available MR compatible ergometer, combined with vendor provided MR imaging sequences, to assess cardiac morphology and function. To assess the impact of movement on image quality, we compared images acquired during exercise, with images acquired immediately (0-4 seconds) post-exercise. To assess the impact of a prolonged pause between exercise and imaging—similar to that encountered when exercise is performed outside of the bore of the MRI— we compared subject hemodynamics, cardiac morphology and cardiac function between exercise and following a 60-second pause. A secondary aim of this study was to perform a comprehensive literature review and meta-analysis of published studies reporting cardiac volumes during exercise cMRI to establish the normative response.

2.1 d) Methods

2.1 d) i: Participants

Eight recreationally active, young, healthy subjects were recruited from the Dallas-Fort Worth community. Participants were eligible for inclusion if they were 18–35 years old, physically active, and had no history of cardiovascular, metabolic or neurological disease. Exclusion criteria included: contraindications to MRI and physical limitations precluding exercise.

All procedures were performed in accordance with the principles of the Helsinki declaration; all participants provided written informed consent and the study was approved by the University of Texas at Arlington and University of Texas Southwestern Medical Center Institutional Review Boards.

2.1 d) ii: Protocol

To address our primary aim, each subject completed three visits. The first visit was used for screening, familiarization with the MRI compatible ergometer (Ergospect Cardio-Stepper, Ergospect, Austria), and determination of target workload and heart rate for exercise cMRI. The second visit was used to characterize the metabolic cost and hemodynamic differences between supine exercise with our MR compatible ergometer and upright exercise with a traditional cycle ergometer (Lode Corival, Lode, Netherlands). The third visit consisted of a resting and exercise stress cMRI.

Supine Cardiopulmonary Exercise Test: Subjects underwent a supine, incremental maximal exercise test, starting at 15 watts at a cadence of 50 revolutions per minute (RPM) and progressing by 15 watts every 2 minutes until volitional exhaustion or significant and continued loss of cadence ($\geq$10 RPM despite verbal encouragement to continue). Heart rate (Polar H1,

Polar, USA), expired gas analysis (TrueOne 2400, Parvomedics, USA) and work-rate (Ergospect, Cardio-Stepper, Ergospect Austria) were recorded continuously.

Upright Cardiopulmonary Exercise Test: Subjects underwent an incremental ramped maximal exercise test, starting at 50 watts at a self-selected cadence (50-90 RPM) and progressing by 20 watts every 2 minutes until volitional exhaustion. Heart rate (Polar H1, Polar, USA), expired gas analysis (TrueOne 2400, Parvomedics, USA) and work-rate (Lode Corival, Lode, Netherlands) were recorded continuously.

Cardiac MRI: Imaging was conducted on a Phillips Achieva 3T scanner. Following resting imaging, subjects exercised at the workload, determined from study visit 1, required to achieve 4 metabolic equivalents (METs, 4 METs ≈ 14 ml/kg/min); the minimal threshold for independent living [95]. High resolution long-axis (4-chamber and 2 chamber) and short axis (mid-ventricular, papillary muscle level) cine images were acquired using balanced fast field echo at rest, during exercise, immediately (0-4 seconds) post-exercise, and 60-seconds post-exercise (to mimic patient transfer from an external exercise device). Typical image parameters include: TR = 3.4 msec; TE = 1.7 msec; flip angle = 45º; acquisition matrix = 195 x 195; field of view = 295 x 295 mm; 8-mm slice thickness; 20-30 phases/cardiac cycle; with one slice acquired per breath hold. Breath-hold time ranged between 7-9 seconds at rest and 3-5 seconds during exercise.

Cardiac MRI data were analyzed using commercially available software (CVI[42], Circle Cardiovascular Imaging Inc., Canada). LV volumes were calculated by Simpson's Biplane method using standard 4 and 2-chamber slices, and cross-sectional area was measured in the short axis plane. All volumes were indexed to body surface area; calculated by the Dubois-Dubois formula, to account for body size variation [96].

2.1 d) iii: Comprehensive Literature Review

To accomplish our secondary aim, we performed a comprehensive literature review, searching articles on PubMed from 1985 to March 2018, using the search terms "exercise AND cardiac MRI", hand searched references, and contacted experts in the field. *A priori,* we included studies reporting left-ventricular cardiac volumes for healthy controls aged 16-35, to best match our participant inclusion criteria. We extracted mean resting and exercise end-diastolic, end-systolic and stroke volumes, heart rates, and ejection fractions from study text, tables and figures (WebPlotDigitizer, V 4.1, Rohatgi, USA). If data were not readily extractable, authors were contacted directly and asked to provide the missing or unattainable data.

2.1 d) iv: Statistical Analysis

Aim 1 – Feasibility Study: Dependent variables were confirmed for normality and homoscedasticity using the Shapiro-Wilk test. Changes in cardiac volumes and global left ventricular function from rest to exercise/post-exercise were assessed using a one-way repeated measures ANOVA with Sidak *post hoc* testing. Hemodynamic and cardiopulmonary data were compared between the supine and upright incremental exercise tests using a paired sample t-test. Statistical analyses were performed using SPSS (version 24 IBM SPSS Statistics, Armonk, USA). All data are expressed as mean (standard deviation) unless otherwise stated, and statistical significance was considered when $P \leq 0.05$.

Aim 2 – Comprehensive Literature Review: Previously published results from the literature review were pooled and analyzed by a random-effect analysis to create a single, more precise estimate of the effect size. The rest/exercise correlation was assumed to be moderate (50%) [97]. Analysis and graphical presentation were performed in R 3.4.2 using the "metaphor"

package (R Core Team (2016), R Foundation for Statistical Computing, Vienna, Austria). Effect sizes were calculated using both Standardized Mean Differences (SMD) and Weighted Mean Differences (WMD) between rest and exercise conditions. A SMD of 0.25 was considered as a small effect size, 0.5 as a medium effect size, and 0.8 and higher as a large effect size. Heterogeneity of studies was explored using the Cochrane's Q test of heterogeneity ($P < 0.05$ was considered statistically significant). Inconsistency in the results of the studies was assessed by I^2 which described the percentage of total variation across studies that was due to heterogeneity. When I^2 was $\geq$ 50%, there was more than moderate inconsistency [98, 99].

2.1 e) Results

2.1 e) i: Aim 1 – Feasibility Study

Eight healthy men and women completed the feasibility study (M/F, 3/5; age, 25 $\pm$ 3 years; BMI, 22.3 $\pm$ 2.7 kg/m^2; BSA, 1.62 $\pm$ 0.14 m^2).

Supine vs. Upright Exercise: The metabolic and hemodynamic data for both the supine and upright cardiopulmonary stress tests are displayed in **Table 1**. Peak oxygen consumption was markedly lower during supine exercise compared to upright cycling, reflective of the smaller muscle mass being utilized with the MR compatible ergometer. Similarly, peak heart rate, peak respiratory exchange ratio, and peak ventilation were also markedly lower. None of the subjects met the criteria for true VO_{2max} during supine exercise, defined as achieving: 1) age predicted maximal heart rate; 2) respiratory exchange ratio > 1.10; or 3) a plateau in oxygen consumption. In contrast, all of the subjects during the upright cycling ramp test met at least two of these criteria. The primary reason for stopping the supine exercise test was an inability to overcome

the change in resistance; none of the subjects reported maximal perceived exertion or breathlessness as a reason for stopping.

cMRI Feasibility: Cardiac imaging was possible during exercise in all of the subjects. None of the subjects reported any discomfort or experienced any adverse events. As expected, image quality was improved post-exercise (0-4 second, 60 seconds), when upper body and chest coil movement were eliminated (**Figure 1**).

Both heart rate and stroke volume increased with exercise, resulting in a 2-fold increase in cardiac index (CI) (**Figure 1**). The increase in stroke volume was mediated entirely by a reduction in end-systolic volume (ESV), as end-diastolic volume (EDV) was unchanged with exercise. Accordingly, ejection fraction was also significantly increased with exercise.

As compared to imaging during exercise, imaging immediately post-exercise had no significant impact on chamber volumes, ejection fraction or cardiac index. In contrast, imaging 60-seconds post-exercise resulted in a significant increase in end-systolic volume, and a significant decrease in heart rate, ejection fraction, and cardiac index (**Figure 1**).

2.1 e) ii: Aim 2 - Literature Review and Data Synthesis

Our search produced 1787 articles; 17 studies reported rest and exercise cardiac volumes. Of the 17 studies, data were extracted from 14 studies (**Table 2)**. One study [72] reported median and interquartile range which could not be used in our synthesis. Two studies [77, 100] were confirmed to have used previously reported data sets and were therefore excluded. . When multiple exercise intensities were reported, the exercise intensity closest to that chosen in our feasibility study was reported.

Characteristics of the studies included in our comprehensive data synthesis are displayed in **Table 2**. The SMD model was used to account for variance in study participants (sex, age, BSA, exercise capacity) and volumetric reporting (standard versus normalized volumes). By combining these datasets, we were able to evaluate the normal physiological response to supine exercise in 226 healthy subjects. The pooled analysis support our proof-of-concept data (reported above), showing a marked and significant increase in ejection fraction during exercise compared to rest (**Figure 2 and Table 3)**. Moreover, we observed no change in left ventricular end-diastolic volume, with changes in stroke volume being entirely driven by reductions in end-systolic volume (**Figure 2 and Table 3)**. Similarly, the magnitude of change in cardiac index was therefore predominantly driven by changes in heart rate (**Table 2**). The Q test revealed significant variation in the exercise response to ejection fraction ($P = 0.004$), end-systolic volume ($P = 0.007$), and stroke volume ($P = 0.005$), although less than moderate ($I^2 = 41\%$, 49% and 27%). There was no heterogeneity in end-diastolic volume response (**Table 3A**).

Visual inspection of **Figure 2** prompted *post-hoc* analysis to determine if the Lafountain study (2016, treadmill exercise) was a statistical outlier. Based on our model, approximately 5% of the externally standardized residuals would be expected to exceed the bounds ±1.96. Externally standardized residuals were calculated for end-systolic volume, stroke volume and ejection fraction as -3.85, 1.92 and 12.24 respectively, indicating the study as a potential outlier. In a sensitivity analysis, removal of Lafountain 2016 from the meta-analysis increased Q test p-values (0.08, 0.05 and 0.26) and reduced I^2 values (38%, 42%, and 26%) for ESV, SV and EF, demonstrating a significant reduction in heterogeneity.

2.1 f) Discussion

The major novel findings of this study are three-fold: First, we demonstrate feasibility of exercise stress cMR using a commercially available leg ergometer, and vendor-provided product sequences at 3T. Second, we demonstrate hemodynamic similarities between imaging during and immediately post-exercise, but marked hemodynamic decline when imaging is delayed for at least 60-seconds post exercise. Finally, by combining our results with previously published data, we were able to define the normal cardiac response to exercise cMRI.

That cardiac imaging was feasible using a commercially available leg ergometer, and vendor-provided product sequences, provides great promise for easy and simple adoption of exercise cMRI in clinical settings. While we observed a marked improvement in image quality by imaging immediately post-exercise, we observed no major differences in cardiac volumes or global left ventricular function between these two conditions. Because inclusion of brief pauses will prolong the exercise stress imaging protocol, we believe imaging during exercise will prove to be the protocol of choice for most clinical sites. Of course, if exercise intensity is increased beyond the submaximal level performed herein, imaging during brief periods of exercise cessation may need to be adopted to overcome the anticipated upper body and chest coil movement. Indeed, as movement artifact increases with higher exercise intensities, spatial resolution may degrade despite consistent imaging parameters. Caution is indeed warranted when interpreting data acquired past 4-seconds post-exercise however, as our results suggest, prolonged periods of rest following exercise are associated with sharp hemodynamic recovery. While it may be argued that the rapid hemodynamic recovery observed in this study is reflective of the younger, healthy population studied herein, clinical patients exercised outside of scanner bore and transferred for imaging share a comparable decline in heart rate [63, 67].

Our feasibility data, together with our meta-analysis, demonstrate that the increase in cardiac output during submaximal supine exercise is driven predominantly by an increase in heart rate, with minimal contribution from stroke volume. The data overwhelmingly suggest that healthy subjects have no end-diastolic volume reserve during supine exercise, despite the expected increase in respiratory and muscle pumps. That this observation is consistent across studies which did not perform breath holds [68, 73, 87-90] argues against intrathoracic pressure (i.e. Valsalva maneuver) mediated reductions in preload. These data must therefore reflect the fact that supine positioning, combined with leg elevation (feet in the ergometer stirrups), produces near maximal end-diastolic volumes.

That we only observed a small reduction in end-systolic volume with exercise was surprising, though consistent with other cMRI studies in healthy populations [68, 69, 73, 75, 78, 86-94]. Admittedly, this investigation was focused on submaximal exercise, and thus we cannot extend these end-systolic volume findings to higher exercise intensities. Indeed, studies which included higher workloads have reported exercise intensity dependent reductions in end-systolic volume [72, 73, 87-90]. This inter-study difference likely contributed to the heterogeneity of findings in our meta-analysis for end-systolic volume, ejection fraction, and stroke volume. Regardless of exercise intensity however, the absolute change in end-systolic volume remains fairly low. This may reflect the fact that supine exercise with MR compatible ergometers may not recruit sufficient muscle mass to challenge cardiac output enough to demand greater end-systolic volume changes. Compared to conventional upright cycling, our data clearly show blunted metabolic demand; also observed in a prior investigation using a different MR compatible ergometer [72].

This proof-of-concept feasibility study is not without limitation. First, we limited our data collection to young healthy participants. Future studies are needed to extend these data to clinical populations. Second, spatial resolution was purposely sacrificed to shorten breath-hold time. As a result, the cine images were not sufficient for tissue deformation (i.e. strain) analysis. Inclusion of quantifiable regional wall motion measurements will be an important future addition, especially in clinical populations.

2.1 g) Conclusion

Taken together, the data herein establish the proof-of-concept that exercise cMRI can be easily adapted to a clinical setting, providing gold-standard non-invasive stress images during exercise. Using commercially available MRI compatible exercise equipment and vendor-provided product sequences, we were able to successfully characterize the cardiac response to submaximal exercise. By combining our feasibility data with previously published results, we also establish the normative cardiac stress response in over 200 healthy subjects. With this background in place, future studies are needed to adapt this approach in clinical populations.

2.1 h) References

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2.1 i) Figure Legends:

2.1 i) i: Figure 1:

A) Representative high resolution cMR images at rest (black circle), during exercise (yellow square), immediately post-exercise (0-4 seconds, green triangle) and 60 seconds post-exercise (red diamond). Left ventricular volumes were indexed to body surface area for determining LV end-diastolic **(B)**, end-systolic **(C)** and stroke volume **(D)** and multiplied by heart rate **(E)** to generate cardiac index **(I)**. LV cavity areas were measured from a mid-short-axis image (papillary muscle level), and indexed to body surface area, for determination of end-diastolic **(F)** and end-systolic **(G)** cavity areas. Ejection fraction **(H)** was calculated from the biplane volumes. Data presented as mean and standard error: † indicates a statistically significant ($p<0.05$) difference from rest, and ‡ from exercise.

2.1 i) ii: Figure 2:

Pooled standardized mean difference (exercise-rest, [95% confidence interval] for left ventricular end-diastolic **(A)**, end-systolic **(B)**, and stroke volume **(C)**, and ejection fraction **(D)** from the available exercise cMR studies. [1]Values taken from graphical representations at moderate, and [2]peak intensity exercise. [3]Adolescent populations, with [4]values taken from graphical representations.

2.1 j) Tables

2.1 j) i: ***Table 1:*** *Metabolic and hemodynamic comparison between supine and upright exercise.*

Parameter	Supine	Upright	Percent of upright	*P* value
VO_{2peak} (ml/kg/min)	26 (3)	39 (5)	67 (10)	0.0007
VO_{2peak} (l/min)	1.24 (0.35)	2.31 (0.53)	67 (10)	0.0004
$Ventilation_{peak}$, (l/min)	57 (15)	106 (24)	56 (13)	0.0003
RER_{peak}	1.07 (0.06)	1.23 (0.09)	87 (6)	0.0007
Heart rate (bpm)	153 (19)	188 (9)	82 (9)	0.0011

VO_{2peak}, peak oxygen consumption; RER, respiratory exchange ratio.

2.1 j) ii: ***Table 2:*** *Characteristics of exercise cMRI studies reporting LV volumes.*

Study	*n* (m/f)	Age	Resting HR	Image HR	Exercise Intensity	Field Strength	Exercise Type	Image Type
Present Study	3/5	25 (3)	67 (8)	115 (9)	Sub-Max	3.0T	Supine, Ergospect CardioStepper	Gated, breath-hold, SAX, Bi-plane LAX
Claessen 2014(a)[89]	14/0	36 (6)	66 (7)	143 (7)	Max*	1.5T	Supine, Lode MRI cycle	Real-time free-breathe SAX/LAX stacks
Claessen 2014(b)[88]	8/1	35 (12)	NR	NR	Max*			
Claessen 2015[90]	14/0	36 (15)	66 (7)	149 (11)	Max			
Gusso 2012[69]	4/4	25 (4)	67 (15)	110 (3)	Sub-Max	1.5T	Supine, Custom MRI cycle	Gated breath-hold SAX stack, 3 LAX
Gusso 2012[91] (2017)[100]	10/12	17 (1)	71 (9)	109 (5)	Sub-Max			
La Gerche 2012[73]	10/0	35 (9)	59 (14)	164 (10)	Max*	1.5T	Supine, Lode MRI cycle	Real-time free-breathe SAX/LAX stacks
LaFountain 2016[68]	5/5	26 (5)	62 (8)	166 (20)	Max*	1.5T	Upright, Custom MRI treadmill	Ungated, free-breathe SAX stack and biplane LAX slices
Lurz 2009[94]	5/7	33 (29-42)	74 (3)	154 (13)	Max*	1.5T	Supine, Lode MRI cycle, flutter kicking motion	Real-time free-breathe SAX stacks
Oosterhof 2005[78]	8/6	27 (4)	66 (8)	121 (8)	Sub-Max	1.5T	Supine, Custom MRI cycle	Turbo field echo planar imaging SAX stack
Pinto 2014[92]	10/9	17 (2)	70 (10)	109 (4)	Sub-Max	1.5T	Supine, Custom MRI cycle	Gated breath-hold SAX stack, 3 LAX
Roest 2001[86] (2002)[77]	8/8	18 (2)	71 (10)	121 (14)	Sub-Max	1.5T	Supine, Custom MRI ergometer	Gated breath-hold SAX stack
Roest 2004[93]	7/7	25 (5)	67 (8)	122 (8)	Sub-Max			
Schnell 2017(a)[87]	15/0	35 (8)	59 (12)	155 (18)	Max	1.5T	Supine, Lode MRI cycle	Real-time free-breathe SAX/LAX stacks
Schnell 2017(b)[87]	12/3	35 (14)	66 (7)	150 (12)	Max			
Steding-Ehrenborg 2013[75]	20/6	30 (8)	60 (11)	94 (13)	Sub-Max	1.5T	Supine, Custom MRI ergometer	Gated breath-hold SAX stack and LAX slice

HR, heart rate; NR, not reported; SAX, short axis; LAX, long axis. *Sub-maximal data used in meta-analysis, see Figure 2 legend.

2.1 j) iii: ***Table 3.*** *Effect size, significance, and heterogeneity for pooled analyses.*

A.	**Measure**	***n*** **(comparisons)**	**SMD [95% CI]**	**Significance (H_0:SMD = 0)**	**Q p-value**	**I^2 (%)**
	EDV	16	-0.14 [-0.31, 0.04]	0.13	1.00	0
	ESV	16	-1.18 [-1.42, -0.95]	<0.0001	0.005	41
	SV	16	0.87 [0.64, 1.09]	<0.0001	0.006	50
	EF	15	1.75 [1.48, 2.03]	<0.0001	0.006	26
B.			**WMD**	**(H_0:WMD = 0)**		
	EDV (ml)	11	-0.33 [-0.61, -0.06]	0.027	0.18	28
	ESV (ml)	11	-3.51 [-4.11, -2.91]	<0.0001	<0.0001	75
	SV (ml)	11	3.86 [3.07, 4.65]	<0.0001	<0.0001	91
	EF (%)	15	6.12 [5.26, 6.98]	<0.0001	0.005	95

SMD, standardized mean difference; WMD, weighted mean difference; 95% CI, 95% confidence intervals; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction. Note: SMD calculated by pooling EDV (ESV, SV) for studies reporting measures in ml, ml/m^2 (BSA normalized) and ml/kg (normalized to fat free mass). WMD calculated by pooling studies reporting EDV (ESV, SV) in ml.

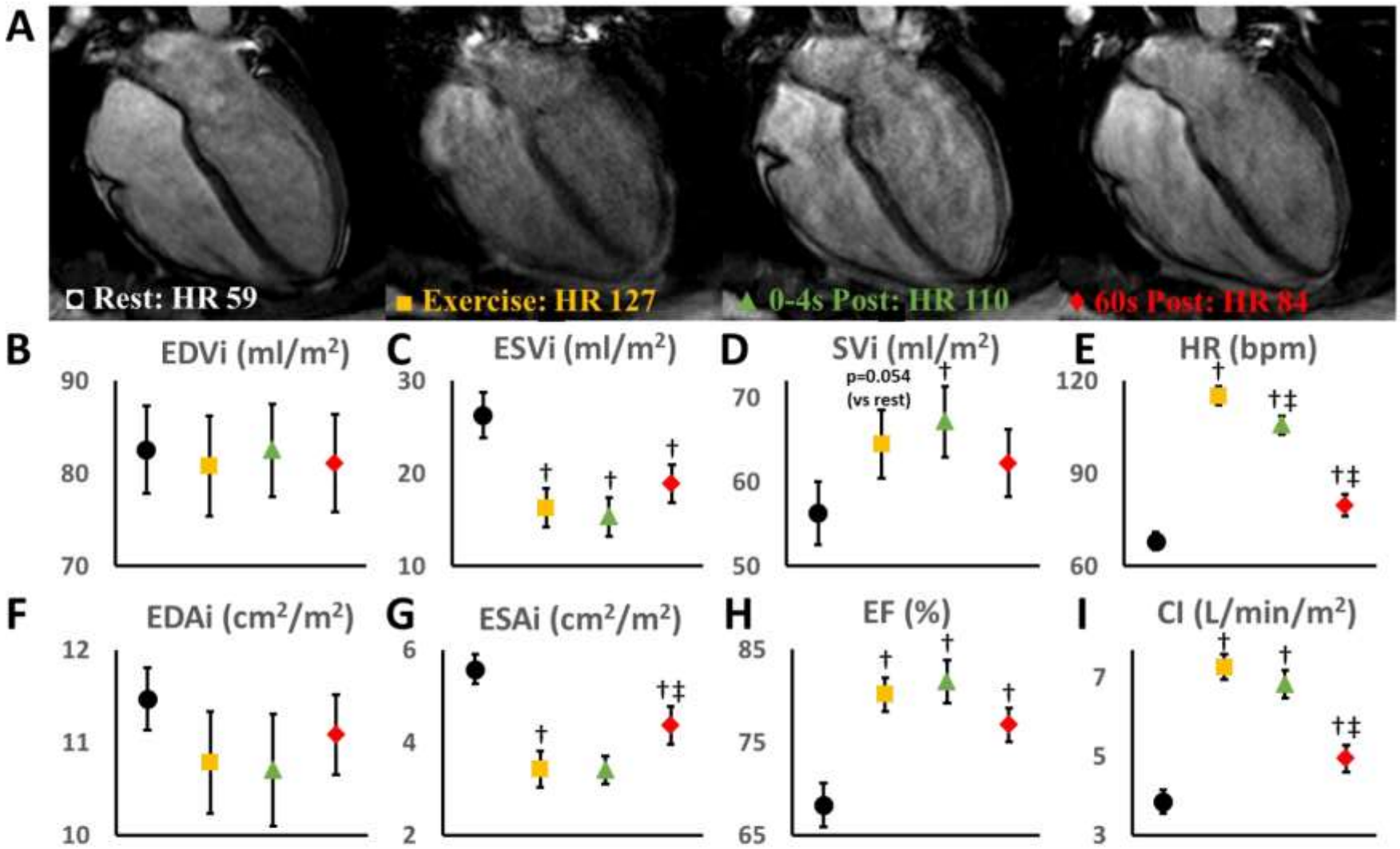

Figure 1: A) Representative high resolution cMR images at rest (black circle), during exercise (yellow square), immediately post-exercise (0-4 seconds, green triangle) and 60 seconds post-exercise (red diamond). Left ventricular volumes were indexed to body surface area for determining LV end-diastolic **(B)**, end-systolic **(C)** and stroke volume **(D)** and multiplied by heart rate **(E)** to generate cardiac index **(I)**. LV cavity areas were measured from a mid-short-axis image (papillary muscle level), and indexed to body surface area, for determination of end-diastolic **(F)** and end-systolic **(G)** cavity areas. Ejection fraction **(H)** was calculated from the biplane volumes. Data presented as mean and standard error: † indicates a statistically significant ($p<0.05$) difference from rest, and ‡ from exercise.

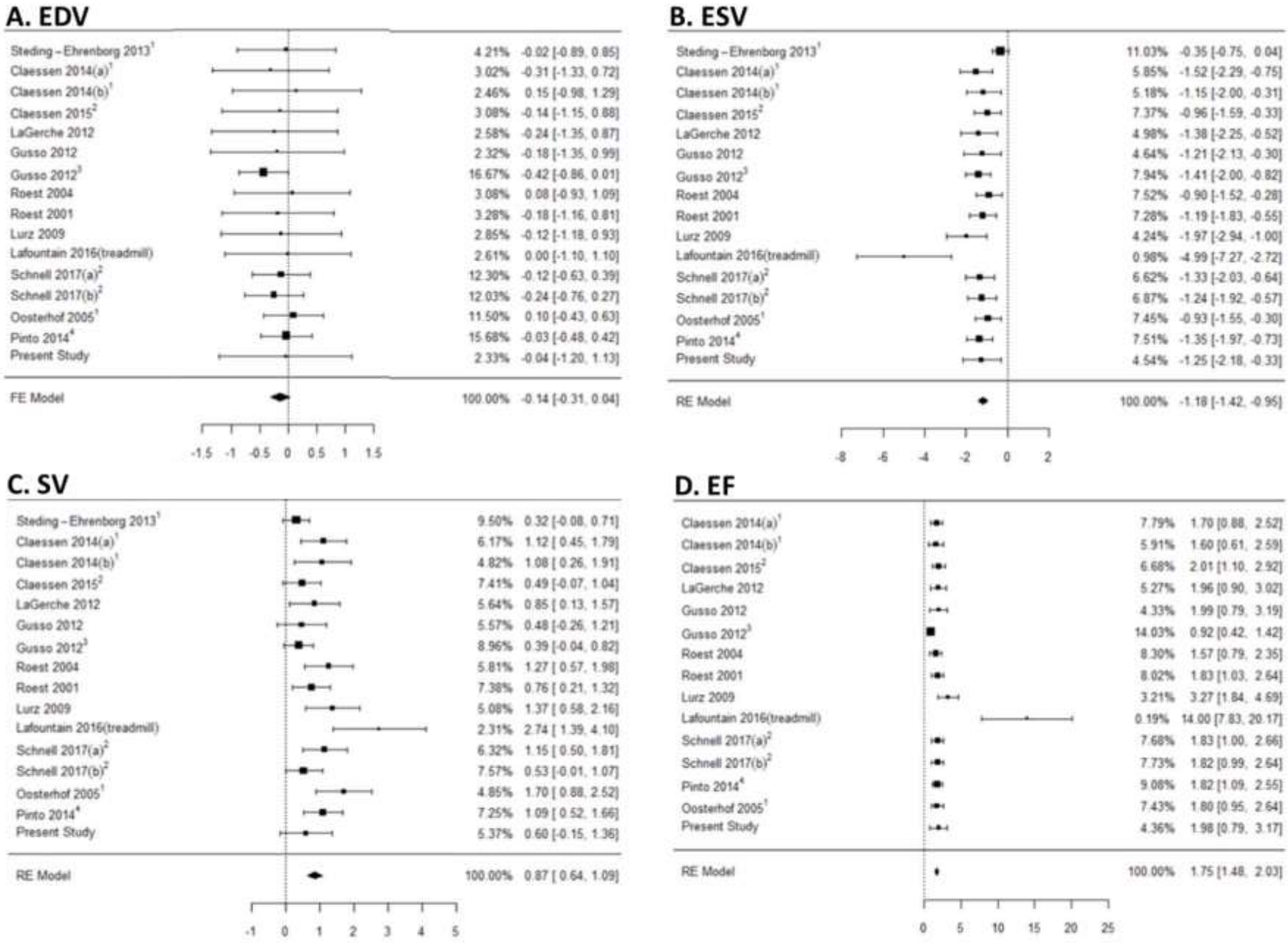

Figure 2: Pooled standardized mean difference (exercise-rest, [95% confidence interval] for left ventricular end-diastolic **(A)**, end-systolic **(B)**, and stroke volume **(C)**, and ejection fraction **(D)** from the available exercise cMR studies. [1]Values taken from graphical representations at moderate, and [2]peak intensity exercise. [3]Adolescent populations, with [4]values taken from graphical representations.

Chapter 3

3.1 Determinants of Exercise Intolerance in Breast Cancer Patients Prior to Anthracycline Chemotherapy

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3.1 a) Abstract

Background: Women with early stage breast cancer have reduced peak exercise oxygen uptake (peak VO_2). The purpose of this study was to evaluate peak VO_2 and right (RV) and left (LV) ventricular function prior to adjuvant chemotherapy.

Methods: Twenty-nine early stage breast cancer patients (mean age: 48 years) and 10 age-matched healthy women were studied. Participants performed an upright cycle exercise test with expired gas analysis to measure peak VO_2. RV and LV volumes and function were measured at rest, sub-maximal and peak supine cycle exercise using cardiac magnetic resonance imaging.

Results: Peak VO_2 was significantly lower in breast cancer patients versus controls (1.7±0.4 vs. 2.3±0.5 l/min, p=0.0013; 25±6 vs. 35±6 ml/kg/min, p=0.00009). No significant difference was found between groups for peak upright exercise heart rate (174±13 vs. 169±16 bpm, p=0.39). Rest, sub-maximal and peak exercise RV and LV end-diastolic and end-systolic volume index, stroke index, and cardiac index were significantly lower in breast cancer patients versus controls ($p<0.05$ for all). No significant difference was found between groups for rest and exercise RV and LV ejection fraction.

Conclusion: Despite preserved RV and LV ejection fraction, the decreased peak VO_2 in early stage breast cancer patients prior to adjuvant chemotherapy is due in part to decreased peak cardiac index secondary to reductions in RV and LV end-diastolic volumes.

3.1 b) Introduction

Breast cancer is the most frequently diagnosed malignancy among women and the second leading cause of cancer mortality in the United States.[101] During the past three decades, the breast cancer mortality rate has decreased by nearly 40% as a result of advances in prevention, early detection and treatment.[2] Despite improved survival, breast cancer survivors are at increased at risk of developing cardiovascular disease that is due, in part, to an unhealthy lifestyle associated with sedentary deconditioning.[102, 103 103-106]

Breast cancer patients have severely reduced exercise tolerance, measured objectively as decreased peak oxygen uptake (peak VO_2), that is associated with decreased quality of life and survival.[13, 17, 107, 108] Jones *et al.* reported that peak VO_2 was 27% lower in breast cancer survivors compared to age and sex-matched sedentary predicted values.[13] Moreover, nearly one-third of survivors had a peak VO_2 below the threshold of independent living.[13] The marked exercise intolerance has been linked to a reduced peak exercise cardiac output secondary to a reduced stroke volume, as peak heart rate (HR) was not different between breast cancer survivors and controls.[17]

It is currently not known whether women with reduced exercise capacity are predisposed to breast cancer or whether exercise intolerance is acquired as a result of cancer therapy. There is a paucity of studies that have measured peak VO_2 in breast cancer patients prior to undergoing adjuvant chemotherapy. A systematic review by Peel *et al.* reported that peak VO_2 was 17% lower in breast cancer patients prior to adjuvant therapy compared to age-matched healthy sedentary predicted values.[109] To date, no study has measured exercise cardiac function in breast cancer patients prior to undergoing chemotherapy. Further, all prior studies have focused

primarily on left ventricular (LV) function, therefore uncertainty remains regarding right ventricular (RV) performance during exercise. Given this uncertainty, the aim of this study was to test the hypothesis that the impaired peak VO_2 in breast cancer patients prior to adjuvant chemotherapy is attributable to decreased exercise cardiac output despite preserved RV and LV ejection fraction.

3.1 c) Methods

3.1 c) i: Subjects

Women aged 18 to 70 years with newly diagnosed, histologically confirmed early stage breast cancer scheduled for anthracycline-based chemotherapy were recruited via collaborating breast surgeons and oncologists from local hospitals in the Melbourne metropolitan area. Patients with structural heart disease, sustained cardiac arrhythmias, or a contraindication to cardiac magnetic resonance imaging were excluded. Age and sex matched control subjects were recruited from an advertisement to staff of the Baker Heart and Diabetes Institute and the wider community. Volunteers involved in competitive sport or enrolled in a structured exercise training program were excluded.

3.1 c) ii: Protocol overview

The study protocol was approved by the Alfred Health Institutional Review Board and written informed consent was obtained from all subjects. Breast cancer patients performed all tests prior to anthracycline treatment.

3.1 c) iii: Cardiopulmonary Exercise Testing

Incremental exercise testing was performed on an electronically braked upright cycle ergometer (Lode, Gronigen, Netherlands) with an initial power output of 10-25 watts (W) and was progressed by 10-30W per minute until volitional exhaustion. Expired gas analysis was performed using a commercially available metabolic measurement system (True One 2400, Parvomedics, UT, USA), with the highest value obtained over 30 seconds used as the peak VO_2 value. HR was continuously measured (1200W RF Wireless System, Norav, FL, USA) while blood pressure (Suntech, Tango, NC, USA) was measured every 2 minutes during exercise.

3.1 c) iv: Biochemistry

Peripheral venous blood samples were taken 10 minutes after completion of exercise cardiac magnetic resonance (cMRI) testing to measure B-type natriuretic peptide (BNP) and troponin I. Hemoglobin values were obtained from patient medical records.

3.1 c) v: Rest and exercise cardiac magnetic resonance imaging cMRI.

Cardiac images were acquired with a Siemens MAGNETOM Prisma 3T scanner with a 5-element phased-array coil (Siemens, MAGNETOM Prisma). Ungated, real-time, steady-state free-precession cine imaging was performed without cardiac/respiratory gating. Forty to 75 consecutive frames were acquired every 36-38 milliseconds at each of 13-18 contiguous 8-mm slices in the short-axis (SAX) plane, and 50 consecutive frames were acquired at approximately the same temporal resolution for 11-15 contiguous 8-mm slices in the horizontal long axis (HLA) plane. Scan duration was approximated to include one full respiratory cycle per slice (~120 and

~90 seconds at rest for SAX and HLA planes respectively, ~4-7 heart beats/slice) for gating of cardiac translation.[73]

RV and LV volumes were generated by manually tracing the endocardium in the SAX plane and using the disk summation method.[73] To minimize cardiac translation error, contours were traced in end diastole and end systole for each slice manually gated to respiration. SAX contour transection points with the HLA plane were displayed for referencing of the atrioventricular valve plane. Trabeculations and papillary muscles were considered part of the ventricular blood pools. HR was determined by generating an anatomical M-mode image from the SAX plane stack. Cardiac index was calculated as the average of RV and LV stroke volume (index to body surface area) multiplied by HR. Physiologic data was synchronized with images for offline data analysis. Images were analyzed with RightVol (Right Volume Leuven, Leuven, Belgium), a MatLab software adjunct.

After resting images were obtained, subjects cycled on a MRI compatible ergometer (MR Ergometer Pedal, Lode, Groningen, Netherlands) at an intensity equal to 20%, 40% and 60% of peak power output obtained during the upright incremental cycle exercise test. These workloads will subsequently be referred to as rest and low, moderate, and peak intensity. Each stage of exercise was maintained for ≈1 to 3 minutes; 30 seconds to 1 minute to achieve a physiological steady-state and 1 to 2 minutes for image acquisition. We previously determined that 66% of the peak power during upright cycling corresponded to peak exercise in a supine position.[86]

3.1 c) vi: Statistical Analysis

Comparison between groups for continuous variables was assessed using student *t* tests. Pearson correlation was used to determine linear correlations between variables. Two-Way ANOVA with post hoc Bonferroni testing was used to compare cardiac volumes and ejection fraction at different exercise intensities. Unless otherwise stated, data are presented as mean (standard deviation). A two-sided p value <0.05 was determined as significant. All statistical analysis were performed with SPSS (version 24 IMB SPSS Statistics, Armonk, NY, USA) statistics software.

3.1 d) Results

3.1 d) i: Participant characteristics

Twenty-nine women with early breast cancer and 10 age and sex-matched healthy controls agreed to participate in this study. No significant difference was found between breast cancer patients and healthy controls for age, height, weight, body mass index, body surface area, hemoglobin, brain natriuretic peptide, or troponin I. However, body mass index tended to be higher in the breast cancer group, as fewer healthy control subjects were overweight, and none were obese (Table 1). Fourteen and fifteen breast cancer patients were scheduled to receive anthracyclines in a neoadjuvant and adjuvant setting respectively.

Cardiopulmonary exercise performance during upright cycle exercise

Peak exercise power output and VO_2 were significantly lower in breast cancer patients compared to healthy controls (38 ± 21% and 29 ± 17% lower respectively, Table 2). Peak exercise systolic and diastolic blood pressure and HR were not significantly different between groups. A sensitivity analysis was conducted to address the potential confounder of surgery prior

peak VO_2 testing. Fifteen breast cancer patients who had undergone breast surgery (local resection or mastectomy, scheduled for adjuvant chemotherapy) were compared with the 14 patients planned for surgery after chemotherapy (neo-adjuvant treatment strategy). No significant difference was found between these groups for age, BMI, BSA, peak power output, VO_2 (L/min or ml/kg/min) or cardiac output (Data supplement).

3.1 d) ii: Cardiac volumes and index at rest, sub-maximal and peak supine cycle exercise

Complete cMRI data was obtained in 26 of 29 breast cancer patients (one subject withdrew from the study prior to cMRI testing, HR could not be measured accurately during peak exercise in one subject- volumes, but not peak cardiac output, are included- one image set was excluded due to artifact) and 10 healthy controls.

No significant difference was found between groups for rest, sub-maximal and maximal RV and LV ejection fraction. A significant main group effect was found for RV and LV end-diastolic volume index, end-systolic volume index, stroke index and cardiac index with values being lower in breast cancer patients compared to controls (Figures 1 to 3). A significant main (exercise) intensity effect was found for RV and LV stroke index and ejection fraction being higher while RV and LV end-systolic volume index were lower during exercise compared to rest in both breast cancer and controls (Figures 1-3). A significant group by intensity interaction was found for cardiac index with breast cancer patients having lower values during sub-maximal and peak exercise compared to controls. Finally, a significant positive relationship was found between peak VO_2 and cardiac output (Figure 4).

3.1 e) Discussion

Consistent with limited prior data, we confirmed that, relative to healthy age-matched controls, women with breast cancer had reduced exercise capacity prior to cancer therapy. We extend prior research by examining the cardiac mechanisms underpinning this exercise impairment by comparing changes in RV and LV volumes measured during peak supine cycle exercise. The principal new finding was that the reduced peak VO_2 in breast cancer patients versus healthy controls was due, in part, to a reduced maximal cardiac index. In turn, the reduced peak exercise cardiac index was due to a reduced RV and LV stroke index and end-diastolic volume index as no significant difference was found between groups for RV and LV ejection fraction.

The mechanisms responsible for reduced biventricular end-diastolic volumes prior to adjuvant chemotherapy were not studied, however, sedentary aging may play a role. Bhella *et al.* (2014) have shown that life-long sedentary (exercise <2 times per week) and casual exercisers (exercise 2-3 times per week) have lower peak oxygen uptake, LV mass and LV distensibility than those who exercise >3 times per week.[110] Despite increased LV stiffness, less active individuals did not have impaired systolic impairment or abnormalities, closely mirrored by our findings.[110] One plausible explanation for our finding of lower functional capacity in breast cancer patients is sedentary deconditioning due to reduced mobility and physical activity following surgery. However, when we compared patients who had undergone surgery versus those who had not received any treatment (surgery, chemotherapy or radiation), no difference in peak VO_2 was observed. This null finding is not inconsistent with patient surveys; patients were referred to the study after surgical recovery and had returned to baseline based on subjective self-report of symptoms. Accordingly, such a substantial decrement in peak VO_2 and ventricular end-

diastolic volumes are better explained by longer-term reductions in physical activity volume and intensity.(4)

Given that the breast cancer patients' in our study had peak VO_2 values that were approximately 30% lower than controls, it is plausible that the reduced biventricular end-diastolic volume may be due to increased LV stiffness. Furthermore, a cluster of co-morbidities associated with a sedentary lifestyle are also linked with increased breast cancer incidence and mortality, it is perhaps reasonable to hypothesize that a lower peak VO_2 may be an additional clinical breast cancer risk factor and be associated with reduced survival after diagnosis.(13) Moreover, obesity, metabolic syndrome and muscle atrophy have been associated with increased breast cancer incidence[111-114] and it has been hypothesized that this may be due to a mild pro-inflammatory state and attenuated immune response.[113, 115] On the other hand, exercise inhibits inflammation in adipose tissue and creates unfavorable conditions for cancer,[116] a reduction in breast cancer incidence and improved survival.[117, 118] Thus, it is perhaps not entirely unexpected that patients diagnosed with breast cancer may have, on average, performed less exercise thereby resulting in reduced cardiac size and lower exercise capacity, as observed in our current study.

In accordance with the '*multiple hit'* hypothesis, the reduced peak VO_2 in breast cancer survivors is attributed, in part, to the adverse cardiac effects of chemotherapy.[5, 17, 119] To date, only one study has measured VO_2 and its determinants during peak exercise in breast cancer survivors (n=47, mean age: 59 years, mean time post chemotherapy: 38 months) and age-matched healthy controls (n=11). The main finding was that the decreased peak VO_2 (20%) in breast cancer survivors versus controls was due to a significantly lower peak exercise cardiac output and stroke volume, as HR and calculated arterial-venous oxygen difference were not significantly different between groups.[17] We extend these findings and show reduced maximal

exercise cardiac output and peak VO_2 prior to undergoing chemotherapy. Importantly, our finding that 45% of the variance in peak VO_2 was explained by cardiac output (Figure 4) suggests that non-cardiac, peripheral factors (eg, muscle blood flow and/or O_2 extraction) also play an important role in limiting peak VO_2 prior to adjuvant chemotherapy.

3.1 e) i: Clinical Implications

Given that peak VO_2 declines by 10% after 12 weeks of chemotherapy (equal to what is observed after a decade of sedentary aging),[120] our finding that breast cancer patients have marked exercise intolerance and impaired exercise cardiac output (and likely impaired peripheral determinants) reserve prior to adjuvant chemotherapy, suggests that interventions that attenuate the decline in peak VO_2 may be an important target of therapy. Indeed, exercise training is an effective therapy to improve peak VO_2 during adjuvant chemotherapy;[107] however, the optimal exercise training program and mechanisms responsible for this favorable adaptation remain uncertain.[121]

3.1 e) ii: Limitations

A limitation of this study was that peak VO_2 testing was performed during upright exercise while cardiac volumes were measured in the supine position, therefore the mechanisms underpinning the reduced maximal cardiac output may differ between groups in the upright position. However, this is unlikely as peak HR was not different between groups during peak upright exercise. Also, breast cancer patients and healthy controls relied on a decrease in RV and LV end-systolic volume to increase stroke index and ejection fraction from rest to peak exercise. Given that breast cancer patients displayed evidence of sedentary aging, we contend that

biventricular end-diastolic volume would remain lower during upright exercise given this remodeling pattern is associated with decreased cardiac distensibility.

3.1 f) Conclusion

Despite preserved rest, sub-maximal and peak exercise RV and LV ejection fraction, breast cancer patients prior to adjuvant chemotherapy have marked exercise intolerance, related to decreased maximal cardiac index, secondary to reduced RV and LV end-diastolic volume. Peripheral, non-cardiac factors may also play a prominent role in limiting exercise tolerance prior to adjuvant chemotherapy. Accordingly, interventions that improve cardiovascular and skeletal muscle function, such as exercise training, initiated at the time of diagnosis may attenuate the decline in peak VO_2 that occurs during adjuvant chemotherapy.

3.1 g) References

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3.1 h) Figure Legends

3.1 h) i: Figure 1:

Left ventricular end-diastolic volume index (A), end-systolic volume index (B), stroke index (C) and ejection fraction (D) at rest, sub-maximal and maximal supine cycle exercise. *, Significant main group (breast cancer vs. control) effect; †, Significant main intensity (low, moderate, and peak exercise vs. rest).

3.1 h) ii: Figure 2:

Right ventricular end-diastolic volume index (A), end-systolic volume index (B), stroke index (C) and ejection fraction (D) at rest, sub-maximal and peak supine cycle exercise. *, Significant main group (breast cancer vs. control) effect; †, Significant main intensity (low, moderate, and peak exercise vs. rest).

3.1 h) iii: Figure 3:

Heart rate and cardiac index at rest, sub-maximal and peak supine cycle exercise. *, Significant main group (breast cancer vs. control) effect; †, Significant main intensity (low, moderate, and peak exercise vs. rest); ‡, Significant group by intensity interaction.

3.1 h) iv: Figure 4:

Relationship between peak VO_2 (upright cycle exercise) and peak cardiac output (Q, supine cycle exercise).

3.1 i) Tables

3.1 i) i: Table 1

Participant Characteristics.

Parameter	BC (n=29)	HC (n=10)	P Value
Age (years) median [range]	48 (12) 51 [19-68]	48 (12) 52 [30-66]	0.98
Height (cm)	165 (9)	166 (7)	0.67
Weight (Kg)	72 (19)	66 (9)	0.31
Body mass index (kg/m^2)	26.7 (6.9)	23.8 (2.1)	0.21
Normal, n (%)	16 (55)	8 (80)	-
Overweight, n (%)	9 (31)	2 (20)	-
Obese, n (%)	4 (14)	-	-
Body surface area (m^2)	1.77 (0.22)	1.73 (0.15)	0.49
Hemoglobin (g/dl)	13.0 (1.4) (n=26)	13.2 (0.5)	0.68
BNP	36.9 (33.6) (n=26)	26.9 (16.5) (n=9)	0.38
Troponin I	2.9 (1.3) (n=26)	3.3 (2.9)	0.58

3.1 i) ii: Table 2

Cardiopulmonary Exercise Performance during peak Upright Cycle Exercise

Parameter	Breast Cancer (n=29)	Healthy Control (n=10)	p Value
Power output (W)	142 (47)	228 (51)	0.00003
VO_2 (l/min)	1.7 (0.4)	2.3 (0.5)	0.0013
VO_2 (ml/kg/min)	25 (6)	35 (6)	0.00009
HR (bpm)	174 (13)	169 (16)	0.39
Systolic blood pressure (mmHg)	185 (27)	180 (28)	0.83
Diastolic blood pressure (mmHg)	93 (16)	84 (19)	0.18

3.1 j) Figures

3.1 j) i: Figure 1.

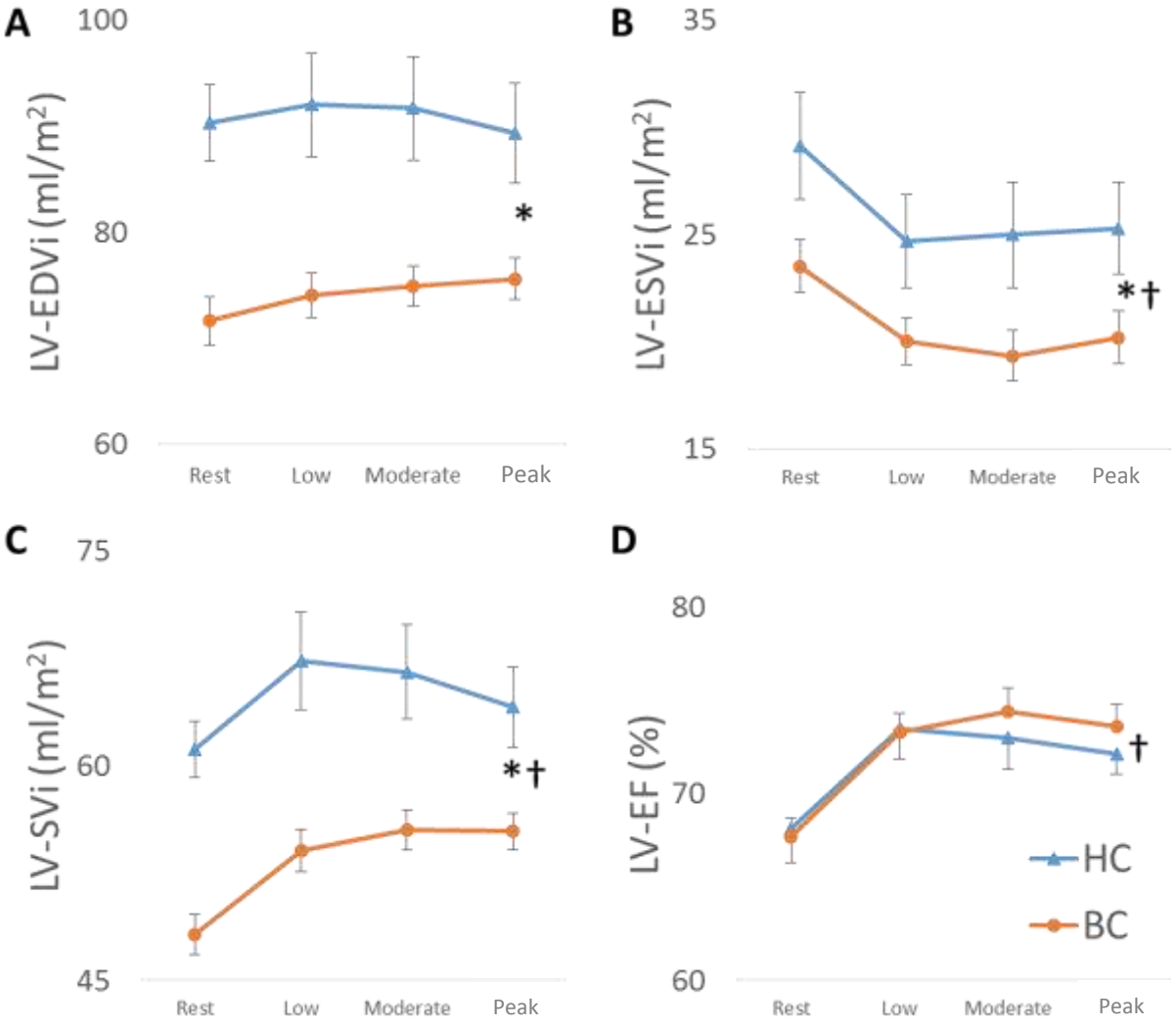

Figure 1: Left ventricular end-diastolic volume index (A), end-systolic volume index (B), stroke index (C) and ejection fraction (D) at rest, sub-maximal and maximal supine cycle exercise. *, Significant main group (breast cancer vs. control) effect; †, Significant main intensity (low, moderate, and peak exercise vs. rest).

3.1 j) ii: Figure 2.

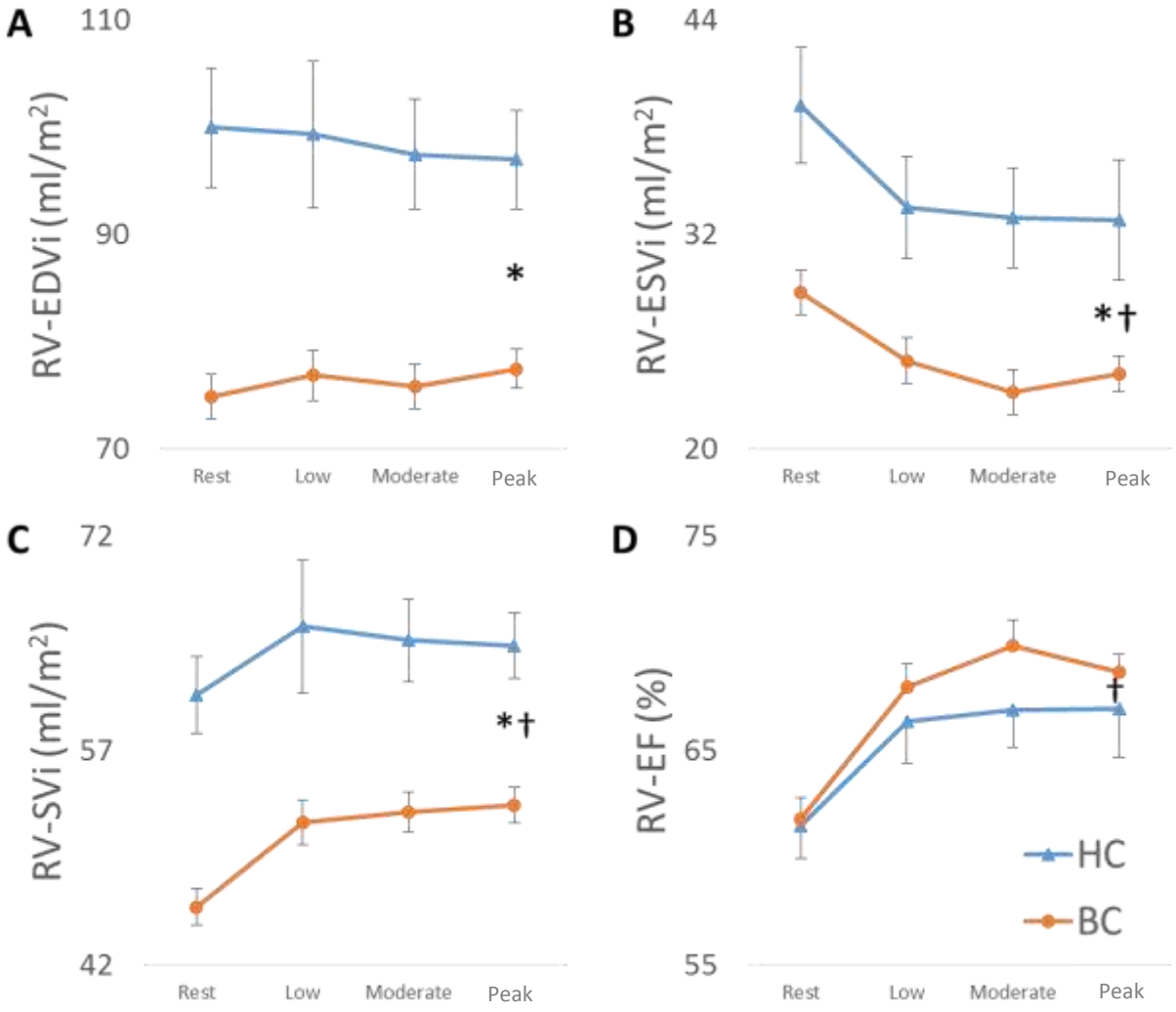

Figure 2: Right ventricular end-diastolic volume index (A), end-systolic volume index (B), stroke index (C) and ejection fraction (D) at rest, sub-maximal and peak supine cycle exercise. *, Significant main group (breast cancer vs. control) effect; †, Significant main intensity (low, moderate, and peak exercise vs. rest).

3.1 j) iii: Figure 3.

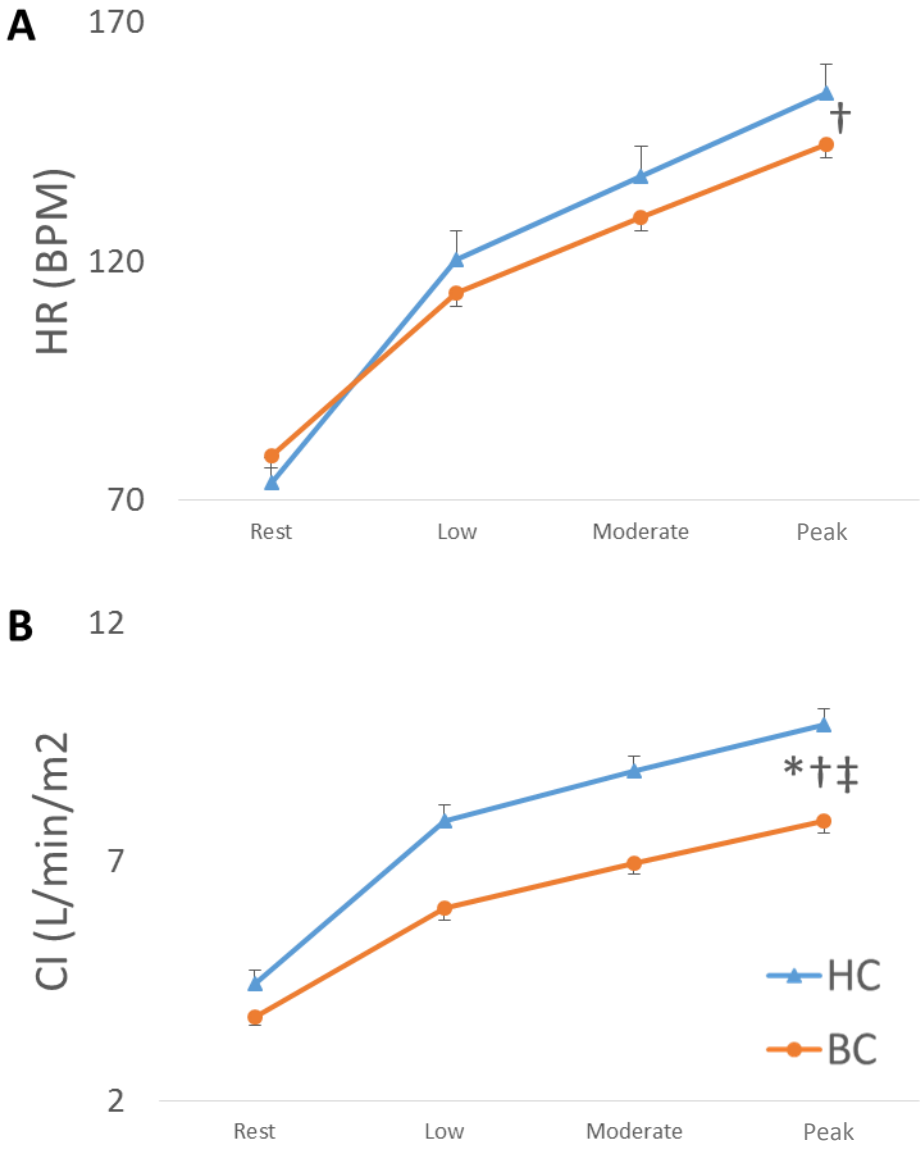

Figure 3: Heart rate and cardiac index at rest, sub-maximal and peak supine cycle exercise. *, Significant main group (breast cancer vs. control) effect; †, Significant main intensity (low, moderate, and peak exercise vs. rest); ‡, Significant group by intensity interaction.

3.1 j) iv: Figure 4.

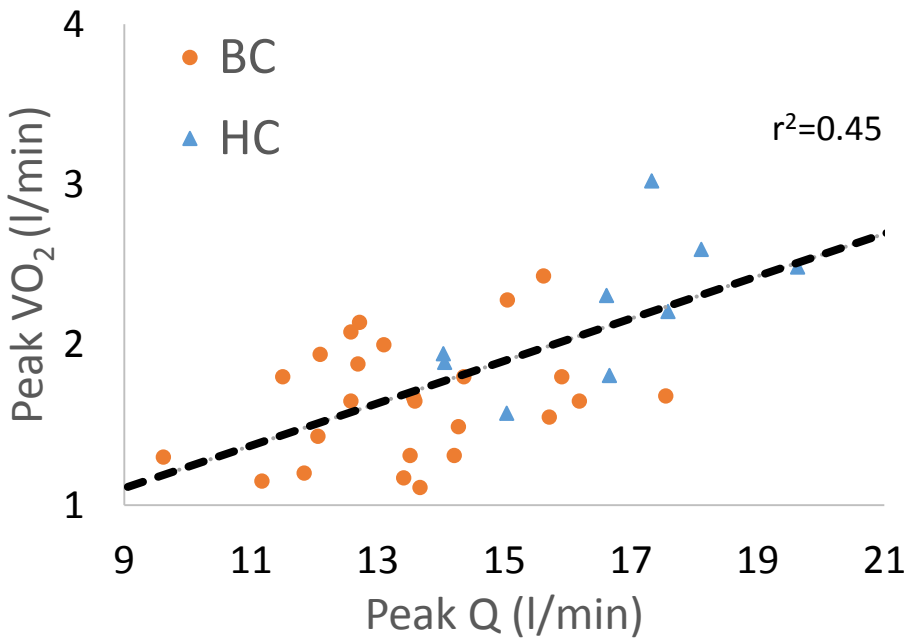

Figure 4: Relationship between peak VO_2 (upright cycle exercise) and peak cardiac output indexed to body surface area (supine cycle exercise).

Chapter 4

4.1 Leg blood flow is preserved during small muscle mass exercise in long-term survivors of anthracycline treated breast cancer.

4.1 a) Abstract*:*

Background: Persistent anthracycline mediated cardiovascular damage to the periphery may contribute to reduced exercise tolerance in women with a history of breast cancer (BC).

Methods: We evaluated the hemodynamic responses during submaximal single leg knee extension (SLKE) exercise in 14 BC survivors (mean age: 61 ± 7 years; mean time post-anthracycline therapy: 12 ± 6 years) and 9 age and sex-matched control (CON) subjects. Heart rate (lead II), blood pressure (finger cuff pressure), leg (femoral artery) blood flow (Doppler ultrasound), vascular conductance and pressure-strain were measured at rest, 25%, 50% and 75% of maximal power output. Quadriceps mass was estimated from thigh volume and skinfold measures.

Results: Estimated quadriceps mass was significantly lower in BC survivors compared to CON (1803 ± 607 vs. 2601 ± 1101 g, p=0.04). No significant difference was found between groups for maximal SLKE power output (28 ± 11 vs. 34 ± 17 Watts, p=0.33), heart rate (109 ± 14 vs. 103 ± 13 bpm, p=0.36) or mean arterial pressure (122 ± 18 vs. 119 ± 26 mmHg, p=0.33). Rest and sub-maximal exercise mean arterial pressure, leg blood flow, leg conductance, pressure-strain were not significantly different between groups.

Conclusion: Resting and sub-maximal exercise leg blood flow, conductance, and femoral artery stiffness (pressure-strain) are not impaired in long-term survivors of anthracycline treated BC. Increasing leg muscle mass may be an important target of interventions aimed at improving cardiovascular health in BC survivors.

4.1 b) Introduction

Advances in prevention, early detection and therapy have contributed to reduced breast cancer (BC) mortality.[122] As a result of improved survivorship, comorbid conditions compete with BC as the leading cause of mortality.[105] Specifically, BC survivors are at increased risk of cardiovascular disease and heart failure that is attributed, in part, to the detrimental cardiovascular effects of anthracycline therapy, including dose-limiting cardiotoxicity.[6, 7]

Cardiorespiratory fitness, measured as peak oxygen uptake (peak VO_2) is the strongest predictor of cardiovascular and all-cause mortality in healthy and clinical populations, and is reduced in BC survivors by 20%.[13, 15, 123, 124] While reduced peak VO_2 is associated with reduced peak cardiac output in BC, emerging evidence suggests that non-cardiac 'peripheral' mechanisms may also contribute to reduced exercise tolerance.[16, 17, 19, 123, 124] Indeed, Howden *et al.* recently reported that the reduced peak VO_2 (15%) from pre to post-anthracycline therapy was due to a decline in arterial-venous oxygen difference, as peak cardiac output did not change during this time. Moreover, Chaosuwannakit *et al.* demonstrated that compared to baseline, resting aortic distensibility decreased (54%) and aortic pulse wave velocity increased (95%) after of 4-months of anthracycline chemotherapy in 40 cancer patients (BC, n=19) with no change in age-and sex-matched controls.[35] Mizia-Stec *et al.* extended these findings by demonstrating that carotid artery stiffness increased from pre-to post anthracycline therapy in 35 BC patients.[34] Finally, Didier and colleagues comparing cancer survivors (n=11, BC n=10, anthracycline treated n=3) and age- and sex-matched controls (n=9) found that the former group had an impaired muscle blood flow response to sub-maximal (20% of maximal voluntary contraction) rhythmic hand-grip exercise.[37]

Taken together, the few studies performed to date suggest that vascular stiffening and reduced muscle blood flow may contribute to the impaired exercise tolerance found in cancer survivors. Currently, no study has examined whether blood flow to major locomotor muscle is impaired, and if this impairment is related to arterial stiffening in BC survivors. Single leg knee extension (SLKE) where the limiting role of the heart is minimized, has been used to identify peripheral blood flow limitations to exercise in non-cancer populations (heart failure patients with preserved or reduced ejection fraction) who have abnormal exercise cardiac function.[125-127] Using SLKE exercise we tested the hypothesis that leg blood flow and vascular conductance would be significantly reduced, and femoral artery stiffness would be significantly increased in anthracycline treated BC survivors compared to age-and sex-matched controls (CON).

4.1 c) Methods:

4.1 c) i: Participants:

Female BC survivors and CON were recruited from the local Dallas/Fort Worth community via recruitment flyers and by word of mouth. Informed, written consent was obtained from all subjects, and the study was approved by the University of Texas at Arlington Institutional Review Board.

Breast cancer survivors had to have completed anthracycline chemotherapy more than 1 year prior to study enrollment. Exclusion criteria for both groups were: orthopedic limitation to exercise, resting blood pressure exceeding 160/100 mmHg for BC survivors or 140/90 mmHg for CON, and inability to acquire a femoral artery ultrasound image of sufficient quality for analysis. Additional exclusion criteria for control participants were presence of cardiovascular disease or current cardio-active medication.

4.1 c) ii: Protocol Overview:

***Visit One*:** *Physical activity and leg muscle mass:* Participants self-reported their level of physical activity (Stanford Leisure Time Activity Category Item) and medical history using a researcher developed questionnaire.[128] Height and weight were measured using a standiometer and electronic scale, and thigh length, circumference (proximal, mid and distal) and skinfold were measured using a flexible measuring tape and skinfold calipers to estimate quadriceps muscle mass using a previously validated equation by Andersen and Saltin.[129]

Incremental SLKE exercise test: Participants were seated on a custom SLKE ergometer during which time a finger blood pressure cuff (Finapress, AD Instruments, Australia) and ECG (lead II) were continuously measured. An image of the right, common femoral artery was optimized (landmarked at the femoral artery bifurication) using B-mode ultrasound. After optimization, a duplex image was acquired, with the pulsed wave velocity sample volume positioned 2 cm proximal to the femoral artery bifurication. The angle of insonation was limited to 60°, and the sample volume was parallel to the artery walls. Two-minutes of resting duplex ultrasound were recorded using a video capture device (Video Capture, Elgato, Germany) for offline analysis.

Participants were then familiarized with SLKE exercise. Specifically, they were instructed to "kick" to a metronome set at 50 beats per minute, and to relax their leg entirely during rebound driven by flywheel momentum. Once participants achieved a cadence of 50 revolutions per minute, the resistance was increased to 10 watts (W) and then increased by 10 W every 2 minutes thereafter until volitional exhaustion or an inability to adhere to the pre-specified cadence.

***Visit Two*:** *Peripheral hemodynamic responses during sub-maximal SLKE exercise:* Participants were instrumented as per Visit 1. After image optimization, 2 minutes of duplex ultrasound were

recorded, and then participants began their first sub-maximal SLKE power output. Workloads were targeted to 25, 50 and 75% of the previously determined maximal power output. Each workload was performed for 5 minutes with concomitant femoral artery imaging. Five minutes of post-exercise recovery were recorded. Workloads were separated by 10 minutes of rest. In a subset of participants, a third visit identical to V*isit 2*, was performed to test repeatability. The average measures interclass correlation was 0.92 for femoral artery blood flow. Data from V*isit 2* are reported.

4.1 c) iii: Measurements:

Duplex ultrasound recordings were analyzed offline using automated edge detection software (CardioSuite, Quipu, Italy). Femoral artery blood flow was calculated as: $\pi(D_{mean}/2)^2$ x V/60 when D_{mean} = femoral artery diameter in cm, V/60 = blood velocity in cm/min. Blood flow during SLKE was calculated using a noise-free 30-60 second velocity average from the final 120 seconds of exercise (stable, steady state), and a 60 second diameter average from post-exercise recovery. Data were cleaned to remove non-physiologic data-points and blood flow was normalized to estimated quadriceps mass. Single leg vascular conductance was calculated as: leg blood flow/mean arterial pressure where mean arterial pressure was averaged over the final minute of each stage. Femoral artery stiffness was quantified as pressure-strain (Young's elastic modulus, E = K[pulse pressure]/strain) where strain = $[D_{diastolic} - D_{systolic}]/D_{diastolic}$ and K = 133.3 $N/m^2/mmHg$, as previously described.[130] Femoral artery systolic and diastolic diameters were measured immediately upon exercise cessation (<20 seconds) using 3 independent cardiac cycles within a 10 second window while pulse pressure was averaged over the same period.

4.1 c) iv: Statistical analysis:

Continuous descriptive and outcome variables were compared between groups using independent t-tests. In the case of non-parametric distribution of continuous variables (confirmed

by the Shapiro-Wilk test) or scale measures, the Mann Whitney U-test was used. A 2-way repeated measures ANOVA was used to compare blood flow, pressure, conductance, heart rate, and pressure-strain responses to SLKE. A Pearson's correlation was used to test for a bivariate relationship between femoral artery stiffness and blood flow. Data are presented as mean ± standard deviation, significance was set at $p<0.05$.

4.1 d) Results:

4.1 d) i: Participants:

Fifteen BC survivors and 11 age- and sex-matched CON were recruited to the study. One participant from each group was excluded prior to Visit 2 with the *post-hoc* exclusion criterion of the presence of an arterial branch superior to the femoral artery bifurcation resulting in an atypical femoral artery blood velocity profile. One CON participant was excluded due to high blood pressure. The BC survivors were on average 12 ± 6 years post anthracycline therapy, and 93% of participants received doxorubicin while 71% underwent radiation therapy and hormone therapy each (Table 1). The BC and CON were well matched for age, body mass index and self-reported physical activity. The BC survivors had a significantly lower estimated quadriceps mass (1803 ± 607 vs. 2601 ± 1002 g, $p=0.04$) and quadriceps mass as a percent of body mass (2.6 ± 0.7 vs 3.6 ± 1.1 %, $p=0.02$) compared to CON.

4.1 d) ii: Incremental to maximal SLKE Exercise:

Power output (BC: 28 ± 11 vs. CON: 34±17 W, $p=0.35$), heart rate (BC: 109 ± 14 vs. CON: 103 ± 13 bpm, $p=0.36$) and mean arterial pressure (BC: 122 ± 18 vs. CON: 119 ± 26 mmHg, $p=0.33$) were not different between groups during maximal SLKE exercise (Figure 1, table 2).

4.1 d) iii: Peripheral hemodynamic at rest and sub-maximal SLKE Exercise:

Resting heart rate, mean arterial pressure, leg blood flow and vascular conductance were similar between BC and CON (Figure 1). Due to video-capture malfunction, duplex ultrasound

records were lost at the 50 and 75% submaximal workloads in one CON participant. As maximal power was not different between groups, relative work rates at 25, 50 and 75% of maximal SLKE exercise were similar between groups. Additionally, heart rate, mean arterial pressure and leg vascular conductance were similar at rest and during submaximal exercise (Figure 1). Femoral artery blood flow was not significantly different between groups at rest or during submaximal exercise (Figure 1). Similarly, no group differences were found in femoral artery strain (Wilks' Lambda, p=0,216 for intensity effect) or pressure-strain at rest and immediately following sub-maximal exercise (Figure 2). Leg blood flow, conductance, mean arterial pressure and pressure-strain increased from rest to submaximal exercise (Figures 1, 2).

4.1 d) iv: Relationship between femoral artery stiffness and blood flow:

No relationship was found between femoral artery blood flow and femoral artery stiffness as measured as pressure-strain at rest, or immediately after 25, 50 or 75% of maximal SLKE (Table 3).

4.1 e) Discussion

Breast cancer survivors have severely reduced exercise tolerance.[109] Emerging evidence suggests that anthracycline chemotherapy may be associated with peripheral dysfunction that may contribute to decreased exercise tolerance.[13, 17] The major new finding of this study was that leg blood flow and conductance during sub-maximal SLKE exercise, where the limiting role of the heart is minimized, was not significantly different between BC and age and sex-matched CON. Further, to our knowledge we provide the first data on femoral artery stiffness at rest and immediately following submaximal exercise in long-term survivors of anthracycline treated BC and report no difference for this outcome between BC and CON.

4.1 e) i: Leg Blood Flow in Long-Term BC Survivors:

Our finding of preserved leg blood flow during non-cardiac limited exercise is contrary to findings from Didier *et al.* who reported that forearm blood flow was attenuated in 11 long-term cancer survivors treated with adjuvant therapy (n=10 BC, mean age=56, mean 3 years post-therapy).[37] The attenuated blood flow was attributed to a blunted arterial blood pressure response, secondary to impaired left ventricular ejection time index derived from an arterial pressure waveform. In contrast, we found no differences in the heart rate or blood pressure responses to exercise, and found a comparable blood flow response when indexed to 100 g of muscle mass during moderate intensity exercise (25 and 50% of peak SLKE, figure 1). Further, at the highest exercise intensity (75% of peak SLKE) we also did not unmask a leg blood flow deficit in BC. The discrepancy in findings may be due to regional differences in muscle mass and adiposity. Critically, we measured blood flow at the level of a conduit artery which supplies the entire leg. Physiologic increases in blood flow should closely match metabolic demand; however, blood may be delivered to vascular beds in close proximity resulting in normal "muscle" blood flow. Metabolite spillover, conductive vasodilation and heat cause increased blood flow to local tissues, including to adipose tissue adjacent to active muscle.[131] Thus, given that we observed reduced quadriceps mass but preserved leg conductance in BC compared to controls, it is possible that blood flow was shunted through non-force producing tissue. In the forearm, this effect may be minimized due to smaller active muscle mass, lower vascular conductance, lower adiposity, and lower work rates producing fewer metabolites and less heat spill over to non-force producing tissue.[132]

A consequence of blood flow shunting through less metabolically active tissue (inactive muscle, adipose tissue, skin) is reduced oxygen uptake during whole body peak exercise. In heart failure with preserved ejection fraction, a syndrome marked by extreme exercise intolerance, the

ratio of intermuscular fat to skeletal muscle is inversely related to peak oxygen uptake.[30] This physiologic relationship is explained by shunting of blood through intermuscular fat, resulting in an impaired matching of cardiac output and peak VO_2. In a study of 14 cancer survivors (mean age 54 years, >12-months post-therapy) and 14 age- and sex-matched controls, the same effect was observed whereby intermuscular fat was associated with reduced exercise capacity.[29]

Our findings demonstrate preserved leg blood flow in response to SLKE despite reduced quadriceps mass in anthracycline treated BC survivors. Preserving or increasing muscle mass is therefore an important target for interventions directed at increasing exercise tolerance in BC. Dietary and exercise interventions have proven efficacy for reducing adiposity and preserving or increasing muscle mass.[133-136] Accordingly, favorable changes in body composition could dramatically improve cardiorespiratory fitness through increasing efficiency of blood flow delivery to active muscle, thus preventing progression of BC survivors towards a HFpEF-like phenotype.

4.1 e) ii: Femoral Artery Stiffness in Long-Term BC Survivorship

Vascular toxicity, specifically vascular stiffening, is a proposed mechanism of anthracycline related decline in cardiovascular health.[6] To our knowledge, only two studies have measured conduit artery stiffness. Mizia-Stec *et al.* reported a decrease in common carotid artery compliance from pre to post-anthracycline therapy (n=35, age: 50 years)[34] while Koelwyn *et al.* report no difference in common carotid artery compliance or distensibility (n=30, 6 years post treatment, age: 61 years) compared to control subjects.[19] Koelwyn *et al.* also reported impaired ventricular-arterial coupling in anthracycline treated BC survivors under stress conditions (25, 50 and 75% of peak cycling work rate). Arterial elastance, estimated from echocardiography derived cardiac volumes and brachial artery end-systolic blood pressures, was not impaired in

BC. Here, we measured arterial stiffness in a large conduit artery feeding major locomotor muscle, and found no decrement in femoral artery pressure-strain. Importantly, pressure strain was not impaired at rest, nor under conditions of increasing blood flow or pressure- consistent with estimated arterial elastance as previously reported.[19] Finally, we found no relationship between leg blood flow and femoral artery pressure-strain (table 3). Therefore, stiffening of conduit arteries does not appear to limit blood flow to major locomotor muscle during sub-maximal SLKE where the limiting role of the heart is minimized.

4.1 e) iii: Limitations:

A limitation of the study is that blood flow was measured in the common femoral artery, rather than at the level of active muscle. While we have interpreted our findings in the context of the available literature, it is unclear whether perturbations in blood flow delivery to active muscle microvasculature exist, or whether muscle oxygen utilization is preserved in long term survivors of anthracycline treated BC. To our knowledge, no data on mitochondrial function are available, nor on active-muscle blood flow and diffusive oxygen conductance- two possible contributors to exercise intolerance and functional disability.

4.1 e) iv: Conclusion:

Leg blood flow, vascular conductance and arterial stiffness during sub-maximal SLKE exercise are not significantly different between BC survivors treated with anthracycline chemotherapy and age and sex-matched CON. Body composition (i.e. increasing lower extremity muscle mass) may be an important target when tailoring dietary and exercise interventions to improve cardiovascular health in long-term survivors of anthracycline treated BC.

4.1 f) References

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4.1 g) Figure Legends:

4.1 g) i: Figure 1:

Single leg knee extension power (A), and blood flow (B), mean arterial pressure (C), leg conductance (D) and heart rate (E) responses. Significant intensity effect ($p<0.05$) indicated by *vs rest, † vs 25%, ‡ vs 50%, # vs 75%.

4.1 g) ii: Figure 2:

Femoral artery pressure-strain at rest and during submaximal exercise. Significant intensity effect ($p<0.05$) indicated by *vs rest, † vs 25%.

4.1 h) Tables:

4.1 h) i: Table 1: Participant characteristics.

Parameter	BC (n=14)	Control (n=9)	P
Age (years)	61 (7)	59 (7)	0.38
BMI (kg/m²)	26.2 (3.8)	27.9 (4.7)	0.54
Estimated Quadriceps Mass (g)	1803 (607)	2601 (1102)	**0.04**
Quadriceps % of Body Mass	2.6 (0.7)	3.6 (1.1)	**0.02**
LTAC Score (1-6)	3 (1)	3 (1)	0.88*
Comorbidities (n, %)			
Arthritis	5 (36)	0 (0)	
Asthma	2 (14)	1 (11)	
Hypothyroid	1 (7)	2 (25)	
High Cholesterol	4 (29)	1 (11)	
Hypertension	2 (14)	0 (0)	
Breast Cancer			
Stage (n, 0/I/II/III)	1/3/7/3	-	
Doxorubicin/Epirubicin (n)	13/1	-	
Time post therapy (years)	12 (6)	-	
Surgery (Lumpectomy/Mastectomy)	5/9	-	
Radiation Therapy (n)	10	-	
Biologic Therapy (n)	3	-	
Hormone Therapy (n)	10	-	

L-TAC: Stanford Leisure Time Activity Category Item. *Mann-Whitney U test.

4.1 h) ii: Table 2: Power output, heart rate and mean arterial pressure during maximal SLKE exercise.

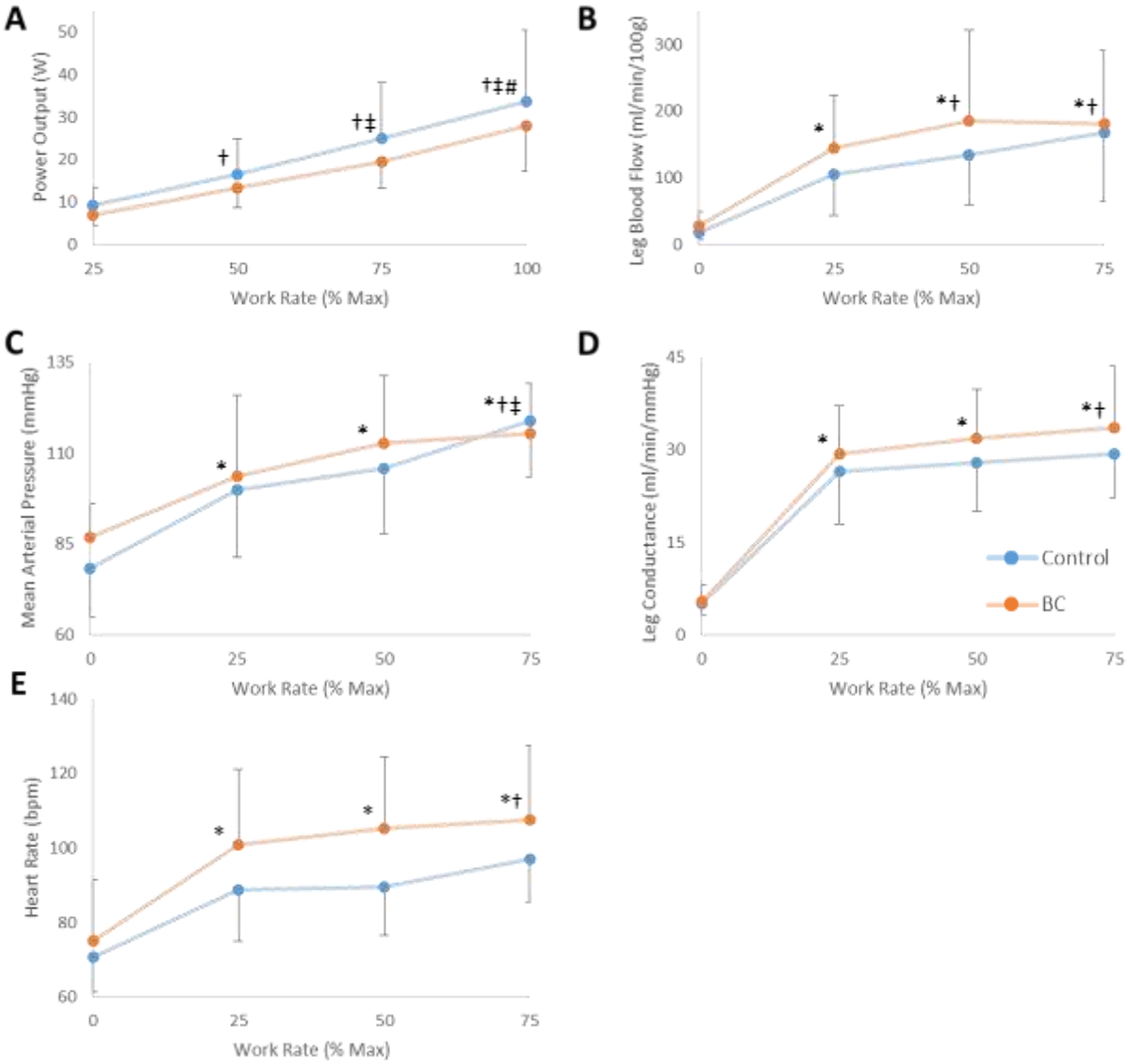
Parameter	BC	Control	P
Power (W)	28 (11)	34 (17)	0.35
Heart Rate (bpm)	109 (14)	103 (13)	0.36
Mean Arterial Pressure	122 (18)	119 (26)	0.33

4.1 h) iii: Table 3: Pearson's correlations between femoral artery blood flow and pressure-strain at rest and immediately after submaximal SLKE exercise.

Blood Flow and Pressure-Strain	Combined Pearson's R (p)	BC Only Pearson's R (p)
Rest	-0.089 (0.688)	-0.157 (0.592)
25% SLKE	0.150 (0.495)	-0.043 (0.883)
50% SLKE	-0.142 (0.528)	-0.203 (0.487)
75% SLKE	-0.253 (0.255)	-0.312 (0.278)

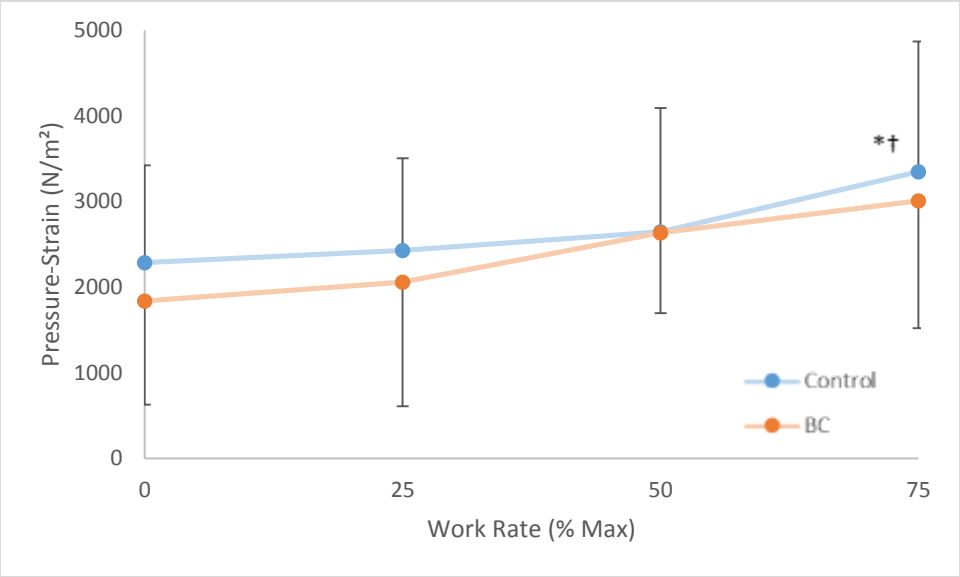
4.1 i) Figures:

4.1 i) i: Figure 1: SLKE power and hemodynamic responses to SLKE exercise.



Single leg knee extension power (A), and blood flow (B), mean arterial pressure (C), leg conductance (D) and heart rate (E) responses. Significant intensity effect ($p<0.05$) indicated by *vs rest, † vs 25%, ‡ vs 50%, # vs 75%.

4.1 i) ii: Figure 2: Femoral artery pressure-strain at rest and immediately after submaximal SLKE exercise



Significant intensity effect ($p<0.05$) indicated by *vs rest, † vs 25%.

Chapter 5

5.1 Exercise intolerance in anthracycline-treated breast cancer survivors: the role of skeletal muscle bioenergetics, oxygenation and composition.

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5.1 a) Abstract

Background: Peak oxygen consumption (VO_2) is reduced in women with a history of breast cancer (BC). We measured leg blood flow, oxygenation, bioenergetics, and muscle composition in women with BC treated with anthracycline chemotherapy (n=16, mean age: 56) and age- and body mass index-matched controls (n=16).

Methods: Whole-body peak VO_2 was measured during cycle exercise. ^{31}Phospohous magnetic resonance (MR) spectroscopy was used to measure muscle bioenergetics during and after incremental to maximal plantar flexion exercise (PFE). MR imaging was used to measure lower leg blood flow, venous saturation (SvO_2) and VO_2 during submaximal PFE, and abdominal, thigh and lower leg intermuscular fat (IMF) and skeletal muscle (SM).

Results: Whole body peak VO_2 was significantly lower in BC survivors vs. controls (23.1±7.5 vs. 29.5±7.7 ml/kg/min). Muscle bioenergetics and mitochondrial oxidative capacity were not different between groups. No group differences were found during submaximal PFE for lower leg blood flow, SvO_2 or VO_2. The IMF:SM ratio was higher in the thigh and lower leg in BC survivors (0.36±0.19 vs 0.22±0.07, p=0.01; 0.10±0.06 vs 0.06±0.02, p=0.03, respectively) and were inversely related to whole-body peak VO_2 (r= -0.71, p=0.002; r=-0.68, p=0.003, respectively). In the lower leg, IMF:SM ratio was inversely related to VO_2 and O_2 extraction during PFE.

Conclusion: SM bioenergetics and oxidative capacity in response to PFE are not impaired following anthracycline treatment. Abnormal SM composition (increased thigh and lower leg IMF:SM ratio) may be an important contributor to reduced peak VO_2 during whole body exercise among anthracycline-treated BC survivors.

5.1 b) Implications for Practice:

Peak oxygen consumption (VO_2) is reduced in breast cancer (BC) survivors and is prognostic of increased risk of cardiovascular disease-related and all-cause mortality. We demonstrated that in the presence of deficits in peak VO_2 one year post-anthracycline therapy, skeletal muscle bioenergetics and oxygenation are not impaired. Rather, body composition deterioration (e.g. increased ratio of intermuscular fat to skeletal muscle) may contribute to reduced exercise tolerance in anthracycline BC survivors. This finding points to the importance of lifestyle interventions including caloric restriction and exercise training to restore body composition and cardiovascular health in the BC survivorship setting.

5.1 c) Introduction

Advances in prevention, early detection, and treatment of early stage breast cancer (BC) have dramatically improved survival, and consequently, other conditions including cardiovascular disease (CVD) now compete with BC as the primary cause of mortality.[1, 2] Women with a history of BC are at greater risk for CVD and related mortality relative to women without BC. It is well established that following treatment for BC, cardiorespiratory fitness, measured as peak oxygen uptake (peak VO_2) during whole-body exercise, is ≈20% lower than age- and sex-matched controls.[13, 16, 17, 19, 124, 137] Low cardiorespiratory fitness is associated with increased heart failure incidence, cardiovascular events, and both CVD disease-related and all-cause mortality.[138]

Several BC therapies can cause cardiovascular injury, including anthracycline chemotherapy, which is associated with a dose-dependent, progressive, myocardial injury.[7] Accordingly, impairments in peak VO_2 could be attributed to 'central' limitations that reduce peak exercise cardiac output.[17, 123] However, 'peripheral' impairments in exercise blood flow, citrate synthase activity, and relative reductions in oxidative muscle fiber and capillarity have been reported in individuals after systemic cancer therapy.[31, 139] Abnormalities in skeletal muscle composition may also limit exercise tolerance; Reding *et al.* reported peak VO_2 was inversely related to the ratio of intermuscular fat (IMF) to skeletal muscle (SM) within the paraspinal muscles in cancer survivors.[29] Given that O_2 consumption during cycle exercise occurs predominantly in the muscles of the thigh and lower leg, skeletal muscle composition in the leg is likely a more important determinant of peak VO_2. Prior reports studied populations heterogeneous for both cancer type and treatment, or only assessed acute changes during active treatment. Accordingly, the aims of this study were to: 1) comprehensively compare non-invasive measures of leg SM composition, rest and exercise blood flow, O_2 extraction, VO_2 and oxidative metabolism during plantar flexion exercise (PFE) using magnetic resonance (MR) imaging, ^{31}P spectroscopy and whole body (cycle)

peak VO_2 in BC survivors approximately one-year post-anthracycline treatment and age- and BMI-matched non-cancer controls; and 2) evaluate the relationship between thigh, lower leg, and paraspinal SM composition and whole-body peak VO_2. We hypothesized that BC survivors would have attenuated exercise leg blood flow, impaired SM oxidative capacity, and unfavorable SM composition compared to controls, and that IMF:SM ratio in the leg would be inversely related to peak VO_2. An exploratory objective was to assess underlying effects of the IMF:SM ratio by examining its relationship with measures of exercise metabolism, blood flow, VO_2, and O_2 extraction in the lower leg of anthracycline-treated BC survivors and non-cancer controls.

5.1 d) Methods

5.1 d) i: Participants

Thirty-two women participated in this cross-sectional study, with sixteen BC survivors and sixteen age and BMI matched non-cancer controls. The women with BC were recruited from a randomly selected cohort of 75 BC survivors who received anthracycline treatment at the Cross Cancer Institute (Edmonton, Canada) in the previous year, by mailing an invitation to participate. The women with BC who responded to the letter were then further screened for eligibility, including an additional inclusion criterion of a minimum of 3.5 months since receipt of their last anthracycline-based chemotherapy treatment to avoid effects of acute cardiotoxicity.[11] The control participants were recruited from the local community via word of mouth and recruitment posters. Exclusion criteria for both groups included a history of cardiovascular disease, diabetes, or lung disease, and contraindications to MRI. Controls were also excluded if they had a history of cancer of any type. Informed, written consent was obtained from all participants, and the study was approved by the University of Alberta Research Ethics Board.

5.1 d) ii: Cardiorespiratory fitness

Exercise testing was performed on an electrically-braked upright cycle ergometer (Ergoselect II 1200, Ergoline, Germany) using a staged protocol that started at 20 watts (W) and increased by 15 W every minute until volitional exhaustion. Expired gas was collected and analysed using a commercially available metabolic measurement system (Encore229 Vmax, SensorMedics, USA) and the highest 20-second average was used as whole-body peak VO_2. Heart rate (lead II ECG) was monitored continuously while rating of perceived exertion (RPE) was recorded every 2 minutes and at peak exercise.

5.1 e) MR Imaging and ^{31}P Magnetic Resonance Spectroscopy

Skeletal muscle function, resting and exercise metabolism, and composition were evaluated using a 3.0 T MRI system (PRISMA, Siemens Healthineers, Erlangen, Germany).

Incremental PFE – ^{31}P Metabolism

Subjects performed an incremental to maximal, unilateral (left leg), supine PFE test using a commercially available MRI ergometer (Trispect Module, Ergospect, Austria; Figure 1) inside the scanner. Plantar flexion exercise utilizes relatively small muscle mass, where the limiting role of the heart is minimized,[140] and elicits a metabolic response similar to the common daily activity of stair climbing.[141] The initial power output was set at 4 W and increased by 2 W every minute until volitional exhaustion. A metronome provided a consistent cadence of 30 contractions/minute and a study member provided feedback and encouragement from inside the scanner room to ensure test consistency. Tests were terminated at volitional exhaustion or technique breakdown (involvement of non-plantar flexor muscle groups or inability to perform 30 contractions/minute).

^{31}P spectra, averaged over 12-second intervals, were acquired continuously from rest, throughout exercise, and for four minutes of recovery after cessation of exercise (TR = 1.5 seconds, flip angle = 30 degrees, 8 cm diameter ^{31}P radiofrequency coil was used for excitation and signal reception). Relative concentrations (area under the individual spectra) of phosphocreatine (PCr) and inorganic phosphate (Pi) were measured to generate Pi:PCr ratios, and intracellular pH was calculated from the chemical shift difference between the Pi and PCr signals.[142] The Pi:PCr ratio and pH were compared at 60% of peak power output as a representative moderate-intensity workload that occurred prior to a marked reduction in pH for all participants. From the time of exercise completion, a mono-exponential curve was fit to the PCr recovery data to calculate the PCr recovery time constant, τ(PCr). The Pi:PCr ratio at the moderate exercise intensities and the PCr recovery τ were measured as indicators of skeletal muscle oxidative metabolism.[143] ^{31}P spectra were analyzed using custom, in-house developed MATLAB code (MATLAB, MathWorks, USA).

Sub-maximal PFE Leg Blood Flow, Oxygen Extraction and VO_2

Following 15-20 minutes of recovery after the incremental test, participants performed four minutes of constant work load sub-maximal PFE using the right leg, at 60% of peak power output. Blood flow and venous oxygen saturation were measured in the right superficial femoral vein just superior to the knee at rest (prior to exercise), and immediately (<1 second) after cessation of exercise, using a previously described method.[140, 144-146] Briefly, MR susceptometry-based oximetry was used to estimate venous oxygen saturation (S_vO_2) based on the magnetic field shift associated with the concentration of deoxyhemoglobin.[145] The same MRI acquisition included simultaneous quantification of blood flow (ml/min) in the superficial femoral vein. Images were acquired perpendicular to the superficial femoral vein (axial image orientation)

proximal to the knee (Figure 1). Together with arterial oxygen saturation (S_aO_2, finger pulse oximetry) and hemoglobin (Hb) concentration (obtained from blood tests immediately prior to the MRI scan), lower leg VO_2 and O_2 extraction were calculated in accordance with the Fick Principle:

Lower leg VO_2 (ml/min) = blood flow (ml/min) · [(S_aO_2 – S_vO_2, %) · Hb g/dL] · 1.34 mL O_2/g Hb.

While Lower leg O_2 extraction was calculated as:

O_2 extraction = (S_aO_2 – S_vO_2)/S_aO_2 x 100%.

Skeletal Muscle and Fat Composition

At each of the lower leg, thigh and abdomen regions, a multi-slice multi-echo acquisition was used to reconstruct water and fat separated images using the modified Dixon approach (Figure 2).[147] Typical image parameters include a 4 mm slice thickness, axial slice orientation, 1.0-1.3 mm in-plane resolution, with 30 slices covering the lower leg, 50 slices covering the thigh and 12 slices in the abdomen, with a sub-set of images selected for analysis at each location. For the lower leg, slices were analysed from 5 cm below the proximal end of tibial plateau, as identified on a sagittal localizer image of the lower leg, to the distal elimination of the gastrocnemius. For the thigh, 10 consecutive slices were analyzed starting 40 mm from the most distal point of the femur, as identified using the sagittal localizer image of femur. In the abdomen, three slices centered at the middle of the third lumbar vertebrae were identified using sagittal localizer images of the spine.

Absolute volumes of muscle (SM), intermuscular fat (IMF) and subcutaneous fat were measured in the lower leg, thigh and abdomen. Visceral fat (VF) was also measured in the abdomen. For image segmentation, custom MATLAB code was used to perform semi-automated

analysis to identify the boundaries of subcutaneous fat, SM, IMF, VF and bones. All regions were manually checked and adjusted in each slice to ensure accuracy of the automated delineation. Volumes for each component were quantified using the disk summation method at each of the three regions (lower leg, thigh and abdomen). The SM and IMF volumes were used to calculate the ratio of IMF to SM as well as the SM fat fraction (IMF/(IMF+SM)*100%) in each region.

5.1 e) i: Descriptive information

Cardiovascular risk factors and usual physical activity in the past month were collected by researcher-developed questionnaire and the Godin Leisure-Time Exercise Questionnaire.[148] Diagnosis and treatment history were extracted from medical records.

Statistical Analysis

Categorical descriptive characteristics were compared between groups using chi square tests or Fisher's exact test for variables with cell size above or below n=5, respectively. Student's t-tests for independent samples were used to compare continuous variables between groups. In the case of non-parametric distribution of continuous variables (confirmed by the Shapiro-Wilk test), the Mann Whitney U-test was used instead. Bivariate associations between IMF:SM and SM fat fraction in each region and relative peak VO_2 were characterized with Pearson's product-moment correlations within both groups combined and confirmed within the BC group alone. Pearson's correlations were also used to characterize associations between our parameters of lower leg function and metabolism and the IMF:SM ratio in the lower leg. Continuous data are presented as mean ± standard deviation.

5.1 f) Results

5.1 f) i: Participants

Twenty-two women with BC responded to recruitment letters, and after further screening, six were excluded due to a history of diabetes (n=2), MRI incompatibility (n=2), and failure to respond to follow-up calls regarding scheduling (n=2). Of the remaining sixteen BC survivors who enrolled, fifteen received epirubicin, one received doxorubicin, fifteen received radiation therapy, and none received Trastuzumab (Table 1). On average, BC survivors were studied 12.8±4.7 months after administration of the final anthracycline treatment. The groups were well-matched for age, weight, body mass index, hemoglobin, cardiovascular risk factors, and self-reported exercise (Table 1).

5.1 f) ii: Cardiorespiratory Fitness

Peak cycle ergometer power output (132±31 vs. 165±30 W, $p<0.01$), absolute peak VO_2 (1.69±0.37 vs. 2.13±0.41 L/min, $p<0.01$) and peak VO_2 indexed to body mass (23.1±7.5 vs. 29.5±7.7 ml/kg/min, $p=0.02$) were significantly lower in BC survivors compared to controls. Peak exercise heart rate, S_aO_2, respiratory exchange ratio, and rating of perceived exertion did not differ between groups (Table 2).

Skeletal muscle oxidative capacity

Resting Pi:PCr ratio and pH were similar in both groups (Table 2). Peak PFE power output, heart rate, rating of perceived exertion, Pi:PCr ratio and pH were not different between groups (Table 2). Skeletal muscle oxidative capacity, measured as Pi:PCr ratio at a moderate-intensity sub-maximal exercise workloads and by post-exercise PCr recovery τ did not differ between BC and controls, with similar results for pH (Table 2)

5.1 f) iii: Rest and sub-maximal exercise lower leg VO_2 and its determinants

Resting lower leg S_vO_2 was lower in the BC as compared to control group (74±5 vs 80±5%, p<0.01), corresponding to a higher O_2 extraction ratio (0.24±0.06 vs 0.18±0.05, p=0.01), while leg blood flow was lower in the BC group (55±23 vs 77±29 ml/min, p=0.02) resulting in no difference between groups for resting leg VO_2 (Table 2). Sub-maximal exercise power output, VO_2, blood flow, S_vO_2 and O_2 extraction were similar between groups (Table 2, Figure 3).

Body composition

Subcutaneous fat volume did not differ between groups for the abdomen, thigh or lower leg regions (Table 3). In the abdomen, visceral fat volume was higher (BC 406±217 vs 273+123 mL, p=0.04), paraspinal SM volume was lower (313±39 vs 340±32 ml, p=0.04), but absolute IMF volume, IMF:SM, and SM fat fraction did not differ between groups. In the thigh, SM volume did not differ between groups, but IMF volume (82±31 vs 58±17 ml, p=0.01), IMF:SM ratio (0.36±0.19 vs 0.22±0.07, p=0.01) and SM fat fraction (25±10 vs 18±5, p=0.02) were all significantly higher in BC compared to controls. In the lower leg SM was reduced (752±149 vs 903±162 ml, p=0.01; Table 3), IMF volume did not differ, while the IMF:SM ratio (0.10±0.06 vs 0.06±0.02, p=0.03) and the SM fat fraction (9±5 vs 5±2%, p=0.02) (Table 3) were significantly higher in BC.

5.1 f) iv: Relationship of skeletal muscle and intermuscular fat with whole-body peak VO_2

On analysis of all study participants combined, the IMF:SM ratio in the paraspinal, thigh, and lower leg muscle groups each had a strong, inverse relationship to whole-body relative peak VO_2 (r=-0.70, p<0.001, r= -0.72, p<0.001, and r=-0.66, p<0.001 respectively; Figure 3). These relationships were similarly strong in the BC group (paraspinal: r=-0.70, p=0.003; thigh: r= -

0.71, p=0.002; lower leg: r=-0.68, p=0.003). The SM fat fraction had a slightly stronger relationship with whole-body peak VO_2 than the IMF:SM ratio, in all regions (paraspinal: r=-0.73, p<0.001; thigh: r= -0.78, p<0.001; lower leg: r=-0.71, p<0.001).

5.1 f) v: Relationship of lower leg muscle composition, resting and exercise metabolism, blood flow, oxygen consumption, and extraction

The lower leg IMF:SM ratio was not related to lower leg peak power output, Pi:PCr ratio, or pH (Table 4). Lower leg IMF:SM ratio correlated strongly and positively with lower leg sub-maximal exercise S_vO_2 (r=0.70, p<0.001). The IMF:SM ratio also had a strong inverse relationship with lower leg sub-maximal exercise VO_2 and O_2 extraction (r=-0.64, p=0.008; r=-0.67, p<0.001) and a moderate inverse relationship with lower leg exercise blood flow (r=-0.35, p=0.05; Table 4). These relationships were similarly evident within the BC group alone, except for blood flow, where the correlation effect size was similar but no longer significant due to greater variability (r=-0.37, p=0.16).

Discussion

Peak VO_2 is a strong independent predictor of CVD, disability and mortality in healthy and clinical populations.[149] Following BC treatment, women have a peak VO_2 that is on average 20% lower than age-matched healthy sedentary women.[13, 16, 17, 19, 124, 137] The mechanisms responsible for the reduced exercise tolerance are not well understood, however recent evidence suggests that 'non-cardiac' peripheral abnormalities associated with chemotherapy may be important contributors to reduced exercise tolerance.[29, 31] The major new finding of this study is that despite a significantly lower peak VO_2 during whole body exercise, BC survivors had preserved lower leg blood flow, leg VO_2 and mitochondrial oxidative capacity relative to controls during unilateral PFE. A second novel finding is that the IMF:SM ratio and SM fat

fraction in the thigh and lower leg are strongly related to peak VO_2, explaining approximately 50% of the variability in cardiorespiratory fitness. We also uncovered potential mediating mechanisms for the impact of the IMF:SM ratio on local VO_2. Specifically, a higher IMF:SM ratio in the lower leg was associated with increased S_vO_2, reduced muscle VO_2, blood flow, and O_2 extraction with moderate-intensity unilateral PFE. Taken together, these results suggest that the primary peripheral impairments evident approximately one-year after anthracyclines are abnormalities in skeletal muscle composition, namely increased IMF, which is an important contributor to the reduced whole-body VO_2 and O_2 extraction in BC survivors.

5.1 f) vi: Skeletal mitochondrial oxidative capacity in BC survivors

Contrary to our hypothesis, we did not observe impaired muscle function or reduced oxidative capacity in our group of BC survivors. Specifically, in response to unilateral PFE for which maximal blood flow is not limited by convective O_2 delivery, the peak PFE workloads achieved by the BC survivors did not differ from the controls. This was unexpected given the significant and marked reduction in cycle ergometer power output achieved by the BC group relative to the control group. Further, the Pi:PCr ratio, which reflects coupling of ATP use and resynthesis, did not differ between groups at rest or at moderate and peak intensity exercise, indicating similar patterns of aerobic and anaerobic metabolism during exercise (Table 2). Notably, the peak pH at the end of exercise was similar between groups, indicating comparable states of metabolic acidosis, allowing for comparison of post-exercise PCr recovery.[150] The PCr post-exercise recovery τ, a measure of mitochondrial ATP synthesis,[151-153] was not different between groups, nor blood flow, a potential confounding factor (Table 2). Taken together, these results suggest that, on average, women who received anthracyclines for BC do not have impaired SM mitochondrial function in response to small muscle mass exercise where the limiting role of the heart is minimized.

Using vastus lateralis skeletal muscle biopsy, Mijwel *et al.* showed that during receipt of anthracycline chemotherapy, women with BC experienced a reduction in oxidative muscle fibers, citrate synthase, and capillarity.[31] These deteriorations were attributed to deconditioning rather than a result of chemotherapy as a 16-week exercise intervention consisting of high-intensity aerobic and resistance interval training during adjuvant chemotherapy attenuated the decline in mitochondrial function.[31] In the present study, the women with BC were on average 13 months post-anthracycline treatment, and 75% self-reported performing some physical activity in the previous month. It is possible that this length of time since treatment and/or the physical activity performed may have contributed to recovery or preservation of mitochondrial function.

5.1 f) vii: Skeletal Muscle Composition and its relationship to VO_2

Haykowsky *et al.* previously reported that the reduced peak VO_2 in patients with heart failure and preserved ejection fraction (HFpEF) was associated with a higher thigh IMF:SM ratio.[30] Similarly, Reding *et al.* found inverse correlations between peak VO_2 and paraspinal IMF:SM ratio in 14 cancer survivors with mixed diagnoses and timing since treatment.[29] However, Reding *et al.* did not find differences in absolute amounts of IMF or the IMF:SM ratio of the paraspinal muscles between cancer survivors and non-cancer controls. In our study, we used a similar approach to assess the paraspinal muscle and found a similarly strong relationship between IMF:SM ratio and whole-body peak VO_2. We also assessed composition of the thigh and lower leg muscles, as the primary muscles driving oxygen uptake during cycle and PFE. We extended prior non-cancer and cancer studies by demonstrating that BC survivors previously treated with anthracycline chemotherapy have increased thigh IMF, IMF:SM ratio, SM fat fraction, and reduced lower leg SM, and increased lower leg IMF:SM and SM fat fraction compared to controls. Moreover, while the average IMF:SM ratio and SM fraction varied among the paraspinal, thigh, and lower leg muscles, it was inversely related to peak whole-body VO_2 during cycle exercise in all three regions,

implicating IMF in the pathophysiology of anthracycline-related VO_2 deterioration. A further illustration of the extent of impairment in SM composition among women with BC is that the IMF:SM ratio in our BC group was similar to that reported by Haykowsky *et al.* in patients with HFpEF.[30]

We have further added to the understanding of the impact of IMF on SM metabolism by using novel MRI techniques to non-invasively assess lower leg local VO_2 and O_2 extraction during submaximal PFE. We did not find any relationship between IMF:SM and SM exercise metabolism as assessed by ^{31}P spectroscopy. However, lower leg IMF:SM ratio was inversely related to local exercise VO_2 and O_2 extraction in both groups (Table 4). Specifically, the greater the amount of IMF relative to the amount of SM, the less O_2 consumed or extracted with exercise. That we found this relationship between IMF:SM and VO_2 for both whole-body and small muscle mass exercise indicates that the impact of IMF on VO_2 is independent of potential 'central' limitations. A potential explanation for this IMF effect can be drawn from a study demonstrating that blood flow to subcutaneous fat adjacent to exercising muscle increases in an intensity-dependent manner.[131] This study of subcutaneous fat proposed potential mechanisms of vasodilation of adipose tissue vessels including: 1) heat conduction from working muscle; 2) adenosine formation that occurs in fat during exercise; and 3) conductive vasodilation up the arterial tree. It is possible that a similar effect occurs in IMF where blood flow is shunted through the metabolically inactive IMF, resulting in increased mixed venous O_2 content.[131] Consequently, this could explain the strong relationship between lower leg IMF:SM ratio and both S_vO_2 and O_2 extraction (Table 4). Another potential mechanism for the impact of IMF on oxygen extraction is that the deposits of adipose tissue in the muscle could reduce muscle oxygen diffusive conductance due to a greater distance for O_2 to travel from the capillaries to muscle mitochondria and a limited ability to locally restrict vascular

conductance in adipose tissue in close proximity to active SM.[30] Finally, others have suggested that IMF may result in lower overall blood flow to a tissue[154]; indeed we observed a negative correlation between exercise blood flow to the entire lower leg (i.e., both fat and lean tissue) and IMF:SM in both groups combined (Table 4).

In other populations, increased levels of IMF are associated with inflammation, insulin resistance, reduced muscular activation, and reduced physical function.[133] In combination with the findings from the current study, prevention and reduction of IMF accumulation should be an important goal for individuals with cancer. Both exercise and dietary interventions can reduce IMF, but in either case, it appears that weight loss is necessary to achieve reductions in IMF.[133] Weight loss induced by exercise may more effectively reduce IMF compared to weight loss induced by caloric restriction.[134-136, 155] Furthermore, the combination of caloric restriction and aerobic exercise appears to be substantially more effective for reducing IMF compared to caloric restriction and resistance exercise in premenopausal obese women.[155]

Limitations

A limitation of this study is that lower leg VO_2 and its determinants were only measured at rest and sub-maximal PFE. While caution is warranted in extending these measures to maximal PFE, it is unlikely that group differences would exist, as the Pi:PCr ratio and intracellular pH during and after incremental to maximal exercise were not different between groups. Finally, blood flow was measured in the superficial femoral vein and was assumed to be representative of lower leg venous return. Future studies may consider using positron emission tomography or arterial spin labeling techniques to measure skeletal muscle perfusion during both isolated and whole-body exercise.

5.1 g) Conclusion

In women with BC treated with anthracycline chemotherapy one year earlier, peak VO_2 and power output during whole body (cycle) exercise are significantly reduced relative to age- and BMI-matched controls. However, during small muscle mass exercise that is not limited by cardiac reserve, women with BC were able to perform a similar amount of work with a similar lower leg VO_2, blood flow, O_2 extraction and oxidative capacity to controls. The primary peripheral anomaly in BC compared to controls was in SM composition, with an increased IMF:SM ratio and SM fat fraction in the thigh and lower leg among the women with BC. This is an importance difference as a higher IMF:SM ratio was associated with lower whole-body peak VO_2 and reduced lower leg blood flow, VO_2 and O_2 extraction. Therefore, IMF appears to play an important role in the impaired cardiorespiratory fitness reported following completion of BC treatment. Diet and exercise interventions targeted to reduce the relative amount of IMF and increase the absolute amount of SM in the legs should be investigated as possible therapies to improve cardiorespiratory fitness following cancer therapy.

5.1 h) References

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5.1 a) Figure Legends:

5.1 a) i: Figure 1:

A) Experimental set up for plantarflexion exercise device. B) Image acquisition for determination of lower-leg VO_2, showing the typical location for imaging of the superficial femoral vein and representative SvO_2 and blood velocity images, at rest and immediately post-exercise. In this subject, $SvO_2 \approx 86\%$ at rest and 57% post exercise, with corresponding blood velocities of 7 cm/s and 25 cm/s.

5.1 a) ii: Figure 2:

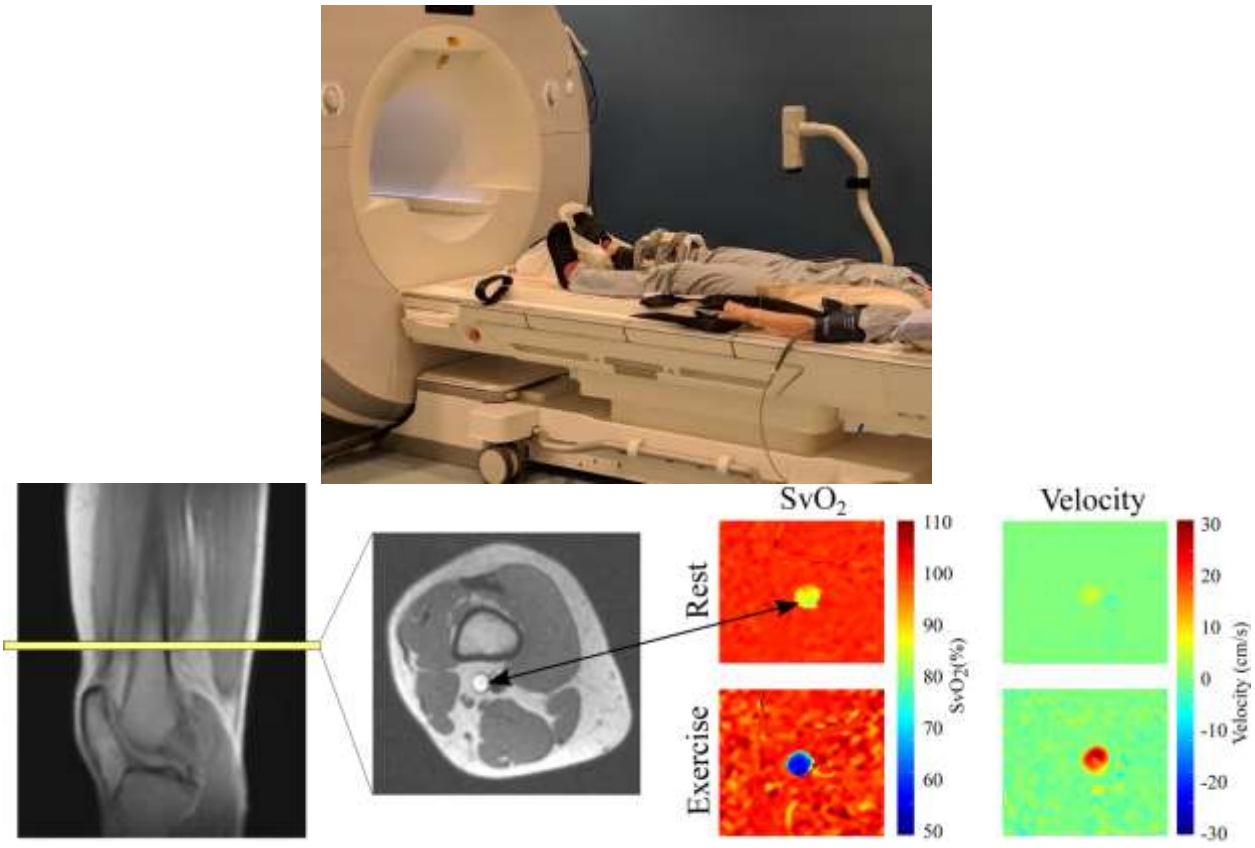
MR body composition acquisition and analysis by the modified Dixon fat-water separation approach in the A) lower leg, B) thigh, and C) abdomen of a representative participant with low intermuscular fat and in the D) lower leg, E) thigh and F) abdomen of a participant with high intermuscular fat.

5.1 a) iii: Figure 3:

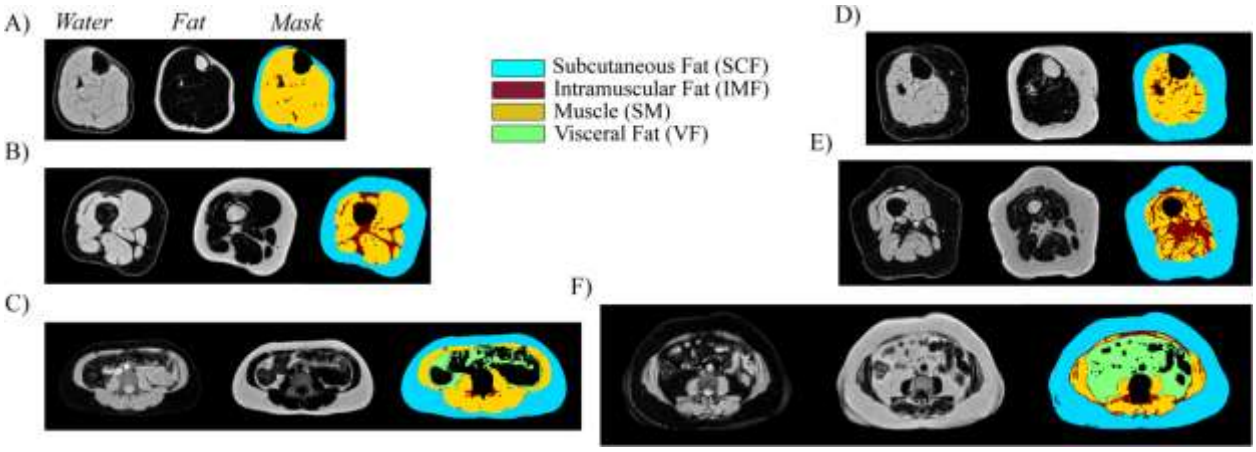
Relationship between IMF: SM ratio and whole-body peak VO_2 for the paraspinal, thigh and lower leg muscle regions.

5.1 b) Figures:

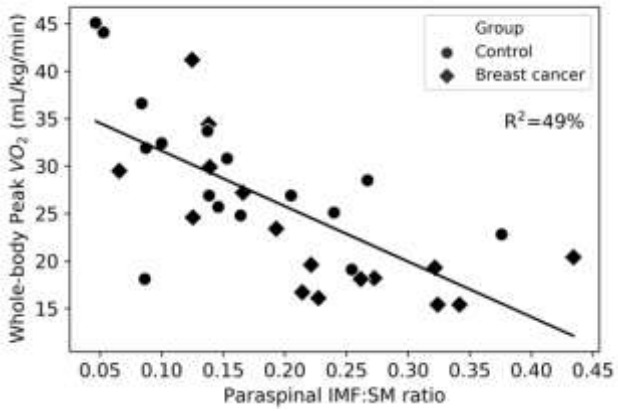
5.1 b) i: Figure 1: A) Experimental set up for plantarflexion exercise device. B) Image acquisition for determination of lower-leg VO_2, showing the typical location for imaging of the superficial femoral vein and representative SvO_2 and blood velocity images, at rest and immediately post-exercise. In this subject, $SvO_2 \approx 86\%$ at rest and 57% post exercise, with corresponding blood velocities of 7 cm/s and 25 cm/s.

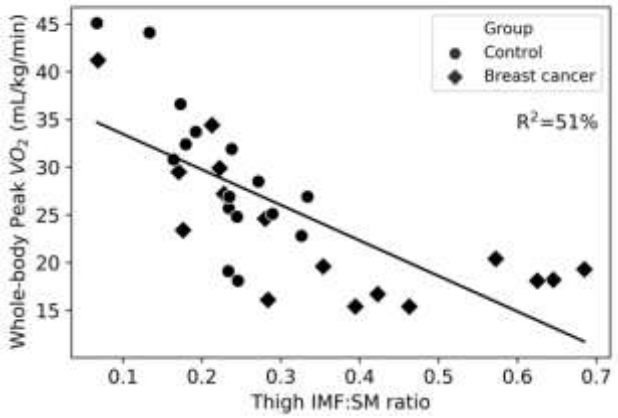


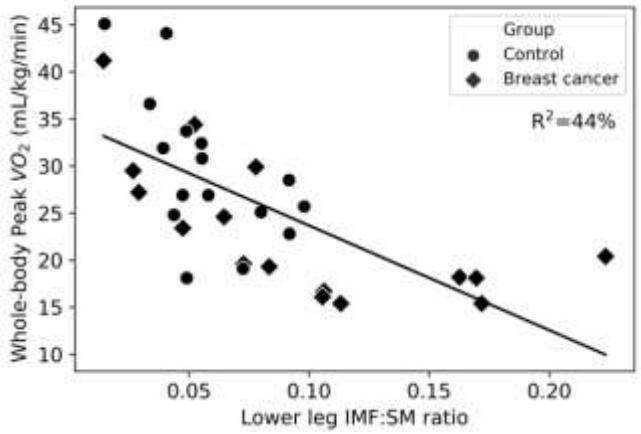
5.1 b) ii: Figure 2: MR body composition acquisition and analysis by the modified Dixon fat-water separation approach in the A) lower leg, B) thigh, and C) abdomen of a representative participant with low intermuscular fat and in the D) lower leg, E) thigh and F) abdomen of a participant with high intermuscular fat.



5.1 b) iii: Figure 3: Relationship between IMF: SM ratio and whole-body peak VO2 for the paraspinal, thigh and lower leg muscle regions.







5.1 c) Tables:

5.1 c) i: Table 1: Participant characteristics.

Parameter	BC (n=16)	Control (n=16)	P value
Age (years)	56 (10)	56 (10)	0.95
Weight (kg)	75.6 (12.0)	74.9 (11.7)	0.87
Body Mass Index (kg/m^2)	29.0 (3.6)	27.9 (4.9)	0.47
Self-Reported Exercise	225 (275)	313 (208)	0.16
Hemoglobin (g/L)	133 (11)	134 (8)	0.67
Hematocrit (%)	40 (3)	41 (2)	0.51
Cardiovascular Risk Factors			
Sedentary (%)	25%	13%	0.65
Hypertension (%)	13%	0%	0.48
Hypercholesterolemia (%)	0%	13%	0.48
Former Smoker (%)	44%	50%	1
Current Smoker (%)	0%	0%	1
Diabetes (%)	0%	0%	1
Overweight (%)	31%	50%	0.47
Obese (%)	44%	44%	1
Other Comorbid Conditions			
Arthritis (%)	31%	44%	0.72
Osteoporosis (%)	13%	6%	1
Thyroid Disorder (%)	13%	19%	1
Lung Disease (%)	0%	0%	1
Breast Cancer Characteristics			
Stage II/III/IV (n)	8/7/1	-	-
Epirubicin/Doxorubicin (n)	15/1	-	-
Dose (mg/m^2)	307 (75)	-	-
Does Reduction (n)	3	-	-
No Surgery/ Lumpectomy/ Mastectomy (n)	1/6/9	-	-
Radiation Therapy (n)	15	-	-
Hormone Therapy (n)	11	-	-

5.1 c) ii: Table 2: Cardiopulmonary exercise performance during cycle exercise and lower leg muscle metabolism and oxygenation at rest and after plantar flexion exercise in BC survivors and controls.

Parameter	BC	Control	p value
Peak Cycle Ergometry			
Power output (W)	132 (31)	165 (30)	**<0.01**
Oxygen uptake (l/min)	1.69 (0.37)	2.13 (0.41)	**<0.01**
Oxygen uptake (ml/kg/min)	23.1 (7.5)	29.5 (7.7)	**0.02**
Respiratory exchange ratio	1.29 (0.09)	1.27 (0.08)	0.60
S_aO_2 (%)	96 (2)	94 (2)	0.08
Heart rate (bpm)	166 (12)	161 (14)	0.35
Rating of perceived exertion (0-10	9 (1)	9 (1)	0.68
Rest (lower leg)			
Pi:PCr	0.10 (0.03)	0.11 (0.03)	0.24
pH	7.06 (0.06)	7.08 (0.10)	0.44
Blood flow (ml/min)	55 (23)	77 (29)	**0.02**
Pulse oximeter S_aO_2 (%)	97 (2)	98 (2)	0.36
S_VO_2 (%)	74 (5)	80 (5)	**<0.01**
O_2 extraction ratio	0.24±0.06	0.18±0.05	**0.01**
VO_2 (ml/min)	2.1 (0.84)	2.3 (0.54)	0.64
Sub-maximal (60% of peak) plantar flexion exercise			
Power output (W)	10.9 (1.2)	12.1 (2.4)	0.09
Peak blood flow (ml/min)	526 (171)	581 (198)	0.41
Peak S_vO_2 (%)	55 (7)	54 (5)	0.81
Peak O_2 extraction ratio	0.44±0.07	0.45±0.06	0.72

Peak VO_2 (ml/min)	40 (15)	45 (15)	0.31
Peak plantar flexion exercise			
Power output (W)	18.0 (2.4)	19.6 (3.4)	0.13
Heart rate (bpm)	102 (16)	100 (20)	0.84
Rating of perceived exertion (0-10 for lower leg)	10 (1)	10 (0)	0.28
Pi:PCr	0.98 (0.32)	0.90 (0.24)	0.41
pH	6.46 (0.22)	6.45 (0.28)	0.39
Recovery (lower leg)			
PCr τ (s)	36 (11)	33 (9)	0.33

*5.1 c) iii: Table 3: Body composition in the thigh, lower leg and abdomen. *Thigh data is for 4 cm of transverse slices starting at 4 cm from distal end of femur. †Lower leg data is for whole lower leg starting 5 cm below tibial plateau until distal end of gastrocnemius.‡ Lumbar spine data is 1.2 cm section of transverse images at the center of the third lumbar vertebrae.*

Variable	BC (n=16)	Controls (n=16)	P-value
Thigh*			
Total volume (mL)	754±83	771±147	0.70
Subcutaneous fat (g)	397±83	420±127	0.55
Muscle (g)	249±53	266±37	0.27
Intermuscular fat (g)	82±31	58±17	**0.01**
IMF:SM	0.36±0.19	0.22±0.07	**0.01**
SM fat fraction (%)	25±10	18±5	**0.02**
Lower leg†			
Total volume (mL)	1599±285	1884±395	**0.03**
Subcutaneous fat (g)	659±189	787±264	0.13
Muscle (g)	752±149	903±162	**0.01**
Intermuscular fat (g)	65±32	51±23	0.06
IMF:SM	0.10±0.06	0.06±0.02	**0.03**
SM fat fraction (%)	9±5	5±2	**0.02**
Abdomen‡			
Total volume	1684±471	1490±473	0.25
Subcutaneous fat	897±258	823±379	0.52
Muscle	313±39	340±32	**0.04**
Intermuscular fat	69±27	53±28	0.13
IMF:SM	0.22±0.10	0.16±0.09	0.06
SM fat fraction	18±6	13±6	0.06
Visceral fat	406±217	273+123	**0.04**

5.1 c) iv: Table 4: Pearson correlations between lower leg IMF:SM ratio and skeletal muscle function, resting and exercise metabolism.

	BC group (n=16)		**All participants (n=32)**	
	r	**p-value**	**r**	**p-value**
Peak plantar flexion power output	-0.24	0.38	-0.29	0.11
Peak plantar flexion Pi:PCr	-0.20	0.46	-0.17	0.35
Peak plantar flexion pH	0.42	0.11	0.22	0.21
Pi:PCr @ ~60% peak power	0.21	0.44	0.07	0.71
pH @ ~60% peak power	0.12	0.66	0.17	0.35
PCr recovery tau	0.03	0.92	0.14	0.43
Lower leg exercise blood flow	-0.37	0.16	-0.35	**0.05**
Lower leg exercise VO_2	-0.63	**0.008**	-0.64	**0.008**
Lower leg exercise S_vO_2 (%)	0.76	**<0.001**	0.70	**<0.001**
Lower leg exercise O_2 extraction	-0.71	**0.002**	-0.67	**<0.001**

Chapter 6

6.1 Conclusion

Breast cancer (BC) mortality has decreased by more than 40% over the last three decades.[122] Accordingly, cardiovascular health is becoming an increasingly important focus when planning cancer treatment, and care beyond antineoplastic therapy. Anthracyclines are proven to provide a BC survivorship advantage, however, at the potential cost of cardiovascular health.[6] We developed and used non-invasive imaging technology to comprehensively investigate pathophysiologic mechanisms leading to cardiovascular decline in BC survivorship.

The strongest predictor of all-cause and cardiovascular mortality in healthy and clinical populations is $VO_{2\ peak}$, which is reduced by 20% in BC survivors relative to age- and sex-matched control subjects. In Chapter 3, we demonstrate reduced $VO_{2\ peak}$ in BC patients prior to receipt of anthracycline chemotherapy. In these patients, using state of the art exercise cMRI we found that peak cardiac output is reduced relative to controls. Importantly, the lower exercise cardiac output was the result of reduced cardiac size rather than cardiac dysfunction, and was evident in the absence of a cardiotoxic stimulus. Supine peak cardiac output was significantly related to upright $VO_{2\ peak}$, but explained less than half of the variance in effect.

We further investigated $VO_{2\ peak}$ deficits in BC survivors by studying non-cardiac, peripheral determinants in Chapter 4. Using single leg knee extension exercise, where the limiting role of the heart is minimized, we found that long-term BC survivors (12 years post anthracycline therapy) had no decrement in peak work rate or leg blood flow, but had reduced estimated quadriceps mass. The finding of preserved leg blood flow in the face of reduced muscle mass implicates uncoupling of the former with oxygen use (i.e. inefficient delivery of blood to active muscle). In Chapter 5, using novel MR strategies, we further investigated this phenomenon to comprehensively measure leg composition, lower leg blood flow, and muscle oxidative capacity. Specifically, BC survivors (>3 months post anthracycline therapy) and age-

and sex matched controls underwent incremental to maximal plantar flexion exercise, another paradigm whereby the limiting role of the heart is minimized. Peak work rate, peak Pi:PCr ratio, muscle acidity, and mitochondrial oxidative capacity in the lower leg as measured by ^{31P}MRS were not different between BC survivors and controls. Further, in response to submaximal plantarflexion exercise, no differences were found in lower-leg blood flow, oxygen extraction or VO_2, despite a severe reduction in whole-body $VO_{2\ peak}$. However, BC survivors had poor body composition relative to controls, as indicated by reduced muscle mass, increased adiposity, or an unfavorable shift in the ratio of adipose tissue to skeletal muscle. Whole body $VO_{2\ peak}$ was strongly related to muscle composition; specifically, an inverse relationship was found between the intermuscular fat (IMF) to skeletal muscle (SM) ratio and $VO_{2\ peak}$. An increased IMF:SM ratio could impair $VO_{2\ peak}$ through mechanisms including: 1) shunting of blood through IMF, resulting in poor matching of blood flow to metabolic demand; 2) diffusive conductance limitations resulting in impaired oxygen extraction; or 3) insulin resistance mediated perturbations in oxidative SM function resulting in reduced oxygen utilization. The relationship between IMF:SM ratio and $VO_{2\ peak}$ was evident across BC and controls; BC had a greater IMF:SM ratio in the thigh, the predominant source of oxygen consumption during cardiopulmonary (cycle) exercise testing.

Taken together, our findings do not support a causal relationship between anthracycline toxicity and exercise intolerance. Several studies report underlying $VO_{2\ peak}$ deficits in BC prior to receipt of anthracycline therapy, and we demonstrate that reductions in peak cardiac output are present prior to anthracycline administration (Chapter 3).[109, 124] Further, our findings do not support unique peripheral mechanisms of anthracycline related exercise intolerance. Instead, reduced $VO_{2\ peak}$ was strongly linked to skeletal muscle mass and muscle composition, with a

propensity of BC survivors having higher adiposity and lower muscle mass relative to disease and treatment naive control subjects. Importantly, neither this phenotype, nor the cardiopulmonary effects of this phenotype are unique to BC (Chapter 5).

While we observed reduced $VO_{2\ peak}$ prior to administration of anthracycline therapy, there is convincing evidence of further reductions in $VO_{2\ peak}$ from pre-to-post-therapy. Early evidence is available to establish a working theory of physiologic mechanisms leading to reduced $VO_{2\ peak}$ in BC. First, Howden *et al.* reported that $VO_{2\ peak}$ declined by 15% from pre (0 months) to post anthracycline therapy (4 months) in BC patients (n=14).[156] Further, this decline was attributed to a reduction in calculated arterial-venous oxygen content difference (a-vO_2), as peak cardiac output was unchanged by anthracycline therapy. In the same study, a group of BC patients (n=14) underwent an exercise training intervention which maintained $VO_{2\ peak}$, by preserving peak cardiac output and a-vO_2 difference. Moreover, a subset of participants who were followed up at 16 months, at which time changes in $VO_{2\ peak}$ persisted from 4 months, but did not worsen.[123] By 16 months, peak cardiac output was significantly reduced as a result of reduced biventricular ejection fraction and ejection fraction reserve. Notably, this change in peak cardiac output indicates a "compensatory" increase in a-vO_2 difference to preserve $VO_{2\ peak}$. One interpretation of these findings is that aberrant peripheral changes occur, resulting in later cardiac adaptation towards normal cardiac output to $VO_{2\ peak}$ matching.

In support of this theory, one study is available providing information on muscle composition and quality changes from pre-to-post-anthracycline therapy. Mijwel *et al.* provide strong evidence of muscle deterioration using baseline and post-therapy vastus lateralis biopsy.[31] In the usual care group, mitochondrial oxidative capacity and type I (oxidative) muscle fiber cross-sectional area decreased. In the same study, intervention groups underwent resistance or

aerobic exercise training which preserved mitochondrial function and muscle fiber composition. The authors proposed that changes observed in the usual care group were related to anthracycline associated reductions in physical activity, rather than anthracycline myo-toxicity. Indeed, these results align well with a study from nearly 2-decades earlier that found that adjuvant therapy was associated with a reduction in physical activity without an accompanying reduction in caloric intake, leading to muscle loss with net weight gain.[27] The consequence of reduced muscle mass and increased adiposity is reduced $VO_{2\ peak}$.

Taken together with our finding of a physiologic link between an increased IMF:SM ratio and reduced $VO_{2\ peak}$, it is possible that peak cardiac output adapts to the minimal value needed to meet peripheral demands. Indeed, we observed increasing $VO_{2\ peak}$ with increasing cardiac output (Chapter 3) as well as decreasing IMF:SM ratio (Chapter 5) independent of a history of BC. Accordingly, preserving or improving body composition should be an important goal and clinical endpoint when designing interventions to improve cardiovascular function in BC survivorship. Critically, there is already a wealth of data demonstrating the efficacy of exercise training to preserve or improve muscle composition in older women, while cardiac adaptation presents a greater challenge.[157-159] Data from Foulkes *et al.* demonstrating aberrant cardiac changes only 12 months after completing therapy stresses the importance of initiating cardio-protective and/or body composition protective exercise training interventions during, or immediately after completion of adjuvant therapy.[123] To this end, future studies are required to test effectiveness of interventions at increasing $VO_{2\ peak}$ and curving CV risk. In consideration of clinical feasibility of an exercise intervention, strategies should consider the cost effectiveness, the minimal amount of exercise needed to preserve cardiorespiratory fitness, patient feasibility, and long term sustainability.

6.1 a) References

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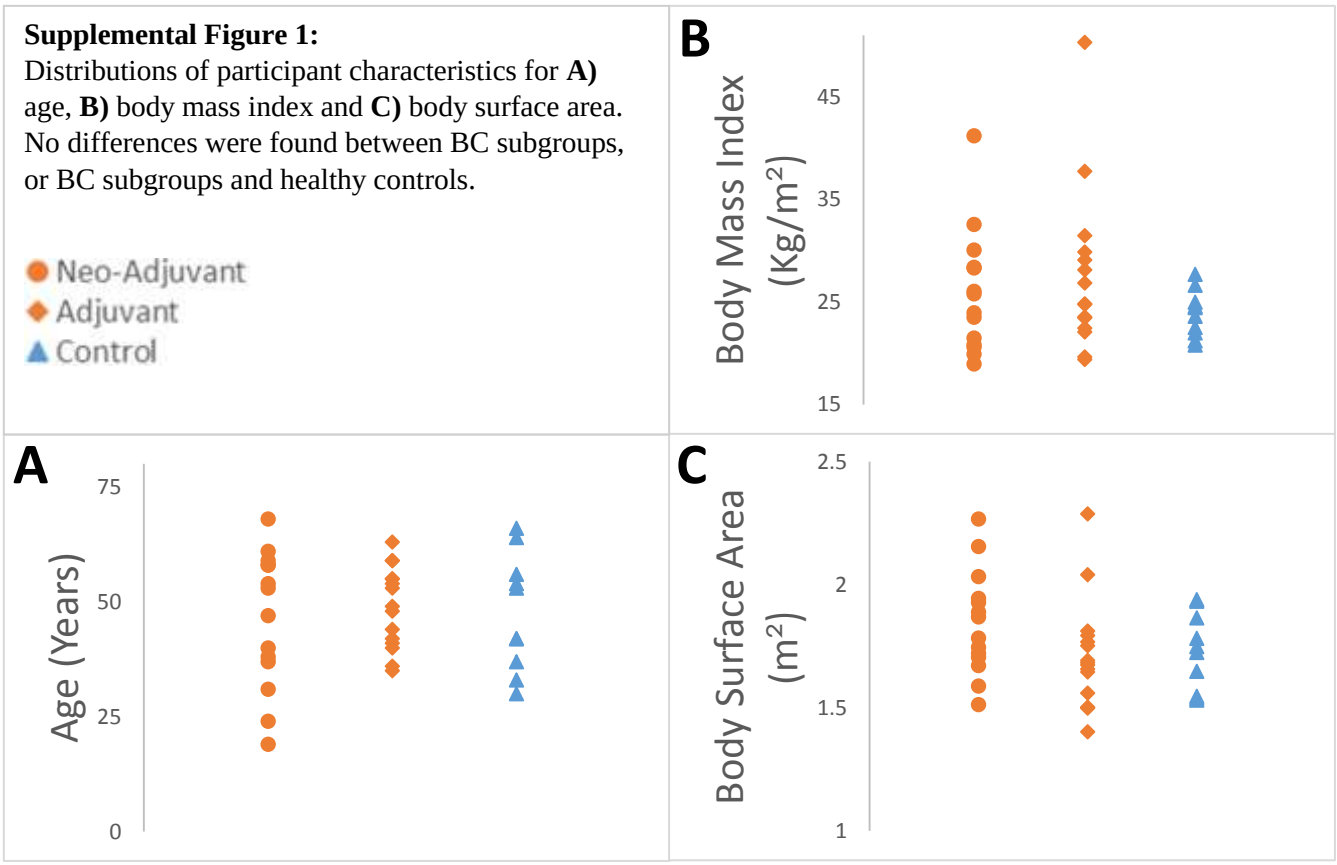
Chapter 7

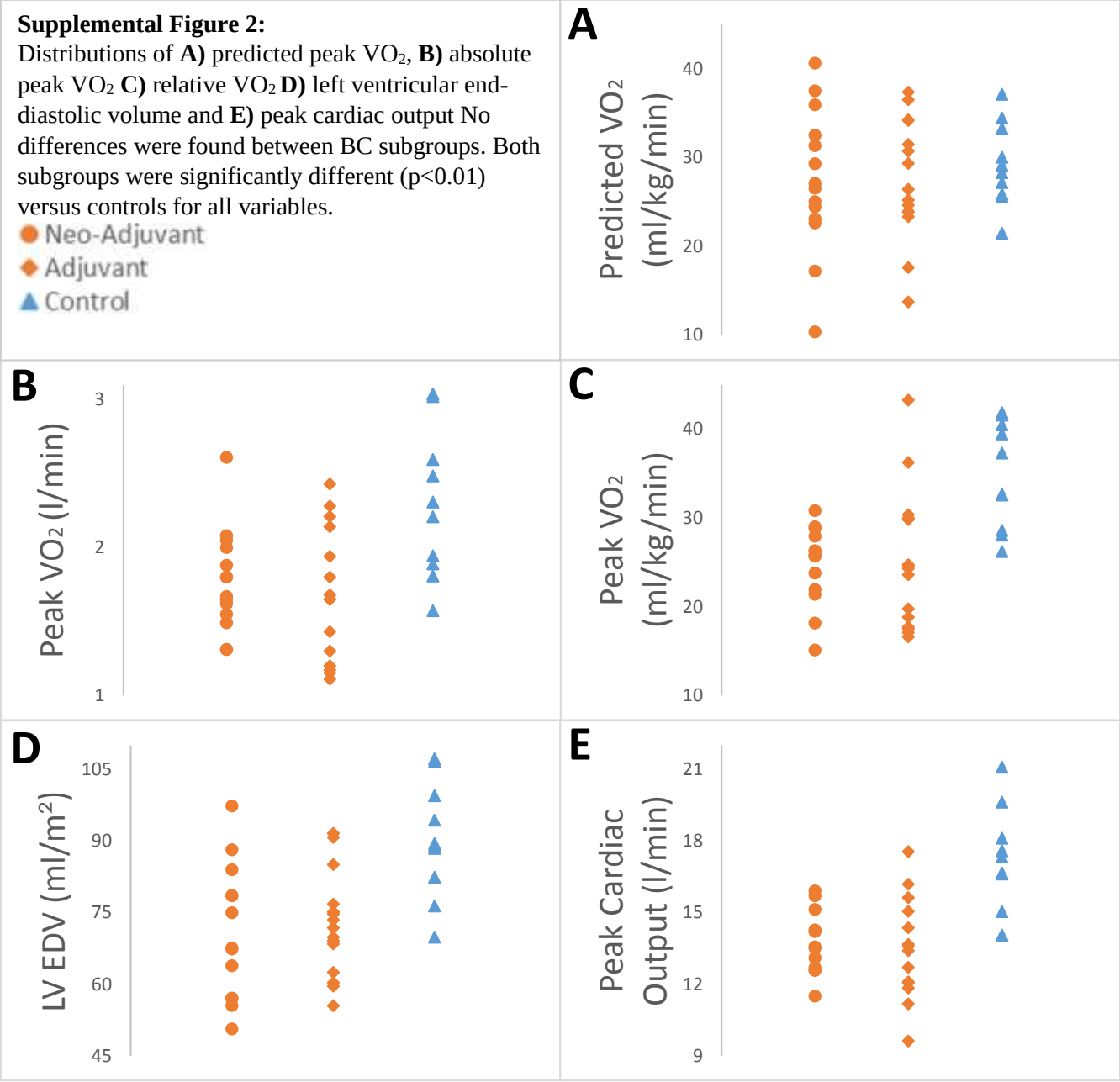
7.1 Appendix

7.1 a) Chapter 3 Data Supplement

Supplemental Figure 1:
Distributions of participant characteristics for **A)** age, **B)** body mass index and **C)** body surface area. No differences were found between BC subgroups, or BC subgroups and healthy controls.



Supplemental Figure 2:
Distributions of **A)** predicted peak VO_2, **B)** absolute peak VO_2 **C)** relative VO_2 **D)** left ventricular end-diastolic volume and **E)** peak cardiac output No differences were found between BC subgroups. Both subgroups were significantly different ($p<0.01$) versus controls for all variables.



Supplemental Table 1:

Mean, standard deviation and p values (student's t-test) for breast cancer subgroups versus healthy controls. Significant values are bolded.

Characteristic	Neoadjuvant Subgroup	Adjuvant Subgroup	Healthy Control	P; Neoadjuvant vs Adjuvant	P; Neoadjuvant vs Healthy Control	P; Adjuvant vs Healthy Control
Age	46 (14)	49 (9)	48 (12)	0.56	0.80	0.79
BMI	25.8 (6)	28 (8)	24 (2)	0.51	0.33	0.16
BSA	1.84 (0.2)	1.72 (0.2)	1.73 (0.1)	0.13	0.15	0.92
VO_2 (ml/kg/min)	25 (4)	25(8)	35 (6)	0.94	**0.00009**	**0.002**
VO_2 (l/min)	1.77 (0.3)	1.68 (0.4)	2.29 (0.5)	0.52	**0.007**	**0.004**
Peak Power (Watts)	146 (39)	134 (51)	228 (51)	0.50	**0.0003**	**0.0002**
Peak Cardiac Output (l/min)	13.7 (1.3)	13.5 (2.1)	17.0 (2.2)	0.75	**0.0005**	**0.0008**