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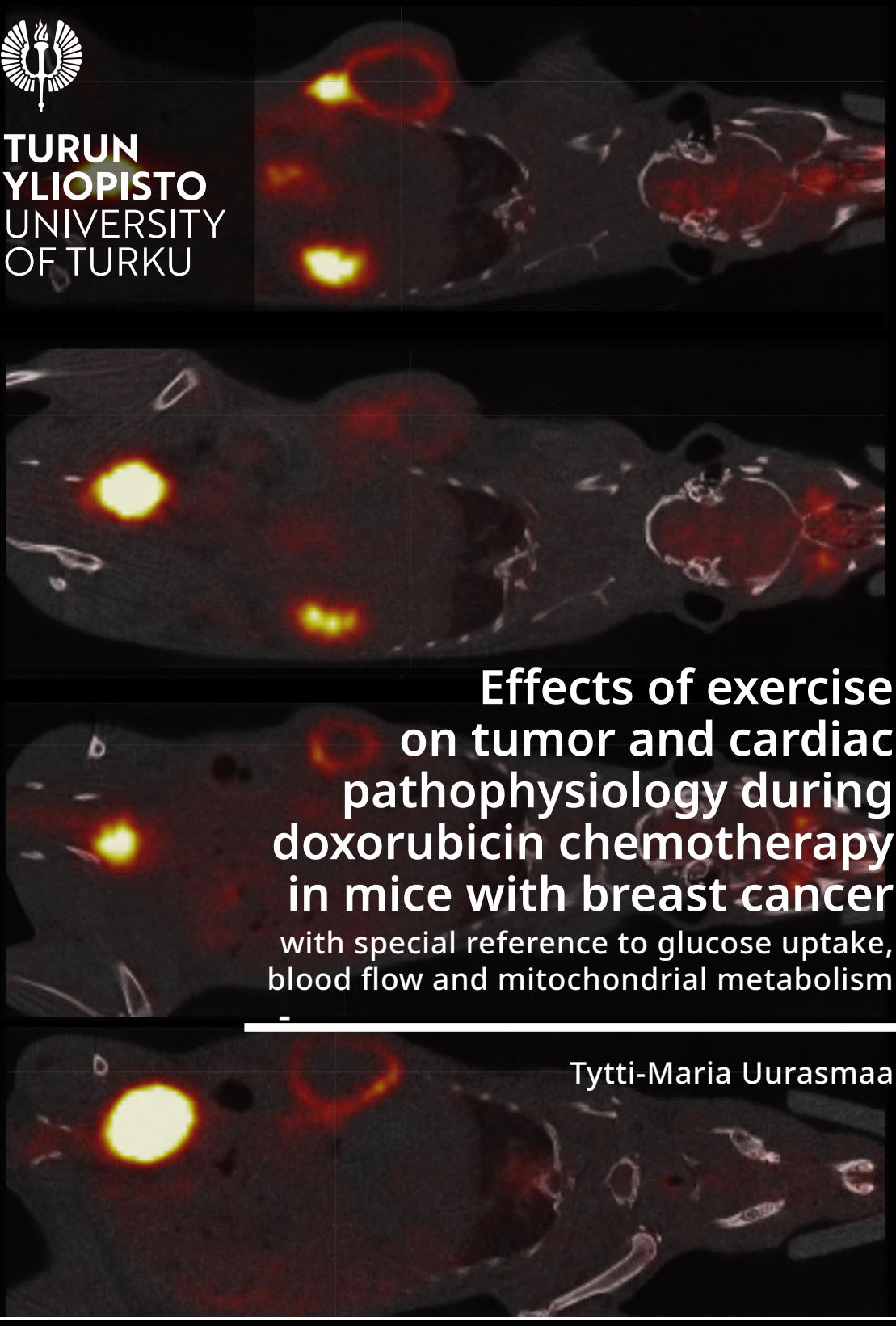

Effects of exercise on tumor and cardiac pathophysiology during doxorubicin chemotherapy in mice with breast cancer

with special reference to glucose uptake, blood flow and mitochondrial metabolism

Tytti-Maria Uurasmaa

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Dedicated to my parents and my brother

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ABSTRACT

Breast cancer patients treated with anthracycline chemotherapeutics such as doxorubicin (DOX) have increased risk of cardiomyopathy. Studies suggest that exercise training (ET) might increase the efficacy of DOX and reduce its cardiotoxic effect, but ET effects on tumor and cardiac pathophysiology are not yet fully known. This thesis aimed to elucidate the effects of voluntary wheel running ET during DOX treatment on the tumor growth and DOX induced cardiotoxicity. The thesis focused particularly on the ET induced alterations in the tumor and heart glucose uptake, mitochondrial metabolism, and blood flow.

Female FVB-mice subcutaneously inoculated with I3TC-mammary tumor cells or saline were given running wheels with DOX or saline treatment. The heart function was measured at baseline and after two (T2) and four DOX doses (T3). Heart and tumor glucose uptake were measured with positron emission tomography (PET) at T2 and T3. Tumor blood flow and blood mean transit time (MTT) were measured with PET at T2. Several molecular variables were also measured from the tumors and hearts. The Study I showed that ET inhibits tumor growth by increasing tumor apoptosis without vascular normalization. ET also increased left ventricle (LV) capillarity and lactate dehydrogenase (LDH) activity. Study II revealed that DOX reduced over time mouse running activity, body weight, LV mass, ejection fraction, LV capillarity and mitochondrial coupling, and increased LV glucose uptake. ET ameliorated all of these changes except body weight loss and capillary rarefaction. Furthermore, ET increased LV citrate synthase activity of all mice and decreased LV LDH activity in DOX treated mice. Study III revealed that a longer time period ET with lower running activity than in Study I reduces tumor metabolic volume, maximum glucose uptake without affecting total lesion glycolysis, mitochondrial function or tumor growth. Initially at T2 ET without DOX reduced tumor MTT, blood vessel α-smooth muscle actin coverage, larger vessel density and caused a trend towards improved blood flow, but vascular changes did not persist until T3. This thesis clearly reveals beneficial effects of ET on tumor and heart glucose uptake and blood flow warranting further studies with more effective DOX treatment and more intense ET, particularly regarding tumor effects.

KEYWORDS: cardiac dysfunction, physical activity, chemotherapy, heart function, oxidative stress, mitochondrial function, blood flow, glucose uptake

TURUN YLIOPISTO
Matemaattis-luonnontieteellinen tiedekunta
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Tytti-Maria Uurasmaa: Liikunnan vaikutukset kasvaimen ja sydämen patofysiologiaan doksorubisiini kemoterapian aikana hiiren rintasyöpä mallissa – tutkimuksia glukoosinotosta, verenvirtauksesta ja mitokondrioiden toiminnasta
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TIIVISTELMÄ

Rintasyöpäpotilailla, joita on hoidettu antrasykliinikemoterapialla, kuten doksorubisiinilla (DOX), on suurentunut kardiomyopatian riski. Tutkimusten mukaan liikunta voisi tehostaa DOX-hoitoa ja vähentää sen kardiotoksisuutta, mutta liikunnan vaikutuksia kasvainten ja sydämen patofysiologiaan ei täysin tunneta. Väitöskirjassa selvitettiin vapaaehtoisen juoksupyöräliikkunnan vaikutuksia kasvaimen kasvuun ja DOX:n aiheuttamaan kardiotoksisuuteen DOX-hoidon aikana hiirillä, keskittyen etenkin liikunnan vaikutuksiin glukoosinottoon, verenkiertoon ja mitokondrioihin

FVB-hiiri naaraiden ihon alle injektoitiin I3TC-rintasyöpäsoluja tai suolaliuosta ja niille annettiin juoksupyörät. Hiiriä hoidettiin DOX:lla tai suolaliuoksella ja sydämen toimintaa mitattiin lähtötilanteessa sekä kahden (T2) ja neljän (T3) DOX-annoksen jälkeen. Positroniemissiotomografialla (PET) mitattiin sydämen ja kasvaimen glukoosinottoa T2:ssa ja T3:ssa. Kasvaimen verenvirtaus ja veren keskimääräinen läpikulkuaika (MTT) mitattiin PET:lla T2:ssa. Kasvaimista ja sydämistä mitattiin myös molekyylitason muuttujia. **Tutkimus I** osoitti liikunnan estävän kasvaimen kasvua lisäämällä apoptoosia ilman verisuonten normalisoitumista. Liikunta lisäsi myös vasemman kammion (LV) laktaattidehydrogenaasin (LDH) aktiivisuutta ja hiussuonitusta. **Tutkimus II** paljasti DOX:n vähentävän ajan mittaan hiirten juoksemista, painoa, ejektiofraktiota, LV:n massaa, LV:n hiussuonitusta ja mitokondrioiden kytkentää sekä lisäävän LV:n glukoosinottoa. Liikunta lievitti kaikkia näitä muutoksia paitsi painonpudotusta ja hiussuonten harventumista. Liikunta lisäsi myös LV:n sitraattisyntaasin aktiivisuutta kaikilla hiirillä ja vähensi LV:n LDH:n aktiivisuutta DOX-hoidetuilla hiirillä. **Tutkimus III** paljasti, että pidempi liikuntajakso alhaisemmalla juoksumäärällä kuin tutkimuksessa I vähensi kasvaimen maksimi glukoosinottoa ja glukoosinottoa suuren aineenvaihdunnan alueella vaikuttamatta koko kasvaimen glukoosinottoon, mitokondrioiden toimintaan tai kasvuun. Aluksi, T2:ssa, liikunta ilman DOX:ia vähensi kasvaimen MTT:tä, α-sileälihas aktiinin peittävyyttä verisuonissa, suurien verisuonien tiheyttä ja aiheutti lähes merkittävän nousun kasvaimen verenvirtauksessa, mutta verisuonimuutokset eivät säilyneet T3:n asti. Väitöskirja osoittaa selkeästi liikunnan hyödylliset vaikutukset kasvaimen ja sydämen glukoosinottoon ja verenkiertoon. Jatkotutkimuksia tarvitaan tehokkaamman DOX-hoidon ja intensiivisemmän liikunnan vaikutuksista etenkin kasvaimiin.

ASIASANAT: sydämen toimintahäiriö, fyysinen aktiivisuus, kemoterapia, sydämen toiminta, oksidatiivinen stressi, mitokondrioiden toiminta, verenkierto, glukoosinotto.

Table of Contents

Abbreviations

α-SMA	Alpha smooth muscle actin
ATP	Adenosine triphosphate
CA IX	Carbonic anhydrase IX
CAT	Catalase
CS	Citrate synthase
Cyt C	Cythochrome C
DCT	Doxorubicin-induced cardiotoxicity
DOX	Doxorubicin
E/A	Ratio of early diastole to late diastole blood flow peak velocity
EC	Endothelial cell
ER	Estrogen receptor
ET	Exercise training
ETS	Electron transport system
FADH2	Flavin adenine dinucleotide
[^{18}F]FDG	2-deoxy-2-[18F]fluoro-D-glucose
FS	Fractional shortening
GPx	Gluthathione peroxidase
GSH	Gluthathione
HER2	Human epidermal growth factor 2
HIF	Hypoxia inducible factor
HOAD	β-Hydroxyacyl CoA dehydrogenase
LDH	Lactate dehydrogenase
LV	Left ventricle
LVEF	Left ventricle ejection fraction
LV IF	Left ventricle input function
LVOT VTI	Left ventricular outflow tract velocity time integral
mtCK	Mitochondrial creatine kinase
MMPs	Matrix metalloproteinases
MTV	Metabolic tumor volume
MTT	Mean transit time
NADH	Nicotinamide adenine dinucleotide

NADPH	Nicotinamide adenine dinucleotide phosphate
NST	No special type
$[^{15}O]CO$	^{15}O-Carbon monoxide
$[^{15}O]H_2O$	^{15}O-Water
OXPHOS	Oxidative phosphorylation
PET	Positron emission tomography
PR	Progesterone receptor
ROI	Region of interest
SOD	Superoxide dismutase
SUV	Standardized uptake value
TAC	Time activity curve
TCA	Tricarboxylic acid
TLG	Total lesion glycolysis
TNBC	Triple negative breast cancer
TOP IIβ	Topoisomerase IIβ
VEFG	Vascular endothelial growth factor

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Uurasmaa TM, Ricardo C, Autio A, Heinonen IHA, Rundqvist H, Anttila K. Voluntary wheel running reduces tumor growth and increases capillarity in the heart during doxorubicin chemotherapy in a murine model of breast cancer. *Frontiers in Physiology Clinical and Translational Physiology*, 2024; 15:1347347. https://doi.org/10.3389/fphys.2024.1347347

II Uurasmaa TM, Bourdin P, Nammas W, Latifi S, Liljenbäck H, Saraste A, Eskola O, Rajander J, Roivainen A, Rundqvist H, Autio A, Heinonen I, Anttila K. Exercise training partly ameliorates cardiac dysfunction in mice during doxorubicin treatment of breast cancer. *Journal of Translational Medicine,* 2025; 23:89. https://doi.org/10.1186/s12967-025-06108-y

III Uurasmaa TM, Laitinen V, Koivula T, Siekkinen R, Ihalainen J, Shimochi S, Liljenbäck H, Iida H, Roivainen A, Rundqvist H, Autio A, Anttila K, Heinonen I. Glucose uptake and circulatory responses to exercise training in a pre-clinical breast cancer model. (manuscript).

1 Introduction

Breast cancer is the most common cancer in women, as well as the leading cause of cancer death in women (Bray et al., 2024). The incidence of breast cancer is growing in high-income countries, with low-income countries having lower incidence but disproportionally higher breast cancer mortality (Bray et al., 2024). Accordingly, in Finland the yearly breast cancer incidence has been growing while the mortality has been declining, but with breast cancer remaining the most common cause of cancer related death in women under 70 years of age (Pitkäniemi et al., 2024). The increase in breast cancer incidence and the overall higher incidence in high-income countries could be due to lifestyle factors such as exposure to oral contraceptives, alcohol consumption, high-fat diet, and physical inactivity, as well as due to better detection of breast cancer (Bray et al., 2024).

Despite the increasing incidence of breast cancer in high-income countries, the mortality rates of breast cancer have been in the decline since 1990s (Bray et al., 2024). With the improved survival of cancer patients, the prevalence of the long-term health effects of the cancer treatments are becoming more common (Jiang et al., 2022). It has been shown that breast cancer patients who have received anthracycline chemotherapy treatment have a higher risk of heart failure and cardiomyopathy compared to patients without history of anthracycline treatment (Greenlee et al., 2022).

Doxorubicin (DOX) is one of the most commonly used anthracycline chemotherapies and it is used, for example, to treat many solid tumors like breast cancer (Octavia et al., 2012). The exact mechanisms in which anthracyclines like DOX drive cardiotoxic effects are still not fully understood, but it is known that DOX affects the heart via multiple mechanisms and particularly via inhibiting topoisomerase IIβ (TOP IIβ), which is expressed throughout the cell cycle even in post mitotic cells, and for example within the myocardium (Capranico et al., 1992; Zhang et al., 2012). The proposed mechanisms underlying doxorubicin-induced cardiac toxicity (DCT) include cardiac apoptosis, calcium dysregulation, inflammation, oxidative stress and particularly mitochondrial dysfunction (Linders et al., 2024). The cardiotoxic effects of cancer treatments not only affect patient prognosis, but also limit the use of the drug affecting their treatment efficacy.

Treatment efficacy is also affected by development or the presence of chemotherapy resistant cancer cells, with chemotherapy resistance contributing to cancer mortality. Therefore, it is important to find ways to reduce cardiotoxic effects of common cancer treatments like DOX but also to enhance their efficacy.

Higher physical activity is associated with lower risk of cancers such as colon cancer and breast cancer, as well as lower risk of cardiovascular disease (Lee 2003). Exercise has been recognized as a potential adjunct therapy for cancer patients, as it has low risk of side effects, low cost and easy access. Exercise after breast cancer treatment can lower patient fatigue, improve physical activity level, aerobic fitness, muscular strength, functional quality of life, lower anxiety, and increase self-esteem (Speck et al., 2010). Some studies have also suggested that exercise could potentially improve chemotherapy efficacy in clinical and preclinical setting (Yang et al., 2021). Furthermore, exercise has been shown to be effective at combating DCT particularly in the preclinical setting (Dozic et al., 2023). However, the mechanisms in which exercise protects the heart from DCT and boosts chemotherapy efficacy not yet completely understood.

Exercise can likely attenuate DCT and potentially boost the chemotherapy efficacy via multiple mechanisms. So far, the alterations in the vascularity, blood flow and glucose metabolism remain understudied, particularly in breast cancer, and this thesis will aim to increase the knowledge on how these variables change the heart and tumor during exercise intervention done concomitantly with DOX treatment of breast cancer. Particularly, this thesis will focus on investigating how exercise could modify mammary tumor blood flow, vasculature, and mitochondrial function during DOX treatment. In addition, the investigation will focus on DOX induced vascular effects, oxidative stress, mitochondrial function and related metabolic enzyme function in the heart, and how exercise could alter these.

2 Review of the Literature

2.1 Breast cancer

2.1.1 Subtypes

Breast cancers arise in the cells of the collecting ducts in the breast within the terminal duct lobular units (Harbeck et al., 2019). Like all cancers breast cancer cells become cancerous by accumulating genetic mutations that enable them to express the hallmarks of cancer, such as apoptosis evasion, insensitivity to anti-growth signals as well as ability to make their own growth signals, induce angiogenesis, and to metastasize (Harbeck et al., 2019). Metastases are the cause of death in most cancers because they disrupt organ function at the site of metastasis; with breast cancer most often metastasizing into bone, axillary lymph nodes, liver, and lungs (Harbeck et al., 2019). World Health Organization (WHO) has classified breast cancer by histological and molecular classification into 19 different subtypes with ductal carcinoma of no special type (NST) being the most common subtype (Harbeck et al., 2019; Weigelt et al., 2008).

Breast cancers are often divided clinically to five subtypes (**Table 1**) which are the triple negative breast cancer (TNBC), human epidermal growth factor 2 (HER2) enriched non-luminal type, and three luminal-types characterized by being positive for oestrogen receptor-α (ER+) and progesterone receptor (PR+) (Harbeck et al., 2019). The luminal subtypes are luminal B-like positive for HER2 (HER2+), luminal B-like negative for HER2 (HER2–), and luminal A-like, which is also HER2– (Harbeck et al., 2019). Besides the expression status of the ER, PR, and HER2, the classification is further based on proliferation marker Ki67 expression and the grade of the tumor, which is determined according to number of cell divisions, proportion of cells in tubular formation, and variation in cell nuclei size and shape (Harbeck et al., 2019). TNBC has the highest grade and Ki67 expression, while luminal A-like breast cancer has the lowest Ki67 expression, lowest grade, and the best prognosis (Harbeck et al., 2019). Cancer prognosis and tumor growth depend on molecular characteristics of the tumor which affect things such as tumor vascularity and oxygenation.

Table 1. The features of common breast cancer subtypes (Harbeck et al., 2019).

	TNBC	HER2-enriched non-luminal	Luminal B-like HER+	Luminal B-like HER–	Luminal A-like
ERα	–	–	+	++	+++
PR	–	–	+	++	+++
HER2	–	++	+	–	–
Ki67	+++++	++++	+++	++	+
Grade	+++++	++++	+++	++	+
Associated histotypes	NST, metaplastic, adenoid cystic, medullary-like, secretory	NST	NST, pleiomorphic	NST, micropapillary, lobular pleiomorphic	NST, tubular cribriform, classic lobular

TNBC = Triple negative breast cancer, HER2 = human epidermal growth factor 2, ERα = oestrogen receptor α, PR = progesterone receptor, Ki67 = proliferation marker antigen Kiel 67, NST = Non-specific type, + = positive expression or grade level, – = negative/no expression.

2.1.2 Tumor vascularity and hypoxia

Many cancer cells can secrete or induce the secretion of pro-angiogenic factors that are used to communicate with the cells of the tumor microenvironment to trigger the formation of new vasculature, termed as angiogenesis. Vasculature is required to allow rapid tumor growth because it allows sufficient delivery of nutrients for cellular growth and proliferation. In cancer cells, and normal cells, the angiogenesis is regulated by anti-angiogenic factors and pro-angiogenic factors, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). These factors regulate the proliferation, survival, and migration of vascular endothelial cells (EC) that form the inner lining of blood vessels (Madu et al., 2020).

The dysregulation of the pro-angiogenic and anti-angiogenic factors that regulate angiogenesis causes tumors to be in a constant pro-angiogenic state which generates improperly matured abnormal vasculature. Tumor blood vessel often have abnormal ECs and abnormal pericyte coverage which causes blood vessel leakiness and dilation which both contribute to poor tumor perfusion and hypoxia (Morikawa et al., 2002a). The increased fluid leakage between tumor cells (excess interstitial fluid) leads to increased tumor interstitial pressure which further hinders tumor perfusion (Morikawa et al., 2002a). Pericytes in tumors are sometimes detected using the contractile filament α-smooth muscle actin (α-SMA) as a marker (Gomes-Santos et al., 2021a) and it is also a marker of smooth muscle and thus blood vessel arterialization. The α-SMA is expressed in pericytes of venules, arterioles, and capillaries in different quantities, and often in abnormal quantities in tumor pericytes

which contributes to abnormal tumor vasculature function (Alarcon-Martinez et al., 2018; Bergers & Song, 2005; Morikawa et al., 2002a).

Due to the abnormal vasculature many cancer regions are poorly perfused and hypoxic despite the presence of blood vessels (Buss et al., 2020). Hypoxia is an important stimulator angiogenesis in tumors and in normal cells. Hypoxia inducible factors (HIFs) function as transcription factors that regulate expression of multiple pro-angiogenic genes like VEGF. Importantly, tumor hypoxia, detected using hypoxia markers such as Carbonic anhydrase IX (CA IX) and HIF1-α, is linked to poor prognosis and treatment resistance in breast cancer (Chia et al., 2001; Generali et al., 2006). The abnormal tumor vasculature promotes cancer metastasis, but also makes it more difficult for most cancer drugs to penetrate the tumor due to inefficient tumor perfusion (Bergers & Song, 2005; Madu et al., 2020). Inefficient tumor perfusion also alters tumor metabolism because HIFs regulate several genes involved in expression of metabolic enzymes.

2.1.3 Tumor metabolism

Increased tissue glucose utilization is common in cancer, and in mammary tumor cells the glycolytic changes have been shown to correlate with the increased glucose uptake (Alvarez et al., 2014). Increased tumor glucose accumulation is usually detected using positron emission tomography (PET) imaging and radioactive tracer, glucose analog 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) which is not fully metabolized and accumulates in cells. Tumor cells often accumulate more [^{18}F]FDG than surrounding tissue because of their high metabolic activity and preference to utilize glycolysis to produce energy over utilizing mitochondrial oxidative phosphorylation (OXPHOS).

The preference of the tumor to produce energy through glycolysis even when there is enough oxygen and functional mitochondria has been termed as the Warburg effect. In glycolysis glucose is converted to pyruvate in a series of reactions in cytoplasm and the pyruvate can enter tricarboxylic acid (TCA) cycle or be used to generate lactate. In TCA cycle pyruvate is converted to acetyl-CoA and then to citrate by citrate synthase (CS) followed by series of reactions generating several compounds used in cellular metabolism. Importantly, these reactions generate oxidized nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) which fuel the OXPHOS in mitochondrial electron transport system (ETS) producing ATP in aerobic conditions (**Figure 1**). In Warburg effect there is increased conversion of pyruvate to lactate via lactate dehydrogenase (LDH) without requirement for oxygen. Lactate maintains acidic tumor microenvironment which favors metastasis and angiogenesis and is immunosuppressive (de la Cruz-López et al., 2019). The LDH A preferentially utilizes the cellular pyruvate to generate lactate while LDH B does the opposite with preference to bind with lactate.

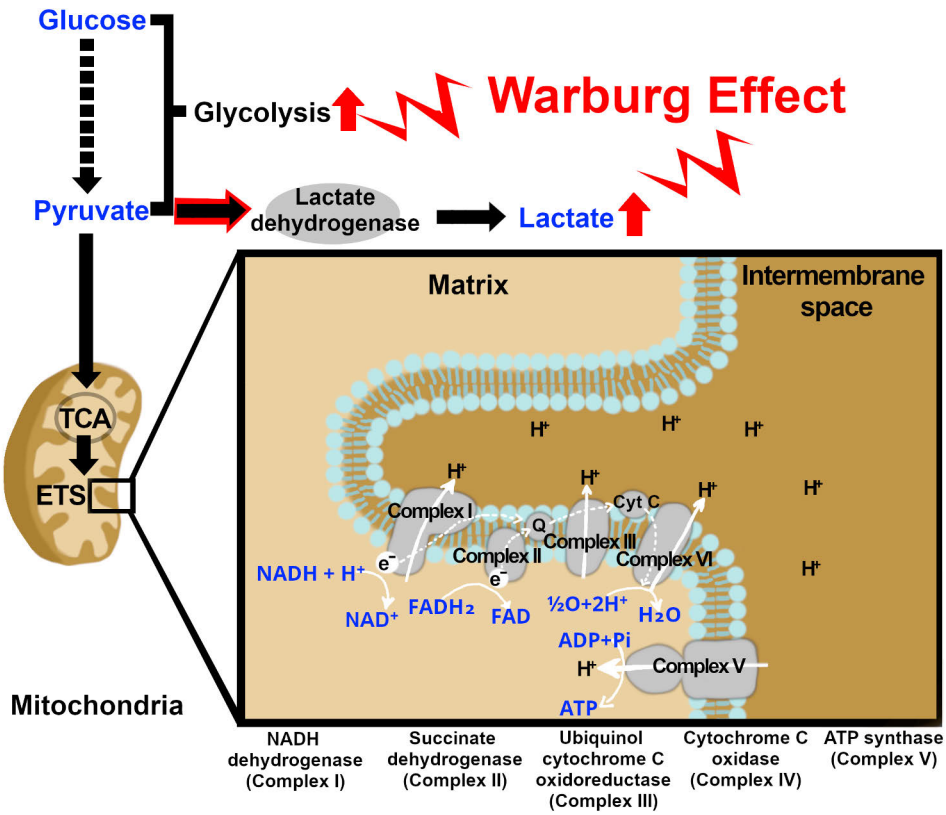

Figure 1. The Warburg effect, the glycolysis and mitochondrial electron transfer system (ETS) and its reactions. First glucose is converted to pyruvate in glycolysis. Pyruvate can be transported to the mitochondria to the tricarboxylic acid cycle (TCA) which produces high energy compounds needed in the ETS. Alternatively, pyruvate can be converted to lactate by lactate dehydrogenase in a reaction that does not require oxygen. In Warburg effect (red arrows) that happens within tumors the glycolysis and lactate production is increased even in the presence of sufficient oxygen. In ETS the hydrogen ions (protons) are pumped from the mitochondrial matrix into the mitochondrial intermembrane space via series of electron transfer reactions done by the mitochondrial complexes I-V and the electron carriers Cytochrome C (Cyt C) and Coenzyme Q (Q). The protons and electrons originate from the tricarboxylic cycle (TCA) where nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) are produced and which are then dehydrogenated by the mitochondrial complexes I and II. The electron transfer ends with reduction of oxygen which generates water. The proton gradient generated between mitochondrial intermembrane space and matrix drives the proton current back to matrix through ATP synthase in a process where ATP is generated from ADP.

How Warburg effect benefits cancer cells remains incompletely understood, considering that glycolysis is less efficient to produce ATP than OXPHOS and that tumors utilizing Warburg effect often still have a significant level of mitochondrial respiration. Some of the proposed benefits of Warburg effect are for example more rapid ATP generation and the lactate production to acidify tumor microenvironment

(Liberti & Locasale, 2016). Glycolysis is faster than mitochondrial OXPHOS thus potentially giving cancer cells a competitive edge over limited substrates (Liberti & Locasale, 2016). Furthermore, it has been suggested that increased glycolysis may affect mitochondrial reactive oxygen species (ROS) generation via altering substrate balance for OXPHOS, which can promote cell signaling that induces inflammation, angiogenesis, and metastasis (Aggarwal et al., 2019; Liberti & Locasale, 2016).

Although ROS is important for cell signaling, particularly in tumor cells, excessive production of ROS can lead to oxidative stress and cellular damage and even apoptosis through mitochondrial release of cytochrome C (Cyt C). Oxidative stress is caused by imbalance between the oxidative species like ROS and the cellular antioxidants. Antioxidant molecules like glutathione (GSH) and antioxidative enzymes like superoxide dismutase (SOD) both scavenge ROS, with SOD generating H_2O_2 which catalase (CAT) then removes generating water and oxygen. Mitochondria are a natural source of cellular ROS and cancer cells have increased production of ROS thanks to their high metabolic activity.

Despite the Warburg effect, many cancer cells still depend on mitochondrial OXPHOS. Accordingly, it has been shown that uncoupling the mitochondrial ETS inhibits breast cancer cell proliferation and breast tumor growth by for example diminishing ATP production and increasing ROS accumulation and apoptosis (Sanchez-Alvarez et al., 2013; Zunica et al., 2021). The mitochondrial uncoupling, often quantified by mitochondrial respiratory control ratio (RCR), is descriptive of mitochondrial coupling efficiency. RCR is the ratio of complex I driven maximal respiration to state 4 respiration uncoupled from ATP production, which is measured in absence of ADP and without uncouplers. State 4 respiration is therefore oxygen consumption not related to ATP generation and it is considered a measure of proton leak through the mitochondrial membrane (LEAK). The mitochondrial uncouplers are a potential cancer treatment, but their use is hindered by their effect on non-cancer cells. For example, uncoupling the tumor associated fibroblasts has been shown to promote tumor growth by increasing the ‘fueling’ of epithelial cancer cells in a paracrine fashion (Sanchez-Alvarez et al., 2013; Zunica et al., 2021).

2.1.4 Breast cancer treatments

Radiation therapy, surgical removal of tumors, and pharmacological treatments are used to treat cancer in different combinations, with neoadjuvant therapy administered before surgery and adjuvant therapy after surgery. Several pharmacological treatments exist for breast cancer, among which are immunotherapies, cytotoxins affecting predominantly fast dividing cells, and targeted cancer therapies, such as hormone therapies. Amongst the commonly used chemotherapeutic treatments are the broad-spectrum anthracycline antibiotics that

can be used to treat haematological cancers and several different solid tumors, such as breast cancer tumors (Octavia et al., 2012). The current recommendation for breast cancer treatment states that endocrine therapy with or without targeted therapy, depending on the other molecular characteristics, is recommended for all luminal-like HER2– breast cancers (Harbeck et al., 2019). For HER2+ breast cancer combination of HER2 inhibitors, blocking both receptor and its dimerization, together with chemotherapy is recommended (Harbeck et al., 2019). Meanwhile chemotherapy or immunotherapy are recommended for TNBC (Harbeck et al., 2019). Chemotherapies, such as anthracyclines, are often recommended later when other options have been tried for TNBC and for luminal-like HER2– breast cancers unresponsive to endocrine treatments (Harbeck et al., 2019).

Amongst the most widely used anthracyclines is DOX, also known as Adriamycin®, isolated from *Streptomyces peucetius* variant *caesius* (Arcamone et al., 1969). DOX tightly binds to DNA interfering with various DNA-related functions causing DNA double-strand breaks through inhibition of topoisomerase II thus eventually leading to cell death (Tewey et al., 1984). DOX particularly affects tumor cells through topoisomerase IIα which is expressed during S/G2/M phase of the cell cycle and often overexpressed in more cell cycle independent manner in tumor cells, such as breast cancer cells (Villman et al., 2002). However, like most anticancer treatments DOX also affects non-cancer cells leading to several side effects, such as nausea, loss of appetite, diarrhea, hair loss and leukopenia. The most severe and perhaps well-known side effect of DOX is DCT that can lead to development of heart failure (Cardinale et al., 2015). DOX also has toxic effects on other organs, such as liver, skeletal muscles, kidneys, brain, and reproductive organs; some of which may indirectly also contribute to DCT (Pugazhendhi et al., 2018). Increasing DOX efficacy would potentially allow the use of lower DOX dose, thus reducing the risk of DCT while also lowering chemotherapy resistance, which can develop through ineffective treatment and subsequent selection for treatment resistant cancer cells.

2.2 Effects of exercise on doxorubicin treatment efficacy of breast cancer

Many preclinical studies have shown that exercise alone could inhibit tumor growth and metastasis via its effect on the immune cell function and distribution (Gomes-Santos et al., 2021a; Miao et al., 2024; Pedersen et al., 2016; Rundqvist et al., 2020). However, not all studies have found exercise alone to inhibit tumor growth, with some preclinical studies even suggesting that exercise could increase metastasis (Schadler et al., 2016; Smeda et al., 2017). Thus far, not very many studies have investigated whether exercise during cancer treatment could enhance treatment

efficacy. Nonetheless, the studies so far seem to support the notion that exercise could boost chemotherapy or tamoxifen efficacy against various tumors including breast cancer (Yang et al., 2021). A review by Yang et al. (2021) on preclinical and clinical studies regarding the effects of exercise on chemotherapy efficacy included only one preclinical breast cancer study utilizing DOX, which however, did not support that exercise could boost DOX efficacy in immunodeficient animals (Jones et al., 2005). Meanwhile, only one of the clinical breast cancer studies included in the review found exercise to affect cancer treatment efficacy (Courneya et al., 2014). Later on, at least one additional study using retired breeder mice found that exercise enhanced DOX anti-tumor efficacy in breast cancer (Wakefield et al., 2021). However, due to the varied results more studies are still needed on whether exercise could affect DOX efficacy against breast cancer and at which stage of the treatment.

Also, the exact mechanisms in which exercise could boost cancer treatment efficacy are not well elucidated and more studies are needed on these mechanisms. Exercise could potentially affect chemotherapy efficacy through boosting the immune system, but exercise could also affect tumor growth via its effects on tumor vascularization and subsequently drug delivery and tumor metabolism.

2.2.1 Tumor glucose uptake as a measure of treatment response

Cancer treatment induced decrease in tumor [^{18}F]FDG accumulation, indicative of decreased glucose uptake and thus also glucose metabolism, has been shown to predict the response of metastatic mammary tumors to anthracycline treatment even after just one cycle of chemotherapy (Dose Schwarz et al., 2005) Accordingly, primary mammary tumor metabolic tumor volume (MTV) and total lesion glycolysis (TLG), which describe metabolically active tumor regions and their [^{18}F]FDG accumulation, are associated with higher risk of adverse effects (Pak et al., 2020). *In vitro* DOX has been shown to inhibit mammary tumor cell [^{18}F]FDG uptake, glucose transport and decrease tumoral ATP content, but paradoxically it was also shown to increase hexokinase activity, the rate limiting enzyme of glycolysis (Sharma et al., 2011). It is known that exercise modulates glucose metabolism and lowers the risk of breast cancer (Zhu et al., 2008), but before this thesis it had not been studied whether exercise could enhance the reduction in tumor glucose uptake during DOX treatment. In absence of treatment exercise has been shown to shift TNBC cells to preferentially utilize carbohydrates, whereas in absence of exercise the tumors used both lipids and carbohydrates as energy source (Vulczak et al., 2020).

One mechanism through which exercise could affect tumor metabolism is via alterations to tumor vasculature and subsequent drug delivery, oxygenation, and nutrient supply.

2.2.2 Modulating tumor blood flow

Some studies have shown that exercise can help improve tumor blood delivery which could potentially improve treatment efficacy via improved drug delivery (Seet-Lee et al., 2022). However, the current evidence is inconclusive due to mixed results which highlights a need for more studies on exercise effects on tumor vasculature (Seet-Lee et al., 2022). Tumor blood delivery could be enhanced either via increasing vascular density or by normalizing the tumor vasculature or through both. Increasing tumor vascularity may seem counterintuitive because typically higher vascular density has been linked with more aggressive tumor growth (Uzzan et al., 2004). However, increased tumor vascularity could improve tumor perfusion and oxygenation, and therefore reduce tumor hypoxia, thus potentially improving prognosis and allowing better drug delivery and immune cell infiltration into tumor.

Few studies have investigated whether exercise can improve treatment delivery to tumors during DOX treatment and these studies have shown that exercise can improve DOX delivery to Ewing's sarcoma and melanoma tumors while improving the efficacy of the treatment (Morrell et al., 2019; Schadler et al., 2016). However, there are not many studies like this on treatment of breast cancer. Nonetheless, many studies have investigated the effect of exercise on mammary tumor blood delivery, vascularity, and hypoxia; although mostly in absence of DOX treatment (Betof et al., 2015; Buss et al., 2020; Buss & Dachs, 2018; Faustino-Rocha et al., 2016; Jones et al., 2005; Wakefield et al., 2021). Few preclinical studies have shown that exercise can increase mammary tumor microvessel density and reduce tumor hypoxia (Betof et al., 2015; Faustino-Rocha et al., 2016), but many studies have also had contrasting results showing that tumor microvessel density is not altered by exercise (Buss et al., 2020; Buss & Dachs, 2018). The differing findings regarding tumor vascularity could be due to the exercise intensity and timing and because tumor blood vessel density is difficult to quantify due to its heterogeneity.

The blood vessel density alone may not reflect extent of tumor perfusion due to presence of poorly perfused blood vessels. Indeed, some studies have found that exercise did not affect quantity of mammary tumor blood vessel markers or their vascular density, but exercise did increase number of perfused tumor blood vessels, normalized tumor vasculature, and reduced tumor hypoxia (Gomes-Santos et al., 2021a; Jones et al., 2010). It has been suggested that exercise may normalize tumor vasculature also via pruning nonfunctional tumor microvessels thus leading to decreased vascular density rather than increased one (Ashcraft et al., 2019). Nonetheless, one study showed that exercise can in some instances both normalize orthotopic mammary tumor vasculature and also increase the tumor microvessel density regardless whether tumors were ER+ or ER– (Betof et al., 2015). Conversely, one study reported that 21 days of voluntary running wheel exercise did not increase

microvessel density nor normalize mammary tumor vasculature as indicated by number of perfused blood vessels (Buss et al., 2020).

Increase in tumor blood vessel perfusion suggests more functional vasculature. Exercise induced increase in tumor blood vessel perfusion is often associated with increased blood vessel maturity, which is often measured as pericyte coverage detected using markers like for example desmin or α-SMA (Betof et al., 2015; Gomes-Santos et al., 2021a). However, stromal expression of α-SMA, found for example in tumor associated fibroblasts, in HER2+ mammary tumors has been linked with tamoxifen treatment resistance (Vathiotis et al., 2021). Interestingly, DOX treatment can also increase stromal α-SMA expression in murine breast cancer tumors which may contribute to development of treatment resistance (Morita et al., 2020). DOX can also slightly downregulate mammary tumor ECs and VEGF while having synergistic effect with anti-angiogenic treatment (Shi et al., 2021).

Due to the varying results regarding exercise effects on mammary tumor vasculature more research on this is needed. Particularly *in vivo* measurements of blood flow are needed as blood vessel tone may also be altered which could affect tumor blood flow. Worryingly, studies on breast cancer and prostate cancer have shown that exercise mediated improvement in vascular density or tumor oxygenation is sometimes accompanied with increased tumor growth (Faustino-Rocha et al., 2016; McCullough et al., 2014). However, in a murine model of melanoma it was shown that exercise normalized tumor vascularity and exacerbated tumor growth in absence of treatment, but inhibited tumor growths when exercise was done concomitantly with DOX treatment (Schadler et al., 2016). This, and the fact that DOX can affect α-SMA and EC expression, highlights need for studies on tumor vasculature with exercise done concomitantly with treatment. Furthermore, since exercise can alter tumor hypoxia and oxygenation it might also alter tumor metabolism which may significantly affect tumor growth.

2.2.3 Modulating tumor metabolism

Exercise has been shown to reduce tumor growth of ER+ breast cancer while also decreasing tumor lactate, lactate transporters and increasing the expression of LDH B (Aveseh et al., 2015). It was also demonstrated that the estrogen related receptor-α modulated the changes in LDH and lactate transporter. These findings fit with previous findings about exercise reducing tumor hypoxia and they also suggest reduction in tumor Warburg effect. However, one other study found that while exercise did boost DOX efficacy against mammary tumors in retired breeder mice and decreased tumoral HIF expression, suggesting reduced tumor hypoxia, it did not alter tumor lactate levels (Wakefield et al., 2021). Nonetheless, in the same study the

tumor citrate species were increased suggesting increased flux through TCA cycle which suggests potential for increased OXPHOS capacity.

HIF expression seems to be a crucial determinant of exercise mediated tumor growth acceleration in mammary tumors in absence of cancer treatment (Glass et al., 2017). Within claudin-low breast cancers a high expression and protein level of HIF in tumors was associated with exercise accelerating tumor growth while the opposite was found with low HIF expression (Glass et al., 2017). In contrast, Vulczak et al. (2020) showed that exercise reduced TNBC tumor growth and increased tumor HIF-1α expression and lactate importer MCT-1 without affecting tumor lactate levels. Furthermore, they also found that exercise decreased mitochondrial respiration linked with maximal electron transfer capacity (Vulczak et al., 2020). These differential findings could be due to cancer subtype and or differences in exercise intensity.

Exercise might also inhibit metastasis and tumor growth by reducing nutrient availability to the tumor through increasing the nutrient demand of the other organs (Sheinboim et al., 2022). This remains to be studied during DOX treatment in breast cancer, which would be of interest considering that previously DOX in combination with other anti-cancer drugs has been shown to decrease resting metabolic rate likely through its systemic toxicity (Demark-Wahnefried et al., 1997). The reduction of the systemic toxicity of DOX, particularly DCT, could increase DOX efficacy as DCT limits the use of DOX hindering its efficacy.

2.3 Doxorubicin induced cardiotoxicity

DCT is often defined as >10% decline in left ventricle ejection fraction (LVEF) to ejection fraction value that indicates lower than half of blood volume in LV being ejected by the heart during systole (Cardinale et al., 2015). DOX-induced cardiac dysfunction occurs in a dose dependent manner, with patients scheduled on receiving a cumulative dose ≥250 mg/m^2 of DOX having high risk of developing cardiac dysfunction (Cardinale et al., 2015; Lyon et al., 2022). Additionally, existing cardiovascular pathologies and lifestyle factors, such as alcohol consumption, high-fat diet, and physical inactivity, that affect incidence of cardiovascular diseases can increase the risk of developing DOX-induced cardiac dysfunction (Lyon et al., 2022).

Although DOX affects several tissues, heart is particularly vulnerable to DOX induced toxicity due to its high mitochondrial density, high energy demands and because it is known that DOX accumulates within the mitochondria disrupting their function (Kavazis et al., 2017; Morton et al., 2019). In the clinical setting the initial plasma concentration of DOX following a bolus injection is typically between 1–2 μM which falls within an hour to a range of only 25–250 nM (Gewirtz, 1999;

Harahap et al., 2020). The cardiac accumulation of the drug can be 50-fold compared to plasma concentration (Timour et al., 1988) or even higher (Zeng et al., 2019) Furthermore, mitochondrial DOX concentration can be two-fold compared cytoplasmic concentration (Morton et al., 2019). Therefore, the cardiac mitochondria are exposed to higher DOX concentrations than expected by the plasma concentrations which explains why heart function is particularly affected by DOX.

2.3.1 Functional impairment and vascular toxicity

DOX-induced reduction in LVEF, cardiac output/stroke volume and left ventricular fractional shortening (LVFS) have been shown in patients and in rodent models (Babaei et al., 2020; Kim et al., 2020; Nousiainen et al., 2002; Pan et al., 2021). LVFS is the percentage reduction of LV diameter in systole, which can be used as an alternative of LVEF to detect systolic dysfunction in DCT. Additionally, DOX can induce arrythmias and decrease E/A which represents the ratio of LV blood flow peak velocities during ventricular relaxation in early diastole (the E wave) and in late diastole due to atrial contraction (the A wave) (Steinberg et al., 1987; Upshaw et al., 2020a). Decreased of E/A ratio can indicate insufficient ventricular filling and diastolic dysfunction (Upshaw et al., 2020a).

Reduced LVEF, FS and cardiac output are signs of impaired cardiac contractility and decreased E/A can be a sign of impaired cardiac relaxation, all of which can be caused by, for example, weakness of the LV, hypertrophy, and stiffening of the myocardium. Indeed, DOX has been shown to increase cardiac fibrosis (Chan et al., 2021; Díaz-Guerra et al., 2024; Zhang et al., 2012) which can stiffen the myocardium making it more difficult for the heart to contract or relax effectively (Migrino et al., 2008; Piek et al., 2016). DOX can cause cardiac fibrosis, myocardial accumulation of connective tissue, by inducing activation of cardiac extracellular remodeling via matrix metalloproteinases (MMPs) or by inducing cardiomyocyte apoptosis which results in reparative processes replacing apoptotic cardiomyocytes with connective tissue (Bulten et al., 2019; Chan et al., 2021; Piek et al., 2016). DOX can also increase cardiac fibrosis by activating cardiac fibroblasts independent of apoptosis (Narikawa et al., 2019). Besides causing cardiac fibrosis, DOX can reduce cardiac contractility by causing reduction in LV mass and cardiac contractile proteins through cardiomyocyte apoptosis, cardiomyocyte atrophy, and through activation of MMPs (Chan et al., 2021; Willis et al., 2019; Xia et al., 2020).

DOX induced perivascular fibrosis can impair also microvessel function which can affect gas exchange and blood vessel stiffness thus contributing to the cardiac dysfunction more indirectly (Pan et al., 2021). In fact, the DOX induced arterial stiffness has been shown to precede the decline in LVEF (Bosman et al., 2023). DOX can also cause myocardial capillary rarefaction and cardiac endothelial dysfunction,

with the latter contributing to blood vessel stiffness (Räsänen et al., 2016). DOX also increases cardiac blood vessel permeability by increasing EC death, with cardiac ECs being particularly vulnerable to DOX (Hoffman et al., 2021; Tao et al., 2021). Blood vessel permeability could also be affected by DOX induced decrease in cardiac blood vessel pericyte coverage, which might compromise efficient vascular function (Tao et al., 2021; Wang et al., 2021). Cardiac EC death likely contributes to the DOX induced cardiac capillary rarefaction, but also the downregulation of VEGF might be involved (Räsänen et al., 2016). However, in contrast one study found that DOX upregulated HIF inducing VEGF A expression likely as a protective mechanism (Refaie et al., 2022). Meanwhile one other study found HIF expression to be unchanged following DOX treatment (Bulten et al., 2019). Due to these varied results, there is a need for more studies on the role of HIF and VEGF in DCT.

Besides the vascular effects, apoptosis, and fibrosis; many other mechanisms are known to affect heart function in DCT. DOX can shift the expression of α- and β-myosin heavy chain expression, which affects the ability of the myocardium to contract (Herron & McDonald, 2002; Hydock et al., 2012, 2009). Additionally, DOX can affect the calcium handling of the heart contributing to the weakened heart function through altered calcium ion currents that determine the strength of the myocardial contraction (Hanna et al., 2014; Zhang et al., 2014). Perhaps most importantly, DOX impairs the cardiac energy metabolism, reducing energy available for cardiac function and affecting generation of oxidative stress that damages cellular components.

2.3.2 Glucose uptake as a measure of cardiotoxicity

Some preclinical studies have shown that DOX causes decreased cardiac glucose uptake which could be expected considering that DOX impairs cardiac function and metabolism (Díaz-Guerra et al., 2024). However, strong evidence from both preclinical and clinical studies suggests that DOX can more often cause instead a significant increase in cardiac glucose uptake as indicated by increased cardiac [^{18}F]FDG SUV (Bauckneht et al., 2017, 2020; Bulten et al., 2019; Guerra et al., 2024; Hrelia et al., 2002; Sarocchi et al., 2018). This increase in glucose uptake is supported further by studies that have shown that DOX causes a shift in cardiac metabolism that favors glucose as energy source over fatty-acid oxidation, which is often downregulated by DOX treatment (Bordoni et al., 1999; Carvalho et al., 2010; Guerra et al., 2024). However, some studies have found DOX to increase cardiac reliance on fatty-acid utilization (Brandão et al., 2023). It is possible that differential findings on cardiac glucose uptake and fatty-acid utilization are due to variability in genetic background, the dose of DOX, and different stage of DCT. The differential

findings regarding DCT highlight need for more studies on DOX induced alterations in cardiac metabolism.

Acutely the increased cardiac glucose uptake may be driven by the DOX induced upregulation of glucose transporter 1 (Hrelia et al., 2002). The increase in cardiac glucose uptake has been shown to correlate positively with the cardiac oxidative stress (Baucknehtet al., 2020) and in preclinical and patient studies it has been associated with decreased cardiac function (Baucknehtet al., 2019, 2017; Sarocchi et al., 2018). There has been evidence that the DOX induced increase in cardiac [^{18}F]FDG uptake could potentially serve to maintain cardiac antioxidative mechanisms by generating reducing agent nicotinamide adenine dinucleotide phosphate (NADPH) via pentose phosphate pathway (Baucknehtet al., 2019). NADPH is needed for the reduction of oxidized GSH which can combat cellular oxidative stress generated by DOX in the mitochondria.

2.3.3 Cardiac mitochondrial function

DOX can inhibit several pathways related cardiac mitochondrial function. Some studies indicate that DOX inhibits glycolysis via inhibition of its rate-limiting enzyme muscle type 6-phosphofrucktokinase or by inhibiting hexokinase 2 and pyruvate dehydrogenase isoenzyme 4 (Brandão et al., 2023; Díaz-Guerra et al., 2024; Zhou et al., 2022). Conversely, some studies found DOX to increase glycolysis, as was indicated by phosphorylation of glycolysis enzymes and by increased lactate production (Carvalho et al., 2010; Gratia et al., 2012). The increased glycolysis could be compensatory effect to generate ATP or NADH when OXPHOS capacity is compromised. These mixed results highlight a need for more DCT studies regarding cardiac glycolysis and lactate production.

DOX can also affect the TCA cycle by inhibiting CS-activity (Pillai et al., 2017; Yao et al., 2011). Reduction in CS can also be indicative of reduced mitochondrial content instead of just decreased enzyme function or content. DOX can indeed decrease mitochondrial content by altering mitochondrial degradation and biogenesis (Díaz-Guerra et al., 2024; Sun et al., 2023). Excessive loss of mitochondria could be detrimental to heart, but removal of mitochondria could also be protective since damaged mitochondria contribute increased oxidative stress. Damaged mitochondria are usually removed from the cell via mitophagy, a form of autophagy that controls the breakdown and recycling of damaged mitochondria (Lee et al., 2023; Toda et al., 2023). However, DOX can cause accumulation of dysfunctional mitochondria via inhibiting mitophagy (Toda et al., 2023). Indeed, one study showed that transiently increased mitophagy during early DCT was associated with preserved mitochondrial structure while persistently upregulated mitophagy was associated with mitochondrial fragmentation (Díaz-Guerra et al., 2024).

DOX particularly damages mitochondria because it accumulates to them by binding to the mitochondrial inner membrane proteins called cardiolipins (Goormaghtigh et al., 1980). Mitochondrial DOX accumulation and the formation of cardiolipin-DOX complex can then lead to inhibition of the mitochondrial energy metabolism enzymes (Goormaghtigh et al., 1982). The DOX accumulation also inhibits the binding of cardiac sarcomeric mitochondrial creatine kinase (mtCK) and Cyt C to mitochondrial membranes thus impairing ETS function (Tokarska-Schlattner et al., 2007). DOX can impair cardiac mitochondrial ETS function both acutely after single high dose or after multiple smaller doses of DOX (Huang et al., 2017; Montaigne et al., 2010; Pointon et al., 2010).

DOX has been shown to be able to inhibit the function of mitochondrial respiratory complexes I-IV in the ETS (Goormaghtigh et al., 1982; Marcillat et al., 1989; Muraoka & Miura, 2003; Pointon et al., 2010). The complex I has been shown to be inhibited through its DOX redox cycling by lower concentrations of DOX than the complex II (Marcillat et al., 1989). Meanwhile, complex II can be directly inhibited by oxidised DOX *in vitro* (Muraoka & Miura, 2003). However, without cellular agents capable of oxidizing DOX, like horseradish peroxidase and its substrate H_2O_2, complex II is only inhibited at high DOX concentrations (Muraoka & Miura, 2003). Removal of such oxidizing agents using CAT and GSH can prevent the DOX inhibition of complex II (Muraoka & Miura, 2003). DOX inhibition of respiratory complex IV, or the ETS in general can exacerbate the formation of ROS if oxygen is not fully reduced (Goormaghtigh et al., 1982).

DOX induced mitochondrial damage can lead to mitochondrial release of Cyt C which triggers apoptosis which contributes to DCT (Childs et al., 2002). Mitochondrial damage can also deprive heart from energy needed for its normal function and multiple studies do show that DOX can decrease cardiac ATP and its regeneration (Díaz-Guerra et al., 2024; Nicolay et al., 1987; Ohhara et al., 1981; Zhou et al., 2001). DOX reduces the cardiac ATP supply by inhibiting ETS function and by inhibiting mtCK, which regulates ADP levels in mitochondrial intermembrane space affecting OXPHOS (Saks et al., 2000; Tokarska-Schlattner et al., 2007).

DOX dose affects degree of mitochondrial damage. However, mode in which cardiomyocytes are exposed to DOX seems to also affect degree in which mitochondria are affected. Cardiomyocytes exposed to DOX *in vitro* appear more sensitive to mitochondrial damage than cardiomyocytes exposed *ex vivo* through isolated organ exposure, with 1 or 5 µM of DOX *in vitro* and 20 µM DOX *ex vivo* being required to impair mitochondrial or heart function (Toda et al., 2023; Tokarska-Schlattner et al., 2005). Compensatory activation of brain-type CK maintains heart function at 2 µM DOX exposure *ex vivo* despite inhibition of muscle-type CK (Tokarska-Schlattner et al., 2005). In contrast, *in vivo* 20 mg/kg of DOX

does not acutely (within 48 hours) decrease mitochondrial complex activity in rats but decreases the mitochondrial RCR (Montalvo et al., 2023). The differential study outcomes highlight need for further studies on mitochondrial effects of DOX *in vivo* to better elucidate mechanisms that might be occurring in clinical setting.

Mitochondrial transplantation has been shown to ameliorate DCT, but it also decreases cardiac glycolysis and increases mitochondrial respiration through enhanced glutamine metabolism, which is essential for the cardioprotection to occur (Sun et al., 2023). Sun et al. (2023) proposed that the enhanced glutamine metabolism was cardioprotective through fueling TCA cycle via generation of α-ketoglutaric acid from glutamine, and because glutamine is also used to generate cellular antioxidant GSH thus helping to combat oxidative stress. However, glutamine supplementation in food alone did not enhance glutamine metabolism nor was it cardioprotective. One proposed main mechanism through which DOX damages cardiac mitochondria is through inducing oxidative stress.

2.3.4 Oxidative stress

Oxidative stress generated by DOX is one of the main mechanisms driving DCT as is evidenced by the well-known DOX mediated increase in oxidative species and increased oxidative damage to proteins and lipids in the myocardium (Burridge et al., 2016; Liao et al., 2023; Rajagopalan et al., 1988; Ye et al., 2022; Zhang et al., 2012). Importantly, DOX induced increase in oxidative species does not have major contribution to the toxic effects against the tumors themselves (Myers et al., 1977).

DOX induces oxidative stress via multiple mechanisms summarized in **Figure 2**, one such mechanism being the redox reactions of the DOX molecule caused by cellular electron carriers (Bachur et al., 1979; Gutiérrez et al., 1983; Kalyanaraman et al., 1984; Vásquez-Vivar et al., 1997). In these reactions DOX molecule is reduced and then reacts with molecular oxygen which leads to formation of highly reactive oxygen radical that can react with cellular components damaging them, or it may be converted to H_2O_2 spontaneously or via antioxidative enzymes (Rajagopalan et al., 1988; Svingen & Powis, 1981). However, more recent studies suggest that DOX redox reactions may not contribute to DOX generated oxidative stress as significantly *in vivo* as has been shown *in vitro*, mainly due to the greater partial oxygen pressure *in vitro* (Pointon et al., 2010). Nonetheless, the DOX mediated formation of H_2O_2 can further induce formation of ROS by reacting with the cellular redox-active iron via the Fenton reaction (Minotti, 1990). Redox-active iron can also directly react with DOX forming complexes that drive formation of more oxidative species by reacting with H_2O_2 and forming hydroxyl radicals (Muindi et al., 1984). Furthermore, DOX exacerbates this formation of oxidative species by causing mitochondrial iron accumulation through interfering with their iron homeostasis

(Ichikawa et al., 2014; Minotti, 1990; Montalvo et al., 2023). The mitochondrial iron accumulation can also cause iron induced apoptosis, ferroptosis, which contributes to DCT (Tadokoro et al., 2020).

Cardiomyocytes are more susceptible to oxidative stress because they possess less antioxidants compared to other cells (Doroshow et al., 1980) and one indirect mechanism in which DOX can also increase cardiac oxidative stress is by decreasing the cellular antioxidants. For example, DOX has been shown to decrease SOD and CAT in heart (Montalvo et al., 2023; Sequeira et al., 2021; Ye et al., 2022). Perhaps the most significant way that DOX induces oxidative stress is indirectly through inhibiting the TOP IIβ (Zhang et al., 2012). DOX mediated TOP IIβ inhibition causes mitochondrial DNA damage affecting the cardiomyocyte transcriptome in a way that drives disturbations in mitochondrial biogenesis and function which induces the formation of ROS (Zhang et al., 2012). It has been shown that as much as 70% of the DOX induced ROS generation in the cardiomyocytes was induced through TOP IIβ and that TOP IIβ was essential for the formation of DCT (Zhang et al., 2012).

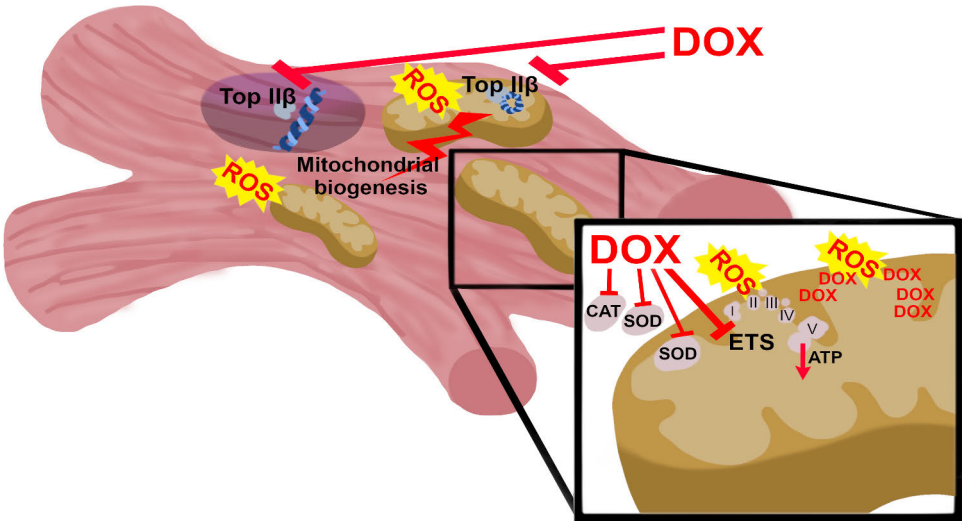

Figure 2. Doxorubicin (DOX) induced oxidative stress within cardiomyocytes. DOX inhibits topoisomerase IIβ (Top IIβ) causing DNA damage in nucleus and mitochondria which induces disruptions in mitochondrial biogenesis and accumulation of damaged mitochondria which produce more reactive oxygen species (ROS). DOX also accumulates to mitochondria and inhibits antioxidative enzymes like catalase (CAT) and superoxide dismutase (SOD) and directly generates ROS through its redox reactions and by inhibiting the electron transfer system (ETS).

2.4 Exercise as a treatment for doxorubicin induced cardiotoxicity

Currently there are not many FDA approved pharmacological treatments against DCT, with dexrazoxane being the only US FDA approved treatment for DCT thus far (Boen et al., 2024). However, some studies have shown that dexrazoxane could possibly reduce treatment response rate (Swain et al., 1997). Therefore, safer treatment options for DCT are needed, exercise possibly being one of them.

2.4.1 Protection of cardiac function and vasculature

Aerobic exercise could be expected to alleviate DCT through its well-known effects to improve cardiac function and vascular function in healthy individuals. However, the cardioprotective effects of exercise against DCT are not well elucidated in clinical setting and the studies thus far have had rather modest findings regarding cardioprotective effects of exercise (Naaktgeboren et al., 2023; Upshaw et al., 2020b). Strong support for cardioprotective effects of exercise, however, comes from multiple preclinical studies. Meta-analyses on preclinical DCT studies with exercise intervention have shown that both forced treadmill exercise and voluntary running wheel exercise can reduce DOX induced decline in cardiac FS measured *in vivo* and the decline in cardiac pressure development measured *ex vivo* (Ghignatti et al., 2021; Naaktgeboren et al., 2021). Only few of the studies found no cardioprotective effect of exercise, with one utilizing ovariectomized rats and other mice with melanoma (Phungphong et al., 2020; Sturgeon et al., 2014a).

The meta-analyses revealed that exercise preconditioning before anthracycline treatment seems to have slightly greater cardioprotective effect compared exercise training performed concomitantly with the treatment. However, preclinical studies with exercise done concomitantly with cancer treatment may better apply to clinical setting since many people do not meet minimal physical activity requirements (Strain et al., 2024). However, one preclinical study and one study on breast cancer patients showed that even a single bout of exercise a day before DOX administration could be cardioprotective at least against some of the acute effects of DOX (Kirkham et al., 2017; Wonders et al., 2008). Currently most animal studies use FS as a measure of the cardiac function *in vivo*, although in clinical setting the LVEF is preferentially used. This could hinder translating the results to the clinical setting.

Few preclinical studies do, however, show that also the reduction in LVEF is mitigated by exercise regardless of whether exercise is done before treatment, concomitantly or after it (Souza et al., 2022; Suthivanich et al., 2024; Wang et al., 2018). Although, one study showed that treatment concomitant exercise preserved LVEF while exercise performed after treatment did not (Wang et al., 2018). Besides systolic function of the heart, the DOX induced decline in diastolic function has also

been shown to be mitigated by exercise (Dolinsky et al., 2013; Parry & Hayward, 2015; Wang et al., 2021). Unfortunately, there are not many preclinical DCT studies with exercise intervention that use tumor bearing models despite the fact that cancer itself can affect the heart. Breast cancer, for example, alters the cardiac capillary vessel permeability and reduces LV longitudinal and circumferential strain which could affect exercise outcomes (Hoffman et al., 2021; Tadic et al., 2018). The few studies using tumor-bearing models have found mixed results possibly due to difference in tumor models, treatments, and exercise regimens used (Parry & Hayward, 2015; Sturgeon et al., 2014a; Wang et al., 2018). To better recapitulate the clinical setting and the molecular mechanisms of DCT more studies are needed on exercise effects against DCT in tumor bearing animal models (Naaktgeboren et al., 2021).

Besides heart function exercise can also mitigate the DOX induced vascular changes. Vascular smooth muscle function is improved by exercise preconditioning without changes in vascular DOX accumulation (Gibson et al., 2013). Exercise done concomitantly with DOX treatment can improve cardiac vascular function by abolishing the DOX mediated reduction in ECs and blood vessel pericyte coverage (Tao et al., 2021; Wang et al., 2021). Recent studies have shown that one way exercise can protect the cardiac vasculature is by promoting bone marrow cell migration to heart which then differentiate to ECs and pericytes repairing damaged vasculature (Tao et al., 2021). This could be assumed to also reduce DOX induced vascular rarefaction, but before this thesis not many studies have looked into this specifically or the factors regulating angiogenesis like HIF-1α and VEGF. However, exercise has been shown to upregulate HIF-1α in rats with heart failure (Xu et al., 2024) as well as VEGF and the capillarity in the aging rat myocardium (Iemitsu et al., 2006). Therefore, exercise could be expected to potentially affect also HIF and VEGF in DCT. Furthermore, exercise has been shown to reduce DOX induced cardiac fibrosis, (Sequeira et al., 2021; Wang et al., 2021; Yang et al., 2020) which could also reduce perivascular fibrosis. Exercise can reduce cardiac fibrosis by directly reducing cardiac fibroblast activation or indirectly by reducing cardiomyocyte apoptosis and cardiac inflammation (Tao et al., 2021; Wang et al., 2024; Yang et al., 2020).

Although it seems clear that exercise can be cardioprotective, the exact mechanisms of exercise induces cardioprotection and the optimal exercise timing and dose in relation to drug dosage are still unclear. One way, physical exercise could potentially mediate its cardioprotective effects is through its well-known benefits to mitochondrial function (Han & Kim, 2013; Laker et al., 2017).

2.4.2 Cardiac energy metabolism

Exercise preconditioning has been shown to decrease cardiac mitochondrial DOX accumulation leading to decreased mitochondrial ROS production and maintained heart function (Morton et al., 2019). However, the cardiac mitochondrial function has been shown to be improved also when the mitochondrial DOX accumulation is not improved, with exercise improving RCR in intramyofibrillar mitochondria and the cardiolipin content in intermyofibrillar and subsarcolemmal mitochondria of DOX treated animals (Montalvo et al., 2023). Indeed, many studies have shown that both voluntary running-wheel exercise and forced treadmill exercise can mitigate DOX induced decrease of RCR when done as preconditioning or concomitantly with DOX treatment (Ascensão et al., 2005b; Kavazis et al., 2010; Marques-Aleixo et al., 2015; Morton et al., 2019).

DOX can decrease RCR through both reducing coupled respiration or by increasing LEAK respiration or by altering both, while exercise has been shown to be able to counter both changes (Ascensão et al., 2005b; Kavazis et al., 2010; Marques-Aleixo et al., 2015; Morton et al., 2019). Marques-Aleixo et al. (2015) found that exercise increases activity levels of mitochondrial complex I and ATP-synthase, but the mitochondrial uncoupling protein 2 content was not altered. They also found that treadmill exercise additionally increased complex IV content whereas voluntary running wheel exercise additionally increased complex III content, which was not increased by treadmill training. This suggests that the intensity of exercise may significantly alter through which complexes exercise improves mitochondrial function. Furthermore, Dolinsky et al. (2013) showed that DOX increases mitochondrial UCP protein content and decreases both complex I and complex II protein levels in mitochondria, with treatment concomitant treadmill exercise maintaining the mitochondrial complex content, but not UCP content, at control levels. However, they did not measure the mitochondrial respiration. There is also evidence that the mitochondrial RCR may be more important than changes in the individual mitochondrial complexes. For example, one study showed that the activities of mitochondrial complexes I-IV or LEAK were not altered, but the DOX induced decrease in RCR was mitigated by exercise (Montalvo et al., 2023). This likely means that smaller cumulative changes in the activities of the complexes were behind the changes in RCR.

What is currently lacking is confirmation whether exercise can improve mitochondrial function also in tumor bearing animals during DOX treatment. Furthermore, it is not known whether specific mitochondrial complexes mediate the exercise induced cardiac protection during DOX treatment. Use of tumor-bearing models in mitochondrial response is particularly important as breast cancer alone has been show to affect the expression of proteins involved in mitochondrial dynamics (Jafari et al., 2021). Additionally, there are not many studies on whether exercise

alters myocardial cell metabolism outside ETS and fatty acid oxidation in DCT, such as the glucose uptake, glycolysis, and TCA cycle enzymes. One study found that neither DOX nor exercise affected cardiac CS-activity, but they did not look into the cardiac glycolysis or related lactate production or LDH activity (Ascensão et al., 2005a). Before this thesis it had not been yet studied whether exercise could alter DOX mediated changes in cardiac glucose uptake in DCT. This could be expected as exercise has been shown to boost cardiac glucose uptake and glycolysis capacity in mice with ischemic induced heart failure (Jiang et al., 2020). Better understanding of the changes in cardiac glucose uptake could be useful as cancer patients are routinely imaged using [^{18}F]FDG PET, which could offer important insight to DCT and benefits of exercise in patients. Exercise might affect cardiac glucose uptake by enhancing the mitochondrial function and thus energy production per molecule of glucose. The mitochondrial function could be improved through exercise mediated reduction in DOX induced oxidative stress.

2.4.3 Mediating doxorubicin induced oxidative stress

Aerobic exercise has been shown to be cardioprotective through its ability to improve cardiac antioxidative capacity and by improving energy metabolism in heart failure patients (Powers et al., 2014; Ventura-Clapier et al., 2007). Reduction in cardiac oxidative stress can reduce mitochondrial damage and reduce cardiac apoptosis and the subsequent loss of cardiac mass thus helping to preserve cardiac function. One way through which exercise can reduce cardiac oxidative stress is via reducing cardiac and mitochondrial accumulation of DOX, which could potentially reduce all cardiotoxic effects of DOX via limiting cardiac exposure to the drug. Indeed, some studies show that exercise preconditioning with or without treatment concomitant exercise can reduce cardiac/mitochondrial DOX accumulation via increasing cardiac expression of multidrug resistance proteins or mitochondria-specific ABC transport proteins able to pump out DOX (Jensen et al., 2013; Morton et al., 2019; Parry & Hayward, 2015; Wang et al., 2018). One study showed that exercise specifically reduced cardiac mitochondrial accumulation without affecting cytosolic fraction of DOX (Morton et al., 2019). Importantly exercise reduced cardiac DOX accumulation, but not its tumor accumulation (Parry & Hayward, 2015). The reduced mitochondrial DOX accumulation was shown to be accompanied by reduced mitochondrial ROS production (Morton et al., 2019). Interestingly it has also been shown that exercise preconditioning can improve DOX redox balance without affecting cardiac accumulation of DOX (Montalvo et al., 2023).

Other way in which exercise can reduce DOX induced oxidative stress is by enhancing the cardiac antioxidant mechanisms, which could reduce mitochondrial damage leading to further decrease in ROS production. Aerobic exercise can

decrease oxidative damage to proteins and lipids by increasing the expression or activity of many cardiac antioxidants like SOD, CAT, glutathione peroxidase (GPx), GSH, and heat shock proteins (Ascensão et al., 2005a, 2005b; Dolinsky et al., 2013; Kavazis et al., 2010; Wang et al., 2024). Particularly cardiac SOD has been shown to be increased by exercise preconditioning and by exercise done at least partially concomitantly with DOX treatment (Ascensão et al., 2005b; Dolinsky et al., 2013; Kavazis et al., 2010; Marques-Aleixo et al., 2015; Sequeira et al., 2021). In contrast, some studies have shown that exercise does not alter SOD expression, but it does upregulate GPx or heat shock proteins or CAT (Ascensão et al., 2005a; Chicco et al., 2006, 2005). One study showed that short term exercise preconditioning specifically increased SOD in subsarcolemmal mitochondria while the intermyofibrillar mitochondria were less protected by exercise (Montalvo et al., 2023). The variability in antioxidative mechanisms altered by exercise suggests that exercise can be cardioprotective through different antioxidative mechanisms depending on exercise timing, modality, and intensity. There are not many studies confirming whether exercise can improve cardiac antioxidative enzyme function also in tumor bearing DOX treated animals.

There is also evidence that exercise mediated abolishment of DOX induced oxidative stress is not always enough and that in female animals the anti-inflammatory effects of estrogen are vital for cardioprotection (Phungphong et al., 2020). Some studies also suggest that exercise can be cardioprotective even in the absence of reduction in cardiac oxidative stress or in absence of enhancement in antioxidative enzymes which suggests that these changes might not be necessary for exercise mediated cardioprotection against DCT (Chicco et al., 2006, 2005).

2.5 Aims

The purpose of my thesis was to investigate how voluntary wheel running exercise training (ET) could affect the tumor physiology to potentially enhance the chemotherapy effect of DOX during the treatment of mouse model of breast cancer. Particularly, the aim was to investigate the changes in tumor growth and the potentially beneficial changes in tumor glucose uptake, blood flow, vasculature, mitochondrial function, and metabolic enzyme function. The purpose of my thesis was also to investigate whether voluntary exercise done at the same time with treatment could also mitigate cardiotoxic effects of DOX and what would be the metabolic and vascular drivers behind the potential exercise benefits. The aim was to investigate the underlying mechanisms driving the cardiac changes relating to glucose uptake, oxidative stress, mitochondrial function, metabolic enzyme function and vasculature. The thesis consists of three separate studies with each having distinct aims:

Study I: Investigation of whether short-term voluntary wheel-running exercise during DOX treatment of breast cancer-bearing mice could induce beneficial cardiac effects and affect tumor growth during DOX treatment. This study focused on vascular changes such as changes in vascular density in tumor and the heart as well as the α-SMA coverage of the tumor blood vessels. Furthermore, this study aimed to measure the factors regulating vascularity like HIF and VEGF in tumors and heart, with HIF being also indicative of hypoxia. The tumor and heart maximal activities of LDH and CS were also measured to see the degree of glycolysis substrates directed to lactate production or degree of lactate utilization in relation to substrates directed to TCA cycle. These factors had not been studied before in response to exercise during DOX treatment of breast cancer. The aim of the Study I was also to evaluate the correct DOX dose and experiment duration for follow up studies of thesis.

Study II: Unlike study I which focused only on exercise effects during DOX treatment, this study aimed to investigate whether voluntary running wheel exercise could reduce DOX and breast cancer effects on the murine heart. Specifically, this study focused on investigating the underlying changes in cardiac oxidative stress, vasculature, metabolic enzyme function and glucose uptake. To do so the cardiac function was measured with ultrasound and the cardiac vascular density, cell size and antioxidative enzyme activities were measured together with markers of oxidative damage to lipids and proteins. The cardiac glucose uptake was measured with [^{18}F]FDG-PET imaging followed by the measurement of maximal activities of cardiac CS, LDH, β-Hydroxyacyl CoA dehydrogenase (HOAD) and mitochondrial complex activities. To best of my knowledge, these variables had not been studied together in response to exercise and DOX before this study, particularly in a tumor bearing model.

Study III: This study aimed to investigate whether voluntary running wheel exercise could affect DOX efficacy or cause potentially beneficial physiological changes in the tumor mitochondrial function, metabolic enzyme function, vascularity, blood flow and glucose uptake. The changes in tumor glucose uptake were measured with [^{18}F]FDG-PET together with the measurement of the maximal activities of tumoral CS, LDH and mitochondrial complexes. Furthermore, the tumor blood flow was measured following exercise and treatment using [^{15}O]H_2O-PET and [^{15}O]CO-PET. In addition, the tumor vascular density changes in capillaries and bigger vessels was measured together with the blood vessel α-SMA coverage and histological tumor hypoxia marker. The measurements are novel since these cellular markers have not been measured before in breast cancer bearing mice treated with DOX during ET and second, the PET imaging of blood flow of tumors has not been done before in best of my knowledge.

3 Materials and Methods

The Studies I-III were conducted using a mouse model of breast cancer as exercise effects cannot be studied using a cell line and certain mechanistic effects cannot be studied in patients. Human breast cancer implanted on animals, i.e. xenograft tumor model was not used in these studies although it would offer a possibility to study human cancer. This was because xenograft model requires a host that is not immunocompetent, which may significantly alter translatability to clinical setting where active immune system is present. Furthermore, exercise has been shown to have significant impact on the immune system and therefore immunocompetent host is a more preferable choice for an exercise intervention study.

Only female animals were used because it is more relevant for breast cancer since male breast cancer is exceedingly rare. Furthermore, majority of preclinical DCT studies have been done utilizing male rodents (Podyacheva et al., 2021), thus important information about females' responses is lacking. Also, DCT studies with exercise intervention have mainly been done in male rodents although there is data also in females (Naaktgeboren et al., 2021). Importantly, differences in DCT susceptibility and mechanisms between sexes have been shown in rodents, with females being more resistant, but it is still poorly understood whether these sex differences translate to clinical setting (Belger et al., 2024; Moulin et al., 2015). In contrast, majority of the very few clinical DCT studies with exercise intervention thus far have been done on female breast cancer patients undergoing chemotherapy (Naaktgeboren et al., 2021). More preclinical studies utilizing female animals are needed to better understand the mechanisms of DCT and exercise interventions happening in clinical setting in female patients.

3.1 Animals and experimental protocol (I, II, III)

The experiments were conducted in Karolinska institute (Study I) and in University of Turku (Studies II, III) with ethical licenses approved by the Swedish Agricultural Agency's regional animal testing committee of Stockholm (permission number N101-16) and national Project Authorization Board (permission number ESAVI/26508/2021). All the experiments were carried out in compliance with the EU Directive 2010/EU/63 on the protection of animals used for scientific purposes.

Study I-III utilized female FVB-mice (Janvier Labs, Inotiv and Charles River Laboratories) of similar age range (8–10 weeks) that were divided to different groups doing voluntary wheel running exercise training (ET) and no-ET groups according to **Figure 3**A. This thesis will refer to the voluntary wheel running exercise used in the studies I–III as ET to clarify that the acute effects of exercise were not studied, although voluntary wheel running exercise is not structured exercise of a specific intensity.

All animals were housed in pairs with *ad libitum* access to food and tap water and with 24/7 access to wireless low-profile running wheels (locked for no-exercise) with the spins of the wheels being recorded. All exercising mice were assumed to run equally per cage, which could underestimate their running activity as the animals have been seen using the wheels also simultaneously. The experiments had similar protocols, with minor variations in timing of imaging and sample collection as shown in **Figure 3**B. The time points in which data were obtained in the studies are: T1, the baseline before cancer inoculation; T2, few days after 2nd dose of DOX treatment (total 10 mg/kg); and T3, few days after 4th DOX dose (total 20 mg/kg).

All the animals were euthanized either day after the last dose of DOX (Study I) or about a week after last dose of DOX (Study II & III) unless if their tumors reached the end-point criteria defined in the ethical permits before experiment end. End-point criteria were wound formation on tumor (total of 2 animals), tumor volume of >1 cm^3 (Study I) or total tumor diameter of 1.5 cm (Study II & III). The hearts and tumors from animals euthanized earlier due to tumor growth were still collected and analysed for pooled T3 results. Organs could not be collected from few animals, but these animals were still included for tumor growth, heart function and running activity analyses. Tumor growth and animal weights were monitored throughout the study period and the tumor-free animals were handled equally and exposed to similar number of times to anaesthetics as the tumor-bearing animals. Tumor volume was measured with calipers (Study I) or with ultrasound under isoflurane anaesthesia (2%, Study II & III). Final tumor dimensions were measured at three dimensions from the dissected tumors using calipers. Tumor volumes were calculated using equation (1) with width and depth assumed equal in caliper measurements.

$$Tumor\ Volume = \frac{lenght \times width \times depth \times 3.14}{6} \quad (1)$$

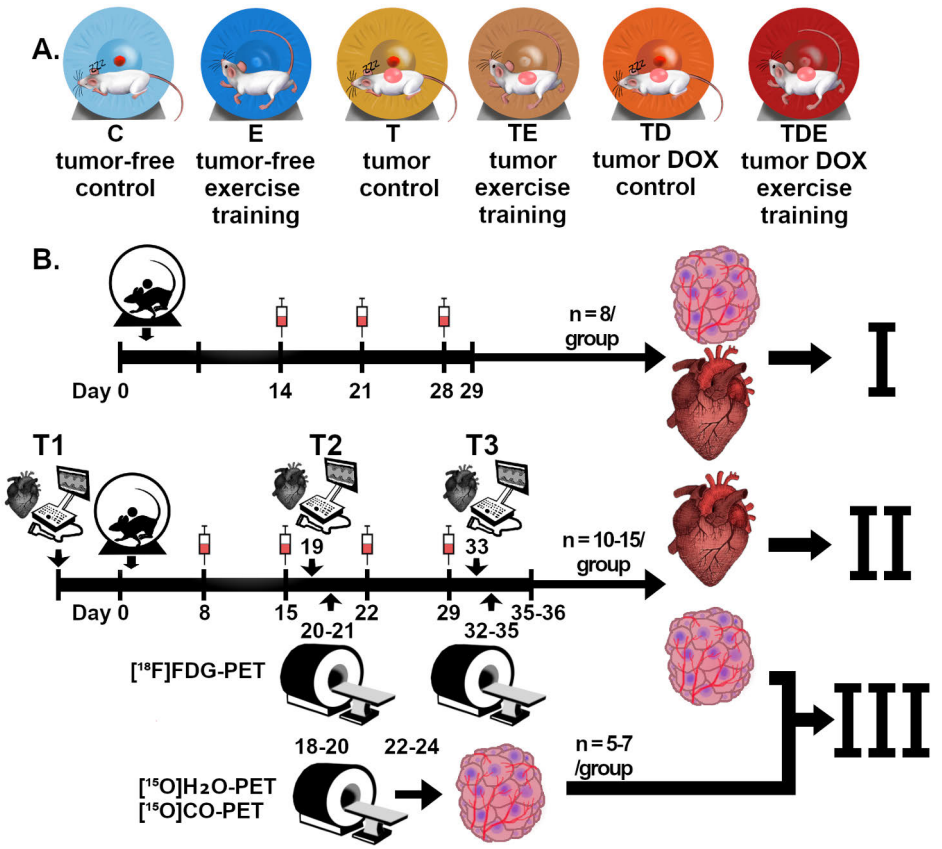

Figure 3. The experimental test groups (A) and experimental protocols (B) used in the studies I–III. Each experiment started with subcutaneous murine mammary tumor cell inoculation or phosphate buffered saline (PBS) injection (day 0) to the mouse flank. At day 1 (day 2 in Study I) all mice received voluntary running wheel (locked or spinning). The mice received weekly administration of DOX (5mg/kg) or PBS indicated by the red syringes. In Study I hearts and tumors were collected from TD and TDE groups. In Study II the heart and gastrocnemius muscle were collected at T3 from the test groups C, CE, T, TE, TD and TDE. The time of ultrasound and [^{18}F]FDG-PET imaging is indicated by picture of the respective machine at different time points (T1-T3). Also the tumors were collected from T, TE, TD and TDE at T3 for the Study III. In Study III the tumors were collected also from a subset of tumor-bearing mice from T, TE, TD, TDE at T2 couple days after imaging them using [^{15}O]H_2O-PET and [^{15}O]CO-PET. Collected tissues were divided for cardiac and tumor mitochondrial respiration measurements (Study II and III) and for enzyme activity, protein quantity and histological analyses (Study I–III). The n-numbers are indicated for each study, with only a subset of the animals imaged in PET at T2 and T3.

3.2 Cell culture and cancer initiation (I, II, III)

I3TC cells used in the experiments were previously isolated from the mammary tumors of transgenic PyMT-MMTV FVB-mice developing spontaneous mammary tumors (Weiland et al., 2012). One reason why the 13TC cells were chosen was because the tumors of the transgenic PyMT-MMTV model have been shown to cluster with

luminal B-like subtype and to mimic the progression of human cancer, with the gradual loss of ER and PR (Attalla et al., 2021). These cells were cultured in the same way in all experiments and inoculated subcutaneously to the animals' flank in the same way. However, in Study I the animals were inoculated without sedation under restraint while in Study II and III the inoculation was done under light isoflurane sedation (5% induction, 2% maintenance). More detailed cell cultivation and inoculation methods can be found from the studies I–III. In experiment I two million cells were inoculated while in experiments II and III 1.8 million cells suspended in phosphate buffered saline were inoculated. The tumor cells were inoculated subcutaneously to the flank of the animals, meaning the tumors would grow non-orthotopically. The flank inoculation was done, because it would prevent tumors from potentially getting mechanical irritation from rubbing against the bedding and from interfering with the running of the animals when growing larger in size if the tumors would be in mammary tissue. Subcutaneous breast cancer implanted near mammary glands mimics a situation where cancer has spread from mammary tissue to nearby tissue regions, and it accounts for some tumor secreted factors despite being non-orthotopic.

3.3 Echocardiography (II)

The transthoracic echocardiography was performed using Vevo 2100 (VisualSonics, Inc., Toronto, ON, Canada) on a heated surface with animal kept under light isoflurane anaesthesia (5% induction, ≈1.5% maintenance) without inducing bradypnea or bradycardia. More detailed description of the protocol is described in the Study II additional file 1. The LVEF was measured in B-mode by detecting the change in LV area between systole and diastole. LVEF was used as a measure of the cardiac contractility and function as it is more accurate than cardiac LVFS measured at M-mode by using only the change in cardiac diameter in the calculation. The LV wall thickness in diastole and systole were analysed from the M-mode images and the estimated LV mass was automatically calculated by the software. Left ventricular outflow tract velocity time integral (LVOT VTI) and the mitral blood flow peak velocities in early and late diastole (E/A) were measured in doppler mode. LVOT VTI reflects the column of blood moving through the left ventricular outflow tract during each systole and thus it can be used as a descriptor of stroke volume and, thus, used to calculate cardiac output.

3.4 Positron emission tomography (II, III)

Glucose analogy [^{18}F]FDG was used to monitor the organ accumulation of glucose using PET imaging with [^{18}F]FDG being commonly used to monitor cancer patients and offering important information of the glucose metabolism of the tissue in

question (**Figure 4**A). Radioactive water with radioactive isotope of oxygen [^{15}O]H_2O and carbon monoxide with the same isotope of oxygen [^{15}O]CO was used to monitor blood flow and blood volume within the organs (**Figure 4**B). Use of [^{15}O]H_2O and [^{15}O]CO to image small animals such as mice has novelty as not many studies have used these techniques in mice due to challenges related to the animal's body size, imaging resolution and the 3 mm positron range of ^{15}O-isotope (Slart et al., 2024). The [^{15}O]H_2O can be injected to the bloodstream, and it is freely diffusible and metabolically inert thus not accumulating into organs but offering information about the perfusion of organs. Meanwhile [^{15}O]CO can be inhaled within a mixture air and once diffused into blood from the lungs it binds to haemoglobin forming carboxyhaemoglobin. [^{15}O]CO remains within the blood giving information about the blood volume within tissues while together blood flow and volume allow the calculation of blood mean transit time (MTT) through tissue.

All PET/CT done in the Studies II and III were performed under light isoflurane anesthesia (3–5% induction, 1–3% maintenance) using small animal PET/CT (Molecubes NV, Ghent, Belgium) with a heated mouse bed. All animals had their tail vein cannulated for tracer injections and data analysis was done using Carimas software (Rainio et al., 2023) and an inhouse program. More detailed imaging protocols are given within the Study II and III.

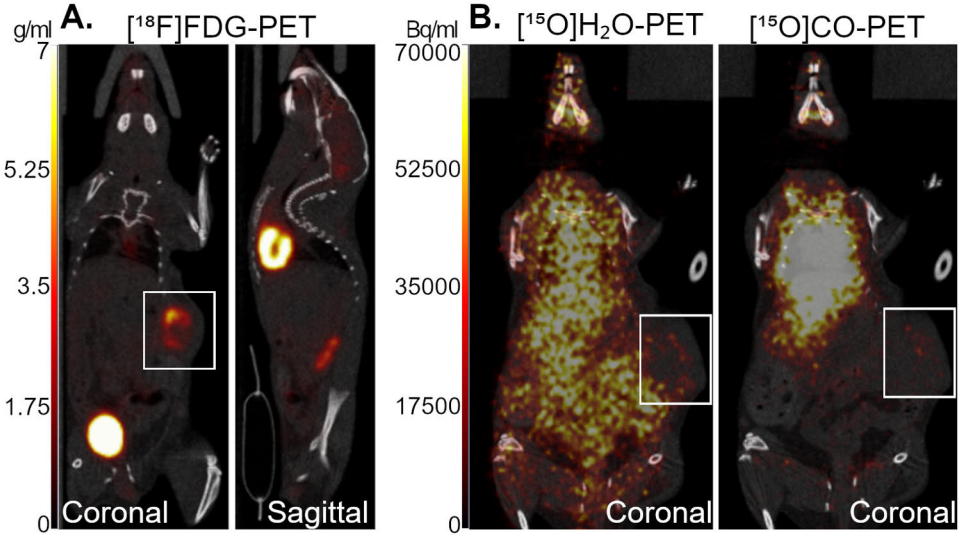

Figure 4. Exemplary mouse positron emission tomography (PET) images. The images are showing the glucose analog [^{18}F]FDG-PET images (A) and the ^{15}O-tracer images (B) with the [^{15}O]H_2O-PET on left and [^{15}O]CO-PET on the right. Exemplary images within A are from the same animal from coronal and sagittal plane. The mean weighted ^{15}O-tracer images are shown from the same animal from last three imaging frames (≈300–480 s). The tumors are indicated with a white rectangle. The heart is clearly visible within [^{18}F]FDG-PET sagittal plane image.

3.4.1 Glucose uptake measurements (II, III)

The [^{18}F]FDG imaging was done for two mice at a time at T2 and T3 according to **Figure 3**. If the mouse was euthanized prior T3 due to tumor growth, another random mouse from the same group was picked for T3 imaging. The mice were fasted for approximately two hours before the imaging, with their blood glucose measured using a glucometer (glucometer, Bayer Contour, Bayer AG, Leverkusen, Germany) from the saphenous vein before and after the [^{18}F]FDG/CT imaging. The animals had 3±0.2 MBq (140±15 MBq/kg) of [^{18}F]FDG tracer injected via tail vein, after which they were CT imaged for attenuation correction and anatomical reference. After 20 minutes from tracer injection the mice were PET imaged using static imaging with framing 1×1200s. The PET [^{18}F]FDG images were analysed by drawing regions of interest (ROI) for each analysed tissue, like LV wall and whole tumor as well as metabolic tumor volume (MTV). MTV was defined as tumor area with a signal >50% of whole tumor standardized uptake value maximum (SUVmax). The standardized uptake value (SUV) for [^{18}F]FDG was calculated for each drawn ROI with equation (2) using decay corrected radioactivity values. For the tumor the whole tumor glucose uptake heterogeneity (GUH) was also calculated using equation (3) and total lesion glycolysis (TLG) using equation (4). The [^{18}F]FDG SUV values (indicative of tissue glucose uptake) were calculated also for other organs such as liver, femur (containing both marrow and compact bone), gastrocnemius muscle and brain.

$$SUV = \frac{Radioactivity\ within\ ROI(Bq/ml)}{Dose(Bq)/Weight(g)} \quad (2)$$

$$GUH = \frac{SUV\ SD\ within\ ROI}{SUV\ within\ ROI} \times 100 \quad (3)$$

$$TLG = whole\ tumor\ mean\ SUV\ \times\ MTV \quad (4)$$

3.4.2 Blood flow measurements (III)

The [^{15}O]H_2O and [^{15}O]CO imaging was done for one mouse at a time at T2 according to **Figure 3**. After being positioned in the bed the mice were injected with [^{15}O]H_2O (10 MBq) via tail vein cannula with injection lasting one-minute. The 7.5-minute dynamic PET-imaging was started at the same time with injection with framing totalling to 450 seconds (frames 20×5s, 3×10s, 4×20s, 4×30s, 2×60s). After radioactive decay another dynamic scan was started at the same time with continuous 3-minute inhalation of gaseous [^{15}O]CO mixed with isoflurane and room air which was delivered using an inhouse developed automated gaseous radiopharmaceutical administration system (Shimochi et al., 2024). The total length of the dynamic

[^{15}O]CO-scan was 480 seconds (frames 8×60s). Finally, the mice were CT imaged for anatomical reference and the correction for attenuation and scatter.

The signals were corrected for radioactive decay and the regions of interest (ROIs) were drawn for the whole tumor and the high flow region determined by drawing ROI covering the highest flow signal areas for each tumor. ROI was also drawn within the LV cavity for the determination of LV input function (LV IF). The LV IF signal was corrected for spill-over radioactivity caused by the limited spatial resolution of PET images relative to the size of LV. LV spill-over was corrected by determination of ROI recovery coefficient done as previously validated for clinical PET imaging (Iida et al., 1992). However, this spill-over correction requires to be validated further in the future studies. The blood flow was determined for each ROI from [^{15}O]-H_2O time activity curves (TACs) as described in Study III. The fitted parameters were the rate constants K_1 blood flow (ml blood × ml volume^{-1}×min^{-1}) and k_2 (min^{-1}) as well as the arterial blood volume fraction (Va, ml arterial-blood ×ml volume^{-1}). Vascular blood volume fraction was calculated from the [^{15}O]-CO TAC using the mean pixel counts from the end of TAC when the distribution reached equilibrium using the same ROIs that were utilized in blood flow determination. Tissue blood vascular volume fraction was calculated using equation (5) and the blood MTT (Mihara et al., 2003) was calculated using equation (6). Similarly, the blood flow, blood volume fraction and MTT was calculated for the mouse brain, liver, femur, and muscle gastrocnemius muscle by drawing ROIs for each tissue.

$$Blood\ vascular\ volume\ fraction = \frac{ROI\ radioactivity\ at\ end\ of\ [^{15}O]CO\ TAC}{\text{Total blood activity concentration mean signal}} \quad (5)$$

$$MTT = \frac{Blood\ vascular\ volume\ fraction}{Tissue\ blood\ flow\ K_1} \quad (6)$$

3.5 Mitochondrial respiration measurements (II, III)

Mitochondrial function of LV and tumor were measured from tissue homogenates in studies II and III. The tissues were kept in cold biopsy preservation solution BIOPS (Study II Additional file 1) until homogenization in mitochondrial respiration medium (Study II Additional file 1) according to the protocol provided in studies II and III. Mitochondrial function was measured by measuring changes in mitochondrial oxygen consumption following addition of substrates and inhibitors using high-resolution respirometry oxygraph 2k (Oroboros Instruments corp., Innsbruck, Austria). The measurement chamber oxygen level was raised at the beginning of each measurement using pure oxygen injected to partially open respirometer chamber and the oxygen level was maintained during the measurements after antimycin-A addition using catalase and H_2O_2 injections to a closed chamber. The mitochondrial respiration was allowed to stabilize before addition of each

substrate and inhibitor to measure the mitochondrial respiration linked to different respiratory states according to **Table 2**. The coupling efficiency was calculated by dividing complex I driven coupled respiration with complex I linked total respiration. For the hearts also the CS/CIV-ratio was calculated by dividing maximal CS activity per grams of tissue with maximal complex IV activity.

Table 2. The substrates and inhibitors in order of addition and mitochondrial respiratory state calculations.

Substrates & Inhibitors	Respiratory state measured	Organ
No exogenous substrates	Residual oxygen consumption: ROX	Tumor
Pyruvate (P) Malate (M) Glutamate* (G)	Complex I driven uncoupled respiration: LEAK = (Respiration with PMG) –ROX	Left ventricle Tumor
ADP-K•H_2O[#] (+0.6 mol $MgCl_2$ / ADP mol)	Complex I driven coupled respiration: CI-OXPHOS = (Respiration at this stage) –LEAK–ROX	Left ventricle Tumor
Cytochrome C	Cytochrome C response: CytC response (%) = (Respiration at this stage) – (Respiration with PMG+ADP) ÷ (Respiration with PMG+ADP) ×100	Left ventricle Tumor
Succinate	Complex I&II driven coupled respiration: CI&CII-OXPHOS = (Respiration at this stage) –LEAK–ROX	Left ventricle Tumor
FCCP	Maximal electron transfer capacity: ETSmax = (Respiration at this stage) –ROX	Left ventricle Tumor
Rotenone	Complex II linked maximal electron transfer capacity CII-ETS = (Respiration at this stage) –ROX	Left ventricle Tumor
	Complex I linked maximal electron transfer capacity: CI-ETS = ETSmax – CII-ETS	Tumor
Antimycin-A	Residual oxygen consumption: ROX	Left ventricle
Ascorbate + TMPD	Maximal activity of complex IV: CIV max = (Respiration at this stage) –ROX, –autoxidation)	Left ventricle Tumor
Sodium azide	Autoxidation	Left ventricle Tumor

*G was only added for LEAK calculation in heart, [#]Two different values of CI-OXPHOS were calculated for the tumors: with G and without G. FCCP = Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone. More details are provided within the Study II and III.

3.5.1 Determination of the mitochondrial quantity (II, III)

In the study II, the cardiac mitochondrial quantity was estimated using mitochondrial index calculated as the ratio of mitochondrial gene (mDNA) for NADH-ubiquinone oxidoreductase chain 1 protein and nuclear gene (nDNA) hexokinase 2 determined using qPCR and the equations (7) and (8) (Quiros et al., 2017). Additionally, CS-activity was used as a proxy for the mitochondrial quantity in hearts and tumors in the Study II and Study III.

$$mtDNA = 2 \times 2^{\Delta Ct} \quad (7)$$

$$\Delta Ct = CT(nDNA\ gene) - CT(mDNA\ gene) \quad (8)$$

3.6 Spectrophotometric assays (I, II, III)

A piece of snap frozen LV, tumor and gastrocnemius muscle was homogenized in 1 mg / 10 µl of homogenization solution (50 mM Hepes, 1 mM EDTA, 0.1% Triton-X, pH 7.4) and in methanol (Study I) or 1 mg / 10 µl of 100 mM K-phosphate buffer containing 150 mM KCl solution with pH 7.4 (studies I, II). The raw homogenate or supernatant from centrifuged homogenate was used for spectrophotometric measurements listed in Table 3.

Table 3. Spectrophotometric techniques used, for further detais see original studies (I,II, III).

Measurement	Assay method	Kit or method source	Organ[Study]
Catalase activity	Chromogenic probe assay	(Vuori & Kanerva, 2018)	LV[I, II]
Citrate synthase activity	Dynamic CoA(SH) + DTNB measurement	(Anttila et al., 2013)	LV[I, II], Tumor[I, III], M Gastro[II]
HOAD activity	Dynamic NADH measurement	(Driedzic & Fonseca de Almeida-Val, 1996)	LV[II]
Lactate dehydrogenase activity	Dynamic NADH measurement	(Anttila et al., 2013)	LV[I, II], Tumor[I, III], M Gastro[II]
Lipid hydroxyl peroxide content	Ferrous Oxidation − Xylenol Orange Assay	(Raja-aho et al., 2012)	LV[I, II], M Gastro[II]
Protein carbonyl content	DNPH method	Sigma-Aldrich MAK094	LV[II]
Superoxide dismutase activity	Indirect xanthine oxidase-based method	Sigma-Aldrich 19160	LV[II]
Total protein concentration	Bicinchoninic acid assay	Thermo Scientific™ Pierce™ Cat. 23225	LV[I, II] Tumor[I, III], M Gastro[II]

I, II, III Original study in which the measurement was used, HOAD = 3-Hydroxyacyl-CoA dehydrogenase, M Gastro = Muscle gastrocnemius, LV = Left ventricle

3.7 Histology and western blot (I, II, III)

The protein detection was done using western blot and immunostaining of the gel separated proteins according to **Table 4** with images of Tris-Glycine eXtended stain-free fast cast acrylamide kit 12% (Bio-Rad) used to detect the total protein per lane.

All histological staining was done using paraffin embedded tissue sections using either conventional staining or immunohistology staining methods according to **Table 4**. In Study II the heart capillary staining was done using Periodic Acid-Schiff (PAS) staining. The sections were sealed using aqueous Kaiser's glycerol gelatin in Study I (108635 Sigma-Aldrich) while aqueous sealing agent VECTASHIELD® HardSet™ Antifade Mounting Medium with DAPI (H-1500-10) was used for fluorescent stained sections in studies II and III with rest of the sections being sealed with non-aqueous DPX mounting media (Sigma-Aldrich, Merck).

Table 4. Primary antibodies that were used in the experiments. See the original studies for further details such as the concentrations and incubation times.

Antigen	Type and host	Manufacturer	Application	Organ	Study
Anti-Actin α-Smooth Muscle-Cy3™	Mouse monoclonal clone 1A4	Sigma-Aldrich C6198, clone 1A4	Histology	Tumor	I, III
Carbonic anhydrase 9	Rabbit polyclonal	Novus Biologicals Bio Techne NB100-417	Histology[a]	Tumor	III
Cleaved Caspase-3	Polyclonal rabbit	Cell signaling technology® #9661	Histology[a], WB[b]	Tumor	I, III
HIF1-α	Rabbit polyclonal	Abcam ab2185	WB[b]	Heart, Tumor	I
Ki67	Rabbit polyclonal	Sigma-Aldrich AB9260	Histology[a], WB[b]	Tumor	I, III
Podocalyxin	Goat Polyclonal	R&D systems AF1556	Histology[a,c]	Heart, Tumor	I, III
VEGF-A	Rabbit monoclonal	Abcam ab214424	WB[b]	Heart, Tumor	I

[a]Anti-rabbit or anti-goat biotinylated secondary antibodies were used (Invitrogen #31732, Vector laboratories BA-1000), [b]The secondary goat anti-rabbit antibody IRDye® 800CW 926–32211, LI-COR was used, [c]Anti-goatsecondary antibody AlFl.488 LifeTech A11055 was used.

3.8 Statistical methods (I, II, III)

Statistical testing listed in **Table 5** was done using Sigma Plot 15, SAS® Enterprise Guide®, GraphPad Prism 5.01 and SPSS 27. The data normality and equality of variance was tested with Shapiro-Wilk and Brown-Forsythe respectively. LOG

transformed data was used for testing if the data normality and variance was improved, otherwise non-parametric tests were used when possible. If the non-parametric test was not possible then non-transformed data was used with the approximate normality checked with a histogram. Approximate normality and equality of residual variances was confirmed for linear model with residual histogram, residual QQ-plot or linear predictor plot. All correlation tests were done using Pearson or Spearman correlation test.

Table 5. Statistical tests used in studies I,II and III

Variable type	Groups compared	Statistical test	Factors	Study
Single time point variables	TD, TDE	t-test or Mann-Whitney Rank Sum or Welch's t-test	-	I
Single time point variables	T, TE, TD, TDE	Two-way ANOVA	ET & DOX	II, III
Single time point variables	C, E, T, TE	Two-way ANOVA	ET & cancer	II
Running activity	TDE: Running at week 1–2, week 3 & week 4	One way ANOVA	Timeframe	I
Running activity	CE, TE, TDE or TE, TDE	Two-way RM ANOVA	Group & Time	II, III
Tumor growth	TD, TDE	Linear model on repeated measures	Group & Time	I
Tumor growth	T, TE, TD, TDE	Proc Glimmix LM RM, with unstructured covariance	ET, DOX & time	III
Heart PET-FDG	C, E, T, TE, TD, TDE or C, E, T, TE	Proc Glimmix LM RM, with unstructured covariance	ET, Time & *DOX or ET, cancer & time	II
Tumor PET-FDG	T, TE, TD, TDE	Proc Glimmix LM RM, with unstructured covariance	ET, DOX & time, covariate: tumor Vol	III

ET = Exercise training, DOX = Doxorubicin, C = tumor-free control group, E = tumor-free ET group, T = Tumor group, TE = Tumor ET group, TD = Tumor group treated with DOX without ET, TDE = Tumor group treated with DOX and with ET. LM RM = linear model on repeated measures, *DOX nested under cancer.

4 Main Results and Discussion

4.1 Exercise training effects on doxorubicin efficacy and breast cancer tumors

4.1.1 Tumor growth, apoptosis, and proliferation

To investigate whether voluntary wheel running ET affected DOX efficacy, the changes in tumor volume over time were evaluated together with the tumor proliferation and apoptosis using proliferation marker Ki67 and apoptosis marker cleaved caspase 3 (cas3). In Study I four weeks of voluntary running wheel ET significantly slowed tumor growth in DOX treated animals, although the final tumor volumes were similar between the groups (Fig 1 in Study I). ET slowed tumor growth via increasing tumor cell apoptosis without affecting tumor cell proliferation as indicated by significantly increased cas3 positive cells and similar levels of proliferation marker Ki67 (Fig 3 in Study I). However, without test groups lacking DOX treatment, it could not be confirmed whether ET enhanced chemotherapy or sensitized tumors to treatment, or whether ET alone inhibited tumor growth.

In contrast, in study III ET had no effect on tumor growth or animal survival in shorter time frame or in longer time frame (Fig 1 and Fig 4 in Study III). However, in the longer time frame DOX significantly decreased the mouse running activity (Fig 4 in Study III). In accordance with these findings the tumor proliferation and apoptosis markers were similar between the groups in study III (Fig 1 and Fig 4 in Study III). The ET intensity may not have been enough to affect tumor growth in Study III because the average running activity of the animals was lower compared to Study I no matter if mice were treated with DOX or not (**Table 6**).

Besides running intensity, the timing of running program in relation to cancer cell inoculation might influence whether ET can affect tumor growth or not. This is because previously it has been shown that exercise alone 6 km/day for four weeks started two weeks prior cancer initiation could inhibit tumor growth of the 13TC tumors (Rundqvist et al., 2020). This finding also supports that more intense ET might be needed to inhibit tumor growth. Furthermore, Wakefield et al. (2021) showed that exercise 10–12 km/day for 4.2 weeks started concurrently with DOX treatment could sensitize EMT6 intraductal mammary tumors to otherwise

inefficient DOX treatment. ET in Study III did not sensitize tumors to DOX treatment, but more efficient treatment dosage together with the same level of physical activity could have more beneficial effects against the subcutaneously inoculated breast tumors. However, this requires further investigations.

Table 6. Comparison of average running activity and duration of the mice in Study I and III.

	Tumor DOX ET Study I	Tumor DOX ET Study III T3	Tumor ET Study III T3	Tumor DOX ET Study III T2
Running (km/day)	4.7±0.6	2.2±1.73	3.9±3.16	3.6±1.7
Run duration (wk)	3.7±0.1	3.85±0.99	3.80±1.26	3.12±0.13

Study III T3 = mice kept for T3, Study II T2 = mice kept for T2, DOX = Doxorubicin, ET = Exercise training (voluntary wheel running)

Besides running activity and timing, the initial tumor growth rate might also affect the differences in study outcomes. In Study I the tumors grew initially significantly slower than tumors in Study III as indicated by tumor volumes prior chemotherapy (**Figure 5**). However, tumors from Study III which were allowed to grow only until T2 grew, nevertheless, similarly as in Study I before DOX treatment (**Figure 5**). Despite this, all Study III tumors had the DOX treatment started earlier than in Study I. Both the higher initial tumor growth rate as well as longer preconditioning with voluntary wheel running ET prior treatment could affect the differential tumor growth outcomes, although ET did not affect tumor volumes until after DOX was started in Study I. Furthermore, the mice from Study I, Study III T2, and Study III T3 were all ordered from different sources and the Study I was done at a different facility, which could contribute to different initial tumor growth through potentially differential gut microbiota composition. The gut microbiota has been shown to affect tumor growth in several cancers in mice (Sethi et al., 2018). Lastly, it must be noted that the tumor size change may not tell everything about the tumoral response

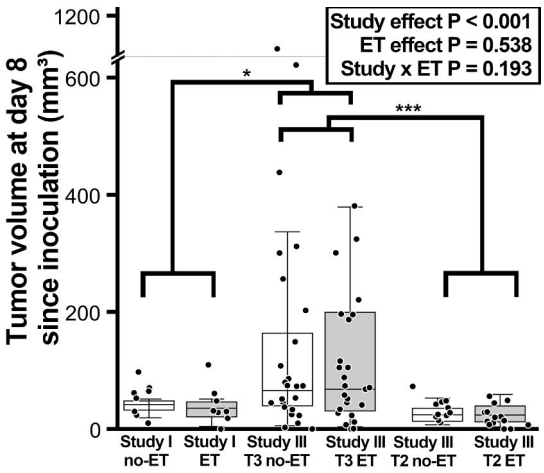

Figure 5. Tumor volume comparison between studies before chemotherapy. Volumes were recalculated similarly using tumor length and width. Two-way ANOVA P values are shown with Holm Sidak post hoc done to compare studies *P<0.05, ***P<0.001. ET = Exercise training, DOX = Doxorubicin. Study III T3 = mice kept for T3, Study II T2 = mice kept for T2.

to DOX and ET as tumors can contain large inactive regions with necrosis or connective tissue (Parihar et al., 2023).

4.1.2 Tumor vascularity and blood flow

The tumor vascularity and blood vessel maturation detected via α-SMA blood vessel coverage were investigated to see whether blood delivery to the tumor would be altered by vascular changes induced by ET, which could in turn potentially affect the drug delivery to the tumor. To investigate changes in tumor perfusion the tumoral hypoxia markers HIF1-α and, CA9 were quantified and the *in vivo* blood flow using PET imaging was measured.

Study I found no evidence of tumor blood vessel normalization indicated by α-SMA coverage or increase in blood vessel density despite ET significantly inhibiting the tumor growth and increasing apoptosis (Fig 3 in Study I). Furthermore, VEGF-A and HIF1-α protein levels were not altered in the tumors (Fig 4 in Study I). This suggests that in Study I the tumor growth was not inhibited through vascular normalization. However, sometimes vascular normalization is not detectable via α-SMA coverage but instead via increased number of visible lumens and longer blood vessels (Schadler et al., 2016). Blood flow measurements *in vivo* would be needed to confirm if tumor blood perfusion was altered *in vivo*. The increased tumor apoptosis in Study I could be caused by other factors, like changes in immune cell infiltration which was not the focus in these studies. Previously ET has been shown to inhibit tumor growth via increasing immune cell infiltration to breast tumors and melanoma tumors (Pedersen et al., 2016; Rundqvist et al., 2020). Some studies have also shown that exercise can both normalize tumor vasculature and increase tumor immune cell infiltration (Gomes-Santos et al., 2021a; Gomes-Santos et al., 2024).

In contrast, we saw effects on tumor vascularity and blood flow in Study III even though in that study the tumor growth was not affected. In Study III at T2, before physical activity decreased in DOX treated groups, the ET decreased the density of blood vessels larger than 8 μm in diameter irrespective of DOX treatment (Fig 2 in Study III). At T2 there was also a non-significant trend of ET increasing tumor blood flow (Fig 2 in Study III), and the tumor blood flow positively correlated with the tumor capillary density in ET groups whereas within the no-ET groups this correlation was not quite significant (Additional file 1 Fig 3 in Study III). Later, the tumor blood vessel α-SMA coverage, overall blood vessel density, capillary vessel density, and the larger vessel density were similar between the groups at T3 (Fig 5 in Study III). These changes suggest that ET slightly improved tumor blood flow initially but not later, without however, affecting the tumor growth or tumor hypoxia as was indicated by similar tumor volumes and unchanged hypoxia marker CA9 coverage (Fig 2 in Study III). The positive correlation between hypoxic tumor area

and tumor capillary density suggests hypoxia induced angiogenesis and large number of poorly functioning blood vessels, which may help explain poor DOX treatment response in study III (Additional file 1 Fig 3 in Study III).

Study III found also that the percentage of α-SMA positive blood vessels negatively correlated with tumor blood flow and that the faster growing tumors tended to have higher proportion of α-SMA positive blood vessels (Additional file Fig 4 in Study III). Meanwhile ET and DOX alone, but not when combined, decreased the α-SMA positive blood vessels at T2 (Fig 2 in Study III). Similarly, DOX and ET both alone, but not when combined, decreased the blood MTT suggesting faster blood passage through the tumor without DOX treatment (Fig 2 in Study III). The combination effects of ET and DOX might have been undetected due to low n-number and greater group variation. Moreover, despite DOX not affecting tumor growth in Study III, DOX decreased tumor blood volume fraction suggesting some treatment effect at T2. Decline in blood flow has been shown to predict patient survival, and the chemotherapy responsive breast tumors have been shown to have decreased blood flow whereas unresponsive tumors had increased tumor blood flow (Mankoff et al., 2003).

However, the faster blood MTT could be indicative of more shunt vessels which do not facilitate efficient oxygen diffusion. Indeed, there was no reduction in tumor hypoxia in Study III, which may partially explain lack of significant correlation between tumor blood flow and tumor hypoxic area (Additional file Fig 3 in Study III). Previously α-SMA quantity has been shown to be associated with number of larger blood vessels, with the larger vessels size being associated with worse breast cancer patient outcomes (Milosevic et al., 2023). Therefore, decrease in α-SMA positive blood vessels may be beneficial. However, in contrast to our findings exercise has been shown to usually increase tumor blood vessel pericyte coverage (measured as α-SMA positive vessels) in breast cancer in association with normalized tumor vascularization (Gomes-Santos et al., 2021a). However, tumor vascularity can be also normalized in absence of changes in α-SMA coverage (Schadler et al., 2016). Some tumors also have increased blood vessel α-SMA coverage as opposed to decreased coverage (Bergers & Song, 2005; Morikawa et al., 2002b). For example, pancreatic ductal adenocarcinoma tumors can acquire high capillary blood vessel expression of α-SMA, that was also associated with blood vessel leakiness (Natarajan et al., 2022). Study III results support that in our breast tumor model the high blood vessel α-SMA coverage was associated with less functional blood vessels. This makes sense also if capillaries vessels acquire α-SMA coverage with increased smooth muscle cell coverage, thus indicating arterialization and indicating vessels with less oxygen exchange.

The initial changes in vascularity and blood flow did not, however, alter tumor growth. The reasons might be the ET intensity and the ineffective DOX treatment.

Recently it was shown that both low and moderate intensity ET can normalize breast tumor vasculature while high intensity ET does not (Gomes-Santos et al., 2024). It was also shown that only the moderate intensity ET increased tumoral immune cell infiltration and inhibited tumor growth (Gomes-Santos et al., 2024). Improved tumor vascularization and blood flow could be expected to improve DOX efficacy even without increased immune cell infiltration, but likely this did not happen as the tumors were DOX unresponsive in the first place. However, the changes in tumor vascularity and blood flow may have altered tumor necrosis and metabolic activity instead of volume.

4.1.3 Tumor glucose uptake and metabolism

To study the tumor metabolism in response to ET, the tumors were crushed, and part of the tumor was homogenized and the maximal activities of CS, LDH as well as mitochondrial respiration were analysed. Furthermore, the tumor glucose uptake was measured *in vivo* using PET [^{18}F]FDG imaging.

The [^{18}F]FDG SUVmax, maximal glucose uptake value within the whole tumor region, was significantly decreased by DOX at T2 in Study III suggesting initial treatment response (Fig 5 in Study III). Over time voluntary wheel running ET and DOX both alone caused a further significant decrease in [^{18}F]FDG SUVmax which was not detected in the ET group also receiving DOX treatment. Furthermore, the MTV describing the glucose uptake within metabolically active tumor region was also decreased over time and both ET and DOX alone caused a further decrease, which was not quite significant in ET group receiving DOX (Fig 5 in Study III). It is possible that together the effect of DOX and ET was not significant due to increased variation and insufficient n-number, but clearly the ET was not sufficient to potentiate the effect of DOX on tumoral glucose uptake. Meanwhile the glucose uptake heterogeneity was significantly reduced by ET irrespective of DOX treatment (Fig 5 in Study III). This corresponds with previous findings in which exercise reduced the heterogeneity in number of perfused blood vessels within orthotopic mammary tumors without altering total perfused vessel number, capillary density, tumor hypoxia, and tumor growth (Buss et al., 2020).

The whole tumor [^{18}F]FDG SUV and TLG describing overall tumoral glucose uptake was not altered by ET or DOX, although they too decreased over time in all groups (Fig 5 Study III). This suggests that over time tumors tended to become less metabolically active or at least utilize less glucose, which is expected for larger tumors with larger portions of their volume containing necrotic regions. In agreement with unaltered whole tumor glucose uptake, the tumoral CS and LDH maximal activities in Study III and in Study I were similar between the groups (Fig 4 in Study I, Additional file Table 3 in Study III). Furthermore, the tumor

mitochondrial function had no detectable changes in Study III T2 in response to ET nor DOX treatment (Additional file Table 4 in Study III). However, these zero findings need to interpret with caution as they are based on only a part of the tumor. Due to tumor heterogeneity, the data obtained from a part of the tumor might not accurately describe the whole tumor or other tumor regions.

Importantly, DOX and ET mediated decrease in some of the tumor glucose uptake parameters suggests that there may have been some beneficial tumor response over time suggesting reduced tumoral glucose utilization. However, these changes did not translate to changes in tumor growth, which may in part be due to the short study timeframe which does not match the longer treatment timeframe and follow up times used in the clinical setting (Mankoff et al., 2003). Future studies are needed to investigate why the glucose uptake was altered by DOX and ET, and whether these changes precede changes in tumor growth in longer term. Furthermore, thesis does not answer whether the tumor cells shift to use more the fatty acids and what is the proportion of the tumoral glucose uptake caused by a presence of non-tumor cells like tumor infiltrating lymphocytes. Nonetheless, the decreased tumor glucose uptake parameters may be beneficial considering that high tumoral [^{18}F]FDG SUVmax and MTV have both been linked with adverse effects in breast cancer patients (AbdElaal et al., 2021; Pak et al., 2020). The [^{18}F]FDG SUVmax has also been shown to correlate with proliferative index of the tumor measured using Ki67 (Bostancı & Hasbek, 2020), but this marker was similar between groups in Study III. Furthermore, changes in tumor glucose uptake have been shown to sometimes precede changes in tumor size, therefore helping to predict treatment responses (Spaepen et al., 2001).

4.2 Cardioprotective effects of exercise training during doxorubicin treatment of breast cancer

4.2.1 Heart function and vasculature

Besides investigating how ET influenced the tumors, study I focused on whether voluntary wheel running ET could cause beneficial structural alterations in heart in DOX treated animals after 15mg/kg cumulative DOX dose. In Study I it was found that ET increased the number of capillaries per cells without affecting the VEGF-A or HIF1-α protein expression nor the cardiomyocyte cross-sectional area (Fig 2,3 in Study I). Previously it has been shown that DOX can lead to cardiac capillary rarefaction, and therefore an increase in capillarity could be a positive change (Räsänen et al., 2016). However, the heart function was not measured in Study I and without control animals untreated with DOX it could not be determined whether at this stage DOX had already caused cardiac toxicity, which the ET could counter.

In Study II it was investigated how the DOX affected heart function over time and whether these changes were mitigated by voluntary running wheel ET. In Study II the heart function was measured at baseline and after 10 mg/kg and 20 mg/kg cumulative DOX dose. As expected, in Study II the cumulative dose of 20 mg/kg of DOX impaired cardiac function by reducing LV EF and LVOT VTI and caused loss of LV mass, number of capillaries per cells and body weight (Fig 1, 2, 5 in Study II). In Study II the decrease in cardiac mass was not due to cardiomyocyte atrophy, as cells size was not different between the groups, thus suggesting loss of cardiac mass may have been due to cardiomyocyte apoptosis. More importantly, Study II found that the decrease in EF, LVOT VTI and LV mass was ameliorated in breast cancer bearing mice after ET of just 2.2±1.73 km/day done for 4 weeks, despite the running activity decreasing as cumulative DOX dose increased (Fig 1,2 in Study II). Furthermore, ET potentially improved diastolic function over time as there was a slight increase in E/A ratio over time (Fig 2 in Study II). Despite ameliorating DOX induced cardiac dysfunction, ET did not fully reverse the decrease in EF and LV mass, while the bodyweight loss and capillary rarefaction were not mitigated (Fig 1,5 in Study II). It is possible that greater ET intensity or longer duration could be even more beneficial.

This is supported by the fact that there was greater physiological cardiac hypertrophy and increase in EF in the healthy and tumor-bearing animals without DOX treatment, which had higher physical activity. Furthermore, the greater physical activity in Study I increased the number capillaries per cardiomyocytes, and previous studies also highlight the importance of ET intensity. Previously, voluntary wheel running ET 9–15km/day for 30 days was shown to ameliorate DOX (18 mg/kg) induced cardiac damage in tumor-free female mice, but also not fully reverse it (Wakefield et al., 2021). Similarly, another study showed that low to moderate intensity treadmill ET for 5 weeks during cumulative DOX dosing of 25 mg/kg only mitigated DOX induced myocardial circumferential strain, but not decline in LVEF in tumor-free male mice (Gomes-Santos et al., 2021b). Meanwhile, in a study on male mice with melanoma, low intensity ET (10 m/min, 45 min/day, 5 days/wk.) of two weeks during DOX treatment (cumulative 4 mg/kg) was not cardioprotective (Sturgeon et al., 2014b). This suggests that there is a threshold of ET that is needed for cardiac protection. Indeed, a study using Ewing's sarcoma bearing immunodeficient male mice showed that just 2-week treadmill ET 45 min/day 12 meters/min fully reversed decrease in cardiac mass and EF during exposure to cumulative DOX dose of 10 mg/kg, but that the loss of body weight was not prevented (Wang et al., 2018). Furthermore, treadmill ET, which could be expected to be more intense than voluntary wheel running, has been shown to prevent DOX mediated decrease in cardiac blood vessel pericyte coverage and number of open lumens, which suggests improved cardiac vascular function (Wang

et al., 2021). Differences in the level of cardioprotection are likely due to differences in ET intensity in relation to the cumulative DOX dose, as well as sex differences and differences in tumor burden.

Indeed, Study II found that the tumor burden itself caused a small but significant reduction in whole cardiac mass and near significant reduction in body weight increase, as well as a blunted ET induced heart hypertrophy (Additional file 1 Fig 1 in Study II). Similarly, a study on Ewing's sarcoma model revealed that DOX caused bodyweight loss and loss of cardiac mass only in the tumor-bearing animals but not in tumor-free animals (Wang et al., 2018). It is possible that, despite the tumor bearing mice without DOX treatment running approximately same distance per day as the tumor-free mice in Study II, the mice could have run at a different intensity thus blunting the ET induced hypertrophy. In support of this, one study previously showed that breast tumor burden reduces exercise tolerance, although it does not reduce VO_{max} nor functional cardiac parameters directly (Weber et al., 2025). Interestingly, one clinical study found that prior chemotherapy breast cancer patients with tumor or after tumor removal surgery, already exhibited relative cardiac remodelling and subclinical cardiac dysfunction associated with the activation of the endothelin system (Maayah et al., 2023). These changes could, however, be characteristics of patients at higher risk of cancer instead of being directly caused by the tumor burden. Nonetheless, these effects of cancer highlight importance of considering the tumor burden in DCT and ET intervention investigations, particularly when the mechanisms of DCT and ET mediated cardiac protection are investigated.

4.2.2 Heart glucose uptake and metabolism

To study how voluntary wheel running ET and DOX modified cardiac glucose metabolism in murine breast cancer model, the cardiac glucose uptake, maximal CS and LDH activity and mitochondrial function were assessed from LV homogenates. Furthermore, in Study II the HOAD activity was also measured to see some indication of changes in fatty acid metabolism, with HOAD catalysing the third reaction of beta-oxidation in which fatty acids are broken down.

In Study I voluntary running wheel ET until 15mg/kg cumulative dose of DOX significantly increased cardiac LDH activity, but not maximal CS activity (Fig 2 in Study I). However, the cardiac HIF-1α was not altered (Fig 2 in Study I). This is an interesting novel finding, as cardiac CS, LDH and HIF1-α have been scarcely studied previously in response to ET during DOX treatment.

In contrast, Study II revealed that 20 mg/kg of DOX significantly increased cardiac glucose uptake, and ET decreased the glucose uptake, while also increasing cardiac maximal CS activity and decreasing cardiac LDH activity in DOX treated

mice (Fig 2 in Study II). It is possible that the ET induced change in LDH activity was different between studies I and II due to the acute effects of DOX in study I, as the hearts were collected day after last DOX dose.

The increased LDH activity suggest increased capacity of the cardiomyocytes to produce lactate, whereas decreased LDH capacity suggests the opposite. Although the measured LDH activity was based on NADH consumption, and thus pyruvate utilization, both LDH A and LDH B can drive the pyruvate conversion to either direction despite their differential substrate preference (Mack et al., 2017). Therefore, increased LDH activity could also indicate increased capacity of the myocardium to utilize lactate as energy source, and a decrease in LDH could signify the opposite. The simultaneous increase in maximal CS activity with decreased cardiac LDH activity in Study II may suggest increased pyruvate usage in the TCA cycle as opposed to potential lactate production or utilization. Increased maximal CS activity could also indicate increased mitochondrial number, as opposed to just increased overall maximal CS activity, however this was not supported by our findings regarding unchanged mitochondrial DNA level (Table 1 in Study II).

Our findings in Study II, but not in Study I, contrast previous findings of a study that found that ET preconditioning does not alter cardiac CS activity in tumor-free DOX treated male mice (Ascensão et al., 2005a). A study on healthy male rats showed that, although ET increases cardiac CS mRNA expression, the activity of CS is not upregulated (Siu et al., 2003). However, studies on heart failure models (induced by myocardial infarction) have shown that ET can increase cardiac CS activity during heart failure, unlike in healthy controls (Jiang et al., 2020; Kemi et al., 2007). In these heart failure studies, the increased CS activity was also accompanied by an increase in LDH A activity. In agreement with this, a study by Todorovic et al. (2021) showed that treadmill ET can increase cardiac LDH2 and LDH4 (but not total LDH) in healthy male rats, whereas the total LDH was also increased in male rats modelling cardiovascular disease through homocysteine administration (Todorovic et al., 2021). The discrepancies between Study II findings and previous studies could be due to different stage of DCT, as well as changes in different isoforms of LDH.

The increase in cardiac CS and simultaneous decrease in LDH in Study II could suggest increased glucose flux through TCA and potentially improved OXPHOS. This is somewhat supported by the fact that in Study II DOX treated animals had significantly reduced mitochondrial RCR (referred to as coupling efficiency in Study 1), and there was a slight trend towards ET improving it (Table I in Study II). There was also a positive correlation between the RCR and EF, suggesting that ET might have somewhat improved mitochondrial function, contributing to the improved cardiac function. Previous studies support the notion that DOX decreases RCR, and that ET can help maintain RCR (Ascensão et al., 2005b; Kavazis et al., 2010;

Marques-Aleixo et al., 2015; Montalvo et al., 2023; Morton et al., 2019). Likely the Study II was unable to detect significant increase in RCR due to the somewhat low n-number.

Furthermore, it seems tempting to conclude that the DOX mediated increase in cardiac glucose uptake could be a result of impaired mitochondrial function, and a compensatory response aiming to maintain cardiac function. Indeed, many previous clinical and preclinical studies support that DOX induces increase in cardiac glucose uptake (Baucknehtet al., 2017, 2020; Bulten et al., 2019; Guerra et al., 2024; Hrelia et al., 2002; Sarocchi et al., 2018). However, Study II found that the cardiac glucose uptake had inverse correlation with the LVEF, and LV mass (Fig 4 in Study II). This suggests that the increased glucose uptake was not a compensatory mechanism able to maintain cardiac function and weight, but instead a measure of cardiac damage. Many studies support this notion, as they have shown that DOX mediated increase in cardiac glucose uptake can be a measure of DOX induced oxidative stress and impaired cardiac function (Baucknehtet al., 2019, 2017, 2020; Sarocchi et al., 2018). The finding that ET can reduce LV glucose uptake which DOX increases, thus blunting DOX effects on glucose uptake is novel and should be investigated further in the clinical setting. More investigation is needed on what precisely decreases LV glucose uptake, particularly in the DOX treated individuals, and what is the role of different LDH isoforms in this, as well as the role of other metabolic mechanisms which Study II did not measure. Although HOAD activity was not altered by DOX nor ET in Study II (Table 2 in Study II), it is possible that fatty acid metabolism could have had changes in its other metabolic enzymes.

4.2.3 Cardiac oxidative stress

To see whether ET modified LV oxidative stress, the lipid damage and protein damage were investigated via measuring the lipid peroxidation and protein carbonylation from LV homogenates. Furthermore, the cardiac antioxidative enzyme activities SOD and CAT were measured. In Study I and Study II ET did not affect LV oxidative damage to lipids or proteins nor the activities of antioxidative enzymes (Fig 2 in Study I, Table 2 in Study II). This suggests that ET did not ameliorate cardiac damage in Study II via increasing antioxidative capacity through changes in overall SOD or CAT activity level. Some previous studies support the notion that exercise can be cardioprotective without affecting cardiac oxidative stress or antioxidative enzymes (Chicco et al., 2006, 2005).

However, findings of Study II contrast with many previous studies as particularly cardiac SODs have been shown to be increased by ET (Ascensão et al., 2005b; Dolinsky et al., 2013; Kavazis et al., 2010; Marques-Aleixo et al., 2015; Sequeira et al., 2021). Nonetheless, some studies have also shown unaltered SOD expression

while GPx or heat shock proteins or CAT were upregulated (Ascensão et al., 2005a; Chicco et al., 2006, 2005). In these studies, ET was done at lower intensity using DOX concomitant ET, voluntary wheel running ET preconditioning, or swimming exercise. Since ET can specifically increase SOD in subsarcolemmal mitochondria and not in the intermyofibrillar mitochondria (Montalvo et al., 2023), it might be that changes in specific antioxidative mechanisms or antioxidative damage markers may be more evident at the mitochondrial level but not at tissue level. Considering that previous studies have had a lot of variability regarding which antioxidative mechanisms are altered by exercise, it may stand to reason that some other antioxidative mechanisms not measured in the current study could be altered instead. The somewhat low n-number may also make it difficult to detect smaller ET induced changes.

Interestingly, besides ET not having effect on LV antioxidative enzymes in Study II, there was also no increase in lipid peroxidation, protein carbonylation nor down regulation of any of the antioxidative enzymes following DOX treatment (Table 2 in Study II). This contrasts with previous studies that have found DOX to increase oxidative damage to lipids and proteins in particular (Ascensão et al., 2005a, 2005b; Kavazis et al., 2010; Marques-Aleixo et al., 2015). Despite the strong evidence that DOX increases ROS formation and oxidative stress, and even inhibits anti-oxidative enzymes, not all studies have been able to detect DOX induced increase in oxidative damage or markers of oxidative stress (Cheong et al., 2021; Chicco et al., 2006). This might be because of varying DOX dosing regimens, timing of the measurement from the dosing as well the varying n-numbers in these studies. Some studies have showed that at least acutely the DCT is not mediated by general oxidative stress, but rather through the specific damage and inhibition of the ETS in mitochondria (Pointon et al., 2010).

Although in Study II no DOX mediated increase in oxidative damage to lipids and proteins was found, it cannot be ruled out that DOX could have anyway affected ROS generation and that other than the measured antioxidative mechanisms could have limited the oxidative damage. Furthermore, it is possible that the oxidative damage generated was not visible at the time point investigated, considering that oxidative stress can occur rapidly, and the damaged cells and organelles can be cleared or removed via apoptosis. The fact that in Study II DOX induced increase in cardiac [^{18}F]FDG uptake could be indicative of increased oxidative stress as has been shown by previous study by Bauckneht et al. (2020).

4.3 Future perspectives and limitations

Since the findings from Study I–III come from a mouse model of breast cancer, and therefore cannot be directly translated to humans, further studies are needed using

different preclinical mammary tumor models and clinical studies. Future preclinical studies should employ more intense ET in order to see whether that could enhance the DOX efficacy or offer additional cardioprotection. Particularly the changes in cardiac LDH activity in DCT and in response to ET need further investigation as the subtypes of this enzyme were not studied in Studies I–II but the overall activity of cardiac LDH was changed. Moreover, since there was evidence that at least initially the tumor vasculature and blood flow may have been altered, there is a need for further investigations using more intense ET and orthotopic tumor models. Orthotopic tumors could potentially respond differentially due to the different tumor microenvironment (Fung et al., 2015).

Clinically, the ET induced changes in cardiac and tumor glucose uptake could be easiest to investigate as many cancer patients go through [^{18}F]FDG-PET imaging. The Study II and III have the limitation of some animals having to be euthanized due to tumor growth prior T3 PET imaging which could cause survival bias in T3 PET measurements. Particularly the tumor data might represent slowest growing tumors in each group at T3. The findings of Study II and III on cardiac and tumor glucose uptake need to be verified in breast cancer patients treated with DOX.

Moreover, it has been suggested that the beneficial effects of ET may not last long term with one study finding only a few beneficial effects on life quality parameters remaining in breast cancer patients at 5 year follow up (Anandavadivelan et al., 2024). Despite this, physical activity has been shown to be associated with reduced cancer recurrence and overall mortality in breast cancer patients at two year follow up (Cannioto et al., 2021). Therefore, longer term outcomes need further investigation, both in clinical and preclinical setting. Particularly interesting would be to investigate whether the short-term alterations induced by ET, such as those in the cardiac glucose uptake and tumor glucose uptake, could predict longer term patient outcomes.

5 Conclusions

Based on the studies included in this thesis it can be concluded that voluntary wheel running ET can in some instances inhibit tumor growth in DOX treated animals, but this might be highly ET intensity and DOX efficacy dependent. However, the novel important finding of this thesis was that even low intensity ET, which voluntary running wheel ET likely is, can mitigate the cardiotoxic effects of DOX reducing cardiac glucose uptake which DOX increases while also reducing MTV and maximal glucose uptake suggesting lower tumoral glucose utilization. However, this effect on tumoral glucose uptake was only apparent in the groups without DOX treatment with higher physical activity, and ET did not sensitize subcutaneous 13TC-mammary tumors to DOX treatment or alter total lesion glycolysis at the intensity used. The ET mediated changes in tumor glucose uptake require further investigation as this study found no explanation from the measured metabolic enzyme activities nor mitochondrial function.

Furthermore, it was shown that initially after cumulative DOX dose of just 10 mg/kg ET had some beneficial effects on tumor vascularity, as it decreased number of larger blood vessels and the blood vessel α-SMA coverage, which was correlated with more aggressive tumor growth. Furthermore, at the same time ET seemed to induce a trend of towards improved tumor blood flow with significantly decreased tumor blood MTT, without decrease in tumoral hypoxia. However, the vascular changes did not persist until fourth dose of DOX, even in the groups without DOX treatment and with steady physical activity. It is, however, possible that tumor bearing animals have decreased exercise intensity as indicated the fact that tumor burden can decrease exercise tolerance (Weber et al., 2025). Further studies are warranted on the effects of ET on tumoral blood flow and glucose uptake during more intense exercise and utilizing more effective DOX treatment regimen. However, the results of this thesis also show that it is possible that greater physical activity level can reduce tumor growth during DOX treatment via increasing tumor apoptosis even when there are no changes in tumor blood vessel α-SMA coverage and blood vessel density.

Lastly and importantly, this thesis showed that low intensity ET which ameliorates DOX induced cardiotoxicity not only maintains LV weight and is able

to reduce cardiac glucose uptake which DOX increases, it also modifies the cardiac metabolic enzymes' maximal activities. ET lowers the maximal LV LDH activity and increases the maximal CS activity in DOX treated animals week after receiving about 15-20 mg/kg cumulative DOX dose. However, acutely 24 hours after cumulative DOX dose of 15 mg/kg and in response to greater physical activity, the opposite seems to be true, with ET increasing maximal LV LDH activity. Interestingly the cardiac glucose uptake negatively correlated with the cardiac mass and EF suggesting that increased cardiac glucose uptake is a measure of cardiac damage. Therefore, the alterations in glucose uptake of heart and tumor in response to ET during DOX treatment warrant further investigation in the clinical setting particularly in regard to whether they could predict patient outcomes. Moreover, the changes in LV LDH and CS maximal activities need to be studied more in detail regards to the different subtypes of LDH which could contribute to the changes in cardiac glucose uptake via altered glucose utilization.

In summary, overall voluntary wheel running ET seems to have beneficial effects for mice with breast cancer with or without DOX treatment. The results of this thesis support the notion that ET could be recommended for individuals with breast cancer and DOX treatment. However, these results need to be confirmed in the clinical setting before they can be applied to humans.

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List of References

AbdElaal, A. A., Zaher, A. M., Abdelgawad, M. I., Mekkawy, M. A., Eloteify, L. M., 2021. Correlation of primary tumor metabolic parameters with clinical, histopathological and molecular characteristics in breast cancer patients at pre-operative staging FDG-PET/CT study. *Egyptian Journal of Radiology and Nuclear Medicine*, *52*, 171.

Aggarwal, V., Tuli, H., Varol, A., Thakral, F., Yerer, M., Sak, K., Varol, M., Jain, A., Khan, M., Sethi, G., 2019. Role of Reactive Oxygen Species in Cancer Progression: Molecular Mechanisms and Recent Advancements. *Biomolecules*, *9*, 735.

Alarcon-Martinez, L., Yilmaz-Ozcan, S., Yemisci, M., Schallek, J., Kılıç, K., Can, A., Di Polo, A., Dalkara, T., 2018. Capillary pericytes express α-smooth muscle actin, which requires prevention of filamentous-actin depolymerization for detection. *ELife*, *7*. https://doi.org/10.7554/eLife.34861

Alvarez, J. V, Belka, G. K., Pan, T.-C., Chen, C.-C., Blankemeyer, E., Alavi, A., Karp, J. S., Chodosh, L. A., 2014. Oncogene pathway activation in mammary tumors dictates FDG-PET uptake. *Cancer Research*, *74*, 7583–7598.

Anandavadivelan, P., Mijwel, S., Wiklander, M., Kjoe, P. L. M., Luijendijk, M., Bergh, J., Rundqvist, H., Wengstrom, Y., 2024. Five-year follow-up of the OptiTrain trial on concurrent resistance and high-intensity interval training during chemotherapy for patients with breast cancer. *Scientific Reports*, *14*, 15333.

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R., Schulte, P. M., 2013. Variation in temperature tolerance among families of Atlantic salmon (Salmo salar) is associated with hypoxia tolerance, ventricle size and myoglobin level. *The Journal of Experimental Biology*, *216*, 1183–1190.

Arcamone, F., Franceschi, G., Penco, S., Selva, A., 1969. Adriamycin (14-hydroxydaunomycin), a novel antitumor antibiotic. *Tetrahedron Letters*, *10*, 1007–1010.

Ascensão, A., Magalhães, J., Soares, J., Ferreira, R., Neuparth, M., Marques, F., Oliveira, J., Duarte, J., 2005a. Endurance training attenuates doxorubicin-induced cardiac oxidative damage in mice. *International Journal of Cardiology*, *100*, 451–460.

Ascensão, A., Magalhães, J., Soares, J. M. C., Ferreira, R., Neuparth, M. J., Marques, F., Oliveira, P. J., Duarte, J. A., 2005b. Moderate endurance training prevents doxorubicin-induced in vivo mitochondriopathy and reduces the development of cardiac apoptosis. *American Journal of Physiology-Heart and Circulatory Physiology*, *289*, H722–H731.

Ashcraft, K. A., Warner, A. B., Jones, L. W., Dewhirst, M. W., 2019. Exercise as Adjunct Therapy in Cancer. *Seminars in Radiation Oncology*, *29*, 16–24.

Attalla, S., Taifour, T., Bui, T., Muller, W., 2021. Insights from transgenic mouse models of PyMT-induced breast cancer: recapitulating human breast cancer progression in vivo. *Oncogene*, *40*, 475–491.

Aveseh, M., Nikooie, R., Aminaie, M., 2015. Exercise-induced changes in tumour LDH-B and MCT1 expression are modulated by oestrogen-related receptor alpha in breast cancer-bearing BALB/c mice. *The Journal of Physiology*, *593*, 2635–2648.

Babaei, H., Razmaraii, N., Assadnassab, G., Mohajjel Nayebi, A., Azarmi, Y., Mohammadnejad, D., Azami, A., 2020. Ultrastructural and Echocardiographic Assessment of Chronic Doxorubicin-Induced Cardiotoxicity in Rats. *Archives of Razi Institute*, *75*, 55–62.

Bachur, N. R., Gordon, S. L., Gee, M. V, Kon, H., 1979. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proceedings of the National Academy of Sciences*, *76*, 954–957.

Bauckneht, M., Cossu, V., Miceli, A., Donegani, M. I., Capitanio, S., Morbelli, S., Marini, C., Sambuceti, G., 2019. FDG-PET Imaging of Doxorubicin-Induced Cardiotoxicity: a New Window on an Old Problem. *Current Cardiovascular Imaging Reports*, *12*, 41.

Bauckneht, M., Ferrarazzo, G., Fiz, F., Morbelli, S., Sarocchi, M., Pastorino, F., Ghidella, A., Pomposelli, E., Miglino, M., Ameri, P., Emionite, L., Ticconi, F., Arboscello, E., Buschiazzo, A., Massimelli, E., Fiordoro, S., Borra, A., Cossu, V., Bozzano, A., Ibatici, A., Ponzoni, M., Spallarossa, P. Gallamini, A., Bruzzi, P., Sambuceti, G., Marini, C., 2017. Doxorubicin Effect on Myocardial Metabolism as a Prerequisite for Subsequent Development of Cardiac Toxicity: A Translational ^{18} F-FDG PET/CT Observation. *Journal of Nuclear Medicine*, *58*, 1638–1645.

Bauckneht, M., Pastorino, F., Castellani, P., Cossu, V., Orengo, A. M., Piccioli, P., Emionite, L., Capitanio, S., Yosifov, N., Bruno, S., Lazzarini, E., Ponzoni, M., Ameri, P., Rubartelli, A., Ravera, S., Morbelli, S., Sambuceti, G., Marini, C., 2020. Increased myocardial 18F-FDG uptake as a marker of Doxorubicin-induced oxidative stress. *Journal of Nuclear Cardiology*, *27*, 2183–2194.

Belger, C., Abrahams, C., Imamdin, A., Lecour, S., 2024. Doxorubicin-induced cardiotoxicity and risk factors. *IJC Heart & Vasculature*, *50*, 101332.

Bergers, G., & Song, S., 2005. The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology*, *7*, 452–464.

Betof, A. S., Lascola, C. D., Weitzel, D., Landon, C., Scarbrough, P. M., Devi, G. R., Palmer, G., Jones, L. W., Dewhirst, M. W., 2015. Modulation of Murine Breast Tumor Vascularity, Hypoxia, and Chemotherapeutic Response by Exercise. *JNCI: Journal of the National Cancer Institute*, *107*. https://doi.org/10.1093/jnci/djv040

Boen, H. M., Cherubin, M., Franssen, C., Gevaert, A. B., Witvrouwen, I., Bosman, M., Guns, P.-J., Heidbuchel, H., Loeys, B., Alaerts, M., Van Craenenbroeck, E. M., 2024. Circulating MicroRNA as Biomarkers of Anthracycline-Induced Cardiotoxicity. *JACC: CardioOncology*, *6*, 183–199.

Bordoni, A., Biagi, P., Hrelia, S., 1999. The impairment of essential fatty acid metabolism as a key factor in doxorubicin-induced damage in cultured rat cardiomyocytes. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1440*, 100–106.

Bosman, M., Krüger, D., Van Assche, C., Boen, H., Neutel, C., Favere, K., Franssen, C., Martinet, W., Roth, L., De Meyer, G. R. Y., Cillero-Pastor, B., Delrue, L., Heggermont, W., Van Craenenbroeck, E. M., Guns, P.-J., 2023. Doxorubicin-induced cardiovascular toxicity: a longitudinal evaluation of functional and molecular markers. *Cardiovascular Research*, *119*, 2579–2590.

Bostancı, M. E., & Hasbek, Z., 2020. Relationship Between SUV_{max} and Ki-67 Expression in Breast Cancer. *Medical Bulletin of Haseki*, *58*, 359–363.

Brandão, S., Reis-Mendes, A., Duarte-Araújo, M., Neuparth, M., Rocha, H., Carvalho, F., Ferreira, R., Costa, V., 2023. Cardiac Molecular Remodeling by Anticancer Drugs: Doxorubicin Affects More Metabolism While Mitoxantrone Impacts More Autophagy in Adult CD-1 Male Mice. *Biomolecules*, *13*, 921.

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., Jemal, A., 2024. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *74*, 229–263.

Bulten, B. F., Sollini, M., Boni, R., Massri, K., de Geus-Oei, L. F., Laarhoven, H. W. M. van, Slart, R. H. J. A., Erba, P. A., 2019. Cardiac molecular pathways influenced by doxorubicin treatment in mice. *Scientific Reports*, *9*. https://doi.org/10.1038/s41598-019-38986-w

Burridge, P. W., Li, Y. F., Matsa, E., Wu, H., Ong, S.-G., Sharma, A., Holmström, A., Chang, A. C., Coronado, M. J., Ebert, A. D., Knowles, J. W., Telli, M. L., Witteles, R. M., Blau, H. M., Bernstein,

D., Altman, R. B., Wu, J. C., 2016. Human induced pluripotent stem cell–derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nature Medicine*, *22*, 547–556.

Buss, L. A., Ang, A. D., Hock, B., Robinson, B. A., Currie, M. J., Dachs, G. U., 2020. Effect of post-implant exercise on tumour growth rate, perfusion and hypoxia in mice. *PLOS ONE*, *15*. https://doi.org/10.1371/journal.pone.0229290

Buss, L. A., & Dachs, G. U., 2018. Voluntary exercise slows breast tumor establishment and reduces tumor hypoxia in *ApoE* $^{-/-}$ mice. *Journal of Applied Physiology*, *124*, 938–949.

Cannioto, R. A., Hutson, A., Dighe, S., McCann, W., McCann, S. E., Zirpoli, G. R., Barlow, W., Kelly, K. M., DeNysschen, C. A., Hershman, D. L., Unger, J. M., Moore, H. C. F., Stewart, J. A., Isaacs, C., Hobday, T. J., Salim, M., Hortobagyi, G. N., Gralow, J. R., Albain, K. S., Budd, G. T., Ambrosone, C. B., 2021. Physical Activity Before, During, and After Chemotherapy for High-Risk Breast Cancer: Relationships With Survival. *JNCI: Journal of the National Cancer Institute*, *113*, 54–63.

Capranico, G., Tinelli, S., Austin, C. A., Fisher, M. L., Zunino, F., 1992. Different patterns of gene expression of topoisomerase II isoforms in differentiated tissues during murine development. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*, *1132*, 43–48.

Cardinale, D., Colombo, A., Bacchiani, G., Tedeschi, I., Meroni, C. A., Veglia, F., Civelli, M., Lamantia, G., Colombo, N., Curigliano, G., Fiorentini, C., Cipolla, C. M., 2015. Early Detection of Anthracycline Cardiotoxicity and Improvement With Heart Failure Therapy. *Circulation*, *131*, 1981–1988.

Carvalho, R. A., Sousa, R. P. B., Cadete, V. J. J., Lopaschuk, G. D., Palmeira, C. M. M., Bjork, J. A., Wallace, K. B., 2010. Metabolic remodeling associated with subchronic doxorubicin cardiomyopathy. *Toxicology*, *270*, 92–98.

Chan, B. Y. H., Roczkowsky, A., Cho, W. J., Poirier, M., Sergi, C., Keschrumrus, V., Churko, J. M., Granzier, H., Schulz, R., 2021. MMP inhibitors attenuate doxorubicin cardiotoxicity by preventing intracellular and extracellular matrix remodelling. *Cardiovascular Research*, *117*, 188–200.

Cheong, A., McGrath, S., Robinson, T., Maliki, R., Spurling, A., Lock, P., Rephaeli, A., Nudelman, A., Parker, B. S., Pepe, S., Cutts, S. M., 2021. A switch in mechanism of action prevents doxorubicin-mediated cardiac damage. *Biochemical Pharmacology*, *185*, 114410.

Chia, S. K., Wykoff, C. C., Watson, P. H., Han, C., Leek, R. D., Pastorek, J., Gatter, K. C., Ratcliffe, P., Harris, A. L., 2001. Prognostic Significance of a Novel Hypoxia-Regulated Marker, Carbonic Anhydrase IX, in Invasive Breast Carcinoma. *Journal of Clinical Oncology*, *19*, 3660–3668.

Chicco, A. J., Hydock, D. S., Schneider, C. M., Hayward, R., 2006. Low-intensity exercise training during doxorubicin treatment protects against cardiotoxicity. *Journal of Applied Physiology*, *100*, 519–527.

Chicco, A. J., Schneider, C. M., Hayward, R., 2005. Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *289*, R424–R431.

Childs, A. C., Phaneuf, S. L., Dirks, A. J., Phillips, T., Leeuwenburgh, C., 2002. Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity, and Bcl-2:Bax ratio. *Cancer Research*, *62*, 4592–4598.

Courneya, K. S., Segal, R. J., Mckenzie, D. C., Dong, H., Gelmon, K., Friedenreich, C. M., Yasui, Y., Reid, R. D., Crawford, J. J., Mackey, J. R., 2014. Effects of Exercise during Adjuvant Chemotherapy on Breast Cancer Outcomes. *Medicine & Science in Sports & Exercise*, *46*, 1744–1751.

de la Cruz-López, K. G., Castro-Muñoz, L. J., Reyes-Hernández, D. O., García-Carrancá, A., Manzo-Merino, J., 2019. Lactate in the Regulation of Tumor Microenvironment and Therapeutic Approaches. *Frontiers in Oncology*, *9*, 1143.

Demark-Wahnefried, W., Hars, V., Conaway, M., Havlin, K., Rimer, B., McElveen, G., Winer, E., 1997. Reduced rates of metabolism and decreased physical activity in breast cancer patients receiving adjuvant chemotherapy. *The American Journal of Clinical Nutrition*, *65*, 1495–1501.

Díaz-Guerra, A., Villena-Gutiérrez, R., Clemente-Moragón, A., Gómez, M., Oliver, E., Fernández-Tocino, M., Galán-Arriola, C., Cádiz, L., Ibáñez, B., 2024. Anthracycline Cardiotoxicity Induces Progressive Changes in Myocardial Metabolism and Mitochondrial Quality Control. *JACC: CardioOncology*, *6*, 217–232.

Dolinsky, V. W., Rogan, K. J., Sung, M. M., Zordoky, B. N., Haykowsky, M. J., Young, M. E., Jones, L. W., Dyck, J. R. B., 2013. Both aerobic exercise and resveratrol supplementation attenuate doxorubicin-induced cardiac injury in mice. *American Journal of Physiology-Endocrinology and Metabolism*, *305*, E243–E253.

Doroshow, J. H., Locker, G. Y., Myers, C. E., 1980. Enzymatic Defenses of the Mouse Heart Against Reactive Oxygen Metabolites. *Journal of Clinical Investigation*, *65*, 128–135.

Dose Schwarz, J., Bader, M., Jenicke, L., Hemminger, G., Jänicke, F., Avril, N., 2005. Early prediction of response to chemotherapy in metastatic breast cancer using sequential 18F-FDG PET. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *46*, 1144–1150.

Dozic, S., Howden, E. J., Bell, J. R., Mellor, K. M., Delbridge, L. M. D., Weeks, K. L., 2023. Cellular Mechanisms Mediating Exercise-Induced Protection against Cardiotoxic Anthracycline Cancer Therapy. *Cells*, *12*, 1312.

Driedzic, W. R., & Fonseca de Almeida-Val, V. M., 1996. Enzymes of cardiac energy metabolism in Amazonian teleosts and the fresh-water stingray (Potamotrygon hystrix). *The Journal of Experimental Zoology*, *274*, 327–333.

Faustino-Rocha, A. I., Silva, A., Gabriel, J., Gil da Costa, R. M., Moutinho, M., Oliveira, P. A., Gama, A., Ferreira, R., Ginja, M., 2016. Long-term exercise training as a modulator of mammary cancer vascularization. *Biomedicine & Pharmacotherapy*, *81*, 273–280.

Fung, A. S., Lee, C., Yu, M., Tannock, I. F., 2015. The effect of chemotherapeutic agents on tumor vasculature in subcutaneous and orthotopic human tumor xenografts. *BMC Cancer*, *15*, 112.

Generali, D., Berruti, A., Brizzi, M. P., Campo, L., Bonardi, S., Wigfield, S., Bersiga, A., Allevi, G., Milani, M., Aguggini, S., Gandolfi, V., Dogliotti, L., Bottini, A., Harris, A. L., Fox, S. B., 2006. Hypoxia-Inducible Factor-1α Expression Predicts a Poor Response to Primary Chemoendocrine Therapy and Disease-Free Survival in Primary Human Breast Cancer. *Clinical Cancer Research*, *12*, 4562–4568.

Gewirtz, D., 1999. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochemical Pharmacology*, *57*, 727–741.

Ghignatti, P. V. da C., Nogueira, L. J., Lehnen, A. M., Leguisamo, N. M., 2021. Cardioprotective effects of exercise training on doxorubicin-induced cardiomyopathy: a systematic review with meta-analysis of preclinical studies. *Scientific Reports*, *11*. https://doi.org/10.1038/s41598-021-83877-8

Gibson, N. M., Greufe, S. E., Hydock, D. S., Hayward, R., 2013. Doxorubicin-Induced Vascular Dysfunction and Its Attenuation by Exercise Preconditioning. *Journal of Cardiovascular Pharmacology*, *62*, 355–360.

Glass, O. K., Bowie, M., Fuller, J., Darr, D., Usary, J., Boss, K., Choudhury, K. R., Liu, X., Zhang, Z., Locasale, J. W., Williams, Cdewhirst, M. W., Jones, L. W., Seewaldt, V., 2017. Differential response to exercise in claudin-low breast cancer. *Oncotarget*, *8*, 100989–101004.

Gomes-Santos, I. L., Amoozgar, Z., Kumar, A. S., Ho, W. W., Roh, K., Talele, N. P., Curtis, H., Kawaguchi, K., Jain, R. K., Fukumura, D., 2021a. Exercise Training Improves Tumor Control by Increasing CD8+ T-cell Infiltration via CXCR3 Signaling and Sensitizes Breast Cancer to Immune Checkpoint Blockade. *Cancer Immunology Research*, *9*, 765–778.

Gomes-Santos, I. L., Jordão, C. P., Passos, C. S., Brum, P. C., Oliveira, E. M., Chammas, R., Camargo, A. A., Negrão, C. E., 2021b. Exercise Training Preserves Myocardial Strain and Improves Exercise

Tolerance in Doxorubicin-Induced Cardiotoxicity. *Frontiers in Cardiovascular Medicine*, *8*. https://doi.org/10.3389/fcvm.2021.605993

Gomes-Santos, I. L., Kumar, A. S., Hausmann, F., Meyer, M. N., Shiferaw, S. Z., Amoozgar, Z., Jain, R. K., Fukumura, D., 2024. Exercise intensity governs tumor control in mice with breast cancer. *Frontiers in Immunology*, *15*. https://doi.org/10.3389/fimmu.2024.1339232

Goormaghtigh, E., Chatelain, P., Caspers, J., Ruysschaert, J. M., 1980. Evidence of a specific complex between adriamycin and negatively-charged phospholipids. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *597*, 1–14.

Goormaghtigh, E, Brasseur, R., Ruysschaert, J.-M., 1982. Adriamycin inactivates cytochrome c oxidase by exclusion of the enzyme from its cardiolipin essential environment. *Biochemical and Biophysical Research Communications*, *104*, 314–320.

Gratia, S., Kay, L., Michelland, S., Sève, M., Schlattner, U., Tokarska-Schlattner, M., 2012. Cardiac phosphoproteome reveals cell signaling events involved in doxorubicin cardiotoxicity. *Journal of Proteomics*, *75*, 4705–4716.

Greenlee, H., Iribarren, C., Rana, J. S., Cheng, R., Nguyen-Huynh, M., Rillamas-Sun, E., Shi, Z., Laurent, C. A., Lee, V. S., Roh, J. M., Santiago-Torres, M., Shen, H., Hershman, D. L., Kushi, L. H., Neugebauer, R., Kwan, M. L., 2022. Risk of Cardiovascular Disease in Women With and Without Breast Cancer: The Pathways Heart Study. *Journal of Clinical Oncology*, *40*, 1647–1658.

Guerra, G., Russo, M., Priolo, R., Riganti, C., Reano, S., Filigheddu, N., Hirsch, E., Ghigo, A., 2024. Exploring the mechanisms of metabolic adaptations to cardiotoxic doxorubicin. *Cardiovascular Research*, *120*. https://doi.org/10.1093/cvr/cvae088.044

Gutiérrez, P. L., Gee, M. V., Bachur, N. R., 1983. Kinetics of anthracycline antibiotic free radical formation and reductive glycosidase activity. *Archives of Biochemistry and Biophysics*, *223*, 68–75.

Han, G.-S., & Kim, S.-R., 2013. Effects of Endurance Exercise on Mitochondrial Function in Mice. *Journal of Physical Therapy Science*, *25*, 1317–1319.

Hanna, A. D., Lam, A., Tham, S., Dulhunty, A. F., Beard, N. A., 2014. Adverse Effects of Doxorubicin and Its Metabolic Product on Cardiac RyR2 and SERCA2A. *Molecular Pharmacology*, *86*, 438–449.

Harahap, Y., Ardiningsih, P., Corintias Winarti, A., Purwanto, D. J., 2020. Analysis of the Doxorubicin and Doxorubicinol in the Plasma of Breast Cancer Patients for Monitoring the Toxicity of Doxorubicin. *Drug Design, Development and Therapy*, *14*, 3469–3475.

Harbeck, N., Penault-Llorca, F., Cortes, J., Gnant, M., Houssami, N., Poortmans, P., Ruddy, K., Tsang, J., Cardoso, F., 2019. Breast cancer. *Nature Reviews Disease Primers*, *5*, 66.

Herron, T. J., & McDonald, K. S., 2002. Small Amounts of α-Myosin Heavy Chain Isoform Expression Significantly Increase Power Output of Rat Cardiac Myocyte Fragments. *Circulation Research*, *90*, 1150–1152.

Hoffman, R. K., Kim, B.-J., Shah, P. D., Carver, J., Ky, B., Ryeom, S., 2021. Damage to cardiac vasculature may be associated with breast cancer treatment-induced cardiotoxicity. *Cardio-Oncology*, *7*, 15.

Hrelia, S., Fiorentini, D., Maraldi, T., Angeloni, C., Bordoni, A., Biagi, P. L., Hakim, G., 2002. Doxorubicin induces early lipid peroxidation associated with changes in glucose transport in cultured cardiomyocytes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1567*. https://doi.org/10.1016/S0005-2736(02)00612-0

Huang, L., Zhang, K., Guo, Y., Huang, F., Yang, K., Chen, L., Huang, K., Zhang, F., Long, Q., Yang, Q., 2017. Honokiol protects against doxorubicin cardiotoxicity via improving mitochondrial function in mouse hearts. *Scientific Reports*, *7*, 11989.

Hydock, D. S., Lien, C.-Y., Jensen, B. T., Parry, T. L., Schneider, C. M., Hayward, R., 2012. Rehabilitative exercise in a rat model of doxorubicin cardiotoxicity. *Experimental Biology and Medicine*, *237*, 1483–1492.

Hydock, D. S., Wonders, K. Y., Schneider, C. M., Hayward, R., 2009. Voluntary wheel running in rats receiving doxorubicin: effects on running activity and cardiac myosin heavy chain. *Anticancer Research*, *29*, 4401–4407.

Ichikawa, Y., Ghanefar, M., Bayeva, M., Wu, R., Khechaduri, A., Prasad, S. V. N., Mutharasan, R. K., Naik, T. J., Ardehali, H., 2014. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *Journal of Clinical Investigation*, *124*, 617–630.

Iemitsu, M., Maeda, S., Jesmin, S., Otsuki, T., Miyauchi, T., 2006. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *American Journal of Physiology-Heart and Circulatory Physiology*, *291*, H1290–H1298.

Iida, H., Rhodes, C. G., de Silva, R., Araujo, L. I., Bloomfield, P. M., Lammertsma, A. A., Jones, T., 1992. Use of the left ventricular time-activity curve as a noninvasive input function in dynamic oxygen-15-water positron emission tomography. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *33*, 1669–1677.

Jafari, A., Sheikholeslami-Vatani, D., Khosrobakhsh, F., Khaledi, N., 2021. Synergistic Effects of Exercise Training and Vitamin D Supplementation on Mitochondrial Function of Cardiac Tissue, Antioxidant Capacity, and Tumor Growth in Breast Cancer in Bearing-4T1 Mice. *Frontiers in Physiology*, *12*. https://doi.org/10.3389/fphys.2021.640237

Jensen, B. T., Lien, C.-Y., Hydock, D. S., Schneider, C. M., Hayward, R., 2013. Exercise mitigates cardiac doxorubicin accumulation and preserves function in the rat. *Journal of Cardiovascular Pharmacology*, *62*, 263–269.

Jiang, C., Deng, L., Karr, M. A., Wen, Y., Wang, Q., Perimbeti, S., Shapiro, C. L., Han, X., 2022. Chronic comorbid conditions among adult cancer survivors in the United States: Results from the National Health Interview Survey, 2002-2018. *Cancer*, *128*, 828–838.

Jiang, H., Jia, D., Zhang, B., Yang, W., Dong, Z., Sun, X., Cui, X., Ma, L., Wu, J., Hu, K., Sun, A., Ge, J., 2020. Exercise improves cardiac function and glucose metabolism in mice with experimental myocardial infarction through inhibiting HDAC4 and upregulating GLUT1 expression. *Basic Research in Cardiology*, *115*, 28.

Jones, L. W., Eves, N. D., Courneya, K. S., Chiu, B. K., Baracos, V. E., Hanson, J., Johnson, L., Mackey, J. R., 2005. Effects of Exercise Training on Antitumor Efficacy of Doxorubicin in MDA-MB-231 Breast Cancer Xenografts. *Clinical Cancer Research*, *11*, 6695–6698.

Jones, L. W., Viglianti, B. L., Tashjian, J. A., Kothadia, S. M., Keir, S. T., Freedland, S. J., Potter, M. Q., Jung Moon, E., Schroeder, T., Herndon, J. E., Dewhirst, M. W., 2010. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. *Journal of Applied Physiology*, *108*, 343–348.

Kalyanaraman, B., Sealy, R., Sinha, B., 1984. An electron spin resonance study of the reduction of peroxides by anthracycline semiquinones. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *799*, 270–275.

Kavazis, A. N., Morton, A. B., Hall, S. E., Smuder, A. J., 2017. Effects of doxorubicin on cardiac muscle subsarcolemmal and intermyofibrillar mitochondria. *Mitochondrion*, *34*, 9–19.

Kavazis, A. N., Smuder, A. J., Min, K., Tümer, N., Powers, S. K., 2010. Short-term exercise training protects against doxorubicin-induced cardiac mitochondrial damage independent of HSP72. *American Journal of Physiology. Heart and Circulatory Physiology*, *299*, H1515-24.

Kemi, O., Hoydal, M., Haram, P., Garnier, A., Fortin, D., Venturaclapier, R., Ellingsen, O., 2007. Exercise training restores aerobic capacity and energy transfer systems in heart failure treated with losartan. *Cardiovascular Research*, *76*, 91–99.

Kim, J., Cho, S.-G., Kang, S.-R., Yoo, S. W., Kwon, S. Y., Min, J.-J., Bom, H.-S., Song, H.-C., 2020. Association between FDG uptake in the right ventricular myocardium and cancer therapy-induced cardiotoxicity. *Journal of Nuclear Cardiology*, *27*, 2154–2163.

Kirkham, A. A., Shave, R. E., Bland, K. A., Bovard, J. M., Eves, N. D., Gelmon, K. A., McKenzie, D. C., Virani, S. A., Stöhr, E. J., Warburton, D. E. R., Campbell, K. L., 2017. Protective effects of

acute exercise prior to doxorubicin on cardiac function of breast cancer patients: A proof-of-concept RCT. *International Journal of Cardiology*, *245*, 263–270.

Laker, R. C., Drake, J. C., Wilson, R. J., Lira, V. A., Lewellen, B. M., Ryall, K. A., Fisher, C. C., Zhang, M., Saucerman, J. J., Goodyear, L. J., Kundu, M., Yan, Z., 2017. Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nature Communications*, *8*, 548.

Lee, I. M., 2003. Physical activity and cancer prevention - Data from epidemiologic studies. *Medicine and Science in Sports and Exercise*, *35*, 1823–1827.

Lee, S., Son, J.-Y., Lee, J., Cheong, H., 2023. Unraveling the Intricacies of Autophagy and Mitophagy: Implications in Cancer Biology. *Cells*, *12*, 2742.

Liao, H.-H., Ding, W., Zhang, N., Zhou, Z.-Y., Ling, Z., Li, W.-J., Chen, S., Tang, Q.-Z., 2023. Activation of AMPKα2 attenuated doxorubicin-induced cardiotoxicity via inhibiting lipid peroxidation associated ferroptosis. *Free Radical Biology and Medicine*, *205*, 275–290.

Liberti, M. V., & Locasale, J. W., 2016. The Warburg Effect: How Does it Benefit Cancer Cells? *Trends in Biochemical Sciences*, *41*, 211–218.

Linders, A. N., Dias, I. B., López Fernández, T., Tocchetti, C. G., Bomer, N., Van der Meer, P., 2024. A review of the pathophysiological mechanisms of doxorubicin-induced cardiotoxicity and aging. *Npj Aging*, *10*, 9.

Lyon, A. R., López-Fernández, T., Couch, L. S., Asteggiano, R., Aznar, M. C., Bergler-Klein, J., … Touyz, R. M., 2022. 2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS). *European Heart Journal*, *43*, 4229–4361.

Maayah, Z. H., Ferdaoussi, M., Boukouris, A. E., Takahara, S., Das, S. K., Khairy, M., Mackey, J. R., Pituskin, E., Sutendra, G., Paterson, D. I., Dyck, J. R. B., 2023. Endothelin Receptor Blocker Reverses Breast Cancer–Induced Cardiac Remodeling. *JACC: CardioOncology*, *5*, 686–700.

Mack, N., Mazzio, E. A., Bauer, D., Flores-Rozas, H., Soliman, K. F. A., 2017. Stable shRNA Silencing of Lactate Dehydrogenase A (LDHA) in Human MDA-MB-231 Breast Cancer Cells Fails to Alter Lactic Acid Production, Glycolytic Activity, ATP or Survival. *Anticancer Research*, *37*, 1205–1212.

Madu, C. O., Wang, S., Madu, C. O., Lu, Y., 2020. Angiogenesis in Breast Cancer Progression, Diagnosis, and Treatment. *Journal of Cancer*, *11*, 4474–4494.

Mankoff, D. A., Dunnwald, L. K., Gralow, J. R., Ellis, G. K., Schubert, E. K., Tseng, J., Lawton, T. J., Linden, H. M., Livingston, R. B., 2003. Changes in blood flow and metabolism in locally advanced breast cancer treated with neoadjuvant chemotherapy. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *44*, 1806–1814.

Marcillat, O., Zhang, Y., Davies, K. J. A., 1989. Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. *Biochemical Journal*, *259*, 181–189.

Marques-Aleixo, I., Santos-Alves, E., Mariani, D., Rizo-Roca, D., Padrão, A. I., Rocha-Rodrigues, S., Viscor, G., Torrella, J. R., Ferreira, R., Oliveira, P. J., Magalhães, J., Ascensão, A., 2015. Physical exercise prior and during treatment reduces sub-chronic doxorubicin-induced mitochondrial toxicity and oxidative stress. *Mitochondrion*, *20*, 22–33.

McCullough, D. J., Stabley, J. N., Siemann, D. W., Behnke, B. J., 2014. Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. *Journal of the National Cancer Institute*, *106*, dju036.

Miao, S.-N., Chai, M.-Q., Liu, X.-Y., Wei, C.-Y., Zhang, C.-C., Sun, N.-N., Fei, Q.-Z., Peng, L.-L., Qiu, H., 2024. Exercise accelerates recruitment of CD8+ T cell to promotes anti-tumor immunity in lung cancer via epinephrine. *BMC Cancer*, *24*, 474.

Migrino, R. Q., Aggarwal, D., Konorev, E., Brahmbhatt, T., Bright, M., Kalyanaraman, B., 2008. Early Detection of Doxorubicin Cardiomyopathy Using Two-Dimensional Strain Echocardiography. *Ultrasound in Medicine & Biology*, *34*, 208–214.

Mihara, F., Kuwabara, Y., Tanaka, A., Yoshiura, T., Sasaki, M., Yoshida, T., Masuda, K., Matsushima, T., 2003. Reliability of mean transit time obtained using perfusion-weighted MR imaging; comparison with positron emission tomography. *Magnetic Resonance Imaging*, *21*, 33–39.

Milosevic, V., Edelmann, R. J., Winge, I., Strell, C., Mezheyeuski, A., Knutsvik, G., Askeland, C., Wik, E., Akslen, L. A., Östman, A., 2023. Vessel size as a marker of survival in estrogen receptor positive breast cancer. *Breast Cancer Research and Treatment*, *200*, 293–304.

Minotti, G., 1990. NADPH- and adriamycin-dependent microsomal release of iron and lipid peroxidation. *Archives of Biochemistry and Biophysics*, *277*, 268–276.

Montaigne, D., Marechal, X., Baccouch, R., Modine, T., Preau, S., Zannis, K., Marchetti, P., Lancel, S., Neviere, R., 2010. Stabilization of mitochondrial membrane potential prevents doxorubicin-induced cardiotoxicity in isolated rat heart. *Toxicology and Applied Pharmacology*, *244*, 300–307.

Montalvo, R. N., Boeno, F. P., Dowllah, I. M., Moritz, C. E. J., Nguyen, B. L., Doerr, V., Bomkamp, M. P., Smuder, A. J., 2023. Exercise and Doxorubicin Modify Markers of Iron Overload and Cardiolipin Deficiency in Cardiac Mitochondria. *International Journal of Molecular Sciences*, *24*, 7689.

Morikawa, S., Baluk, P., Kaidoh, T., Haskell, A., Jain, R. K., McDonald, D. M., 2002a. Abnormalities in Pericytes on Blood Vessels and Endothelial Sprouts in Tumors. *The American Journal of Pathology*, *160*, 985–1000.

Morikawa, S., Baluk, P., Kaidoh, T., Haskell, A., Jain, R. K., McDonald, D. M., 2002b. Abnormalities in Pericytes on Blood Vessels and Endothelial Sprouts in Tumors. *The American Journal of Pathology*, *160*, 985–1000.

Morita, Y., Leslie, M., Kameyama, H., Lokesh, G. L. R., Ichimura, N., Davis, R., Hills, N., Hasan, N., Zhang, R., Kondo, Y., Gorenstein, D. G., Volk, D. E., Chervoneva, I., Rui, H., Tanaka, T., 2020. Functional Blockade of E-Selectin in Tumor-Associated Vessels Enhances Anti-Tumor Effect of Doxorubicin in Breast Cancer. *Cancers*, *12*, 725.

Morrell, M. B. G., Alvarez-Florez, C., Zhang, A., Kleinerman, E. S., Savage, H., Marmonti, E., Park, M., Shaw, A., Schadler, K. L., 2019. Vascular modulation through exercise improves chemotherapy efficacy in Ewing sarcoma. *Pediatric Blood & Cancer*, *66*. https://doi.org/10.1002/pbc.27835

Morton, A. B., Mor Huertas, A., Hinkley, J. M., Ichinoseki-Sekine, N., Christou, D. D., Smuder, A. J., 2019. Mitochondrial accumulation of doxorubicin in cardiac and diaphragm muscle following exercise preconditioning. *Mitochondrion*, *45*, 52–62.

Moulin, M., Piquereau, J., Mateo, P., Fortin, D., Rucker-Martin, C., Gressette, M., Lefebvre, F., Gresikova, M., Solgadi, A., Veksler, V., Garnier, A., Ventura-Clapier, R., 2015. Sexual Dimorphism of Doxorubicin-Mediated Cardiotoxicity. *Circulation: Heart Failure*, *8*, 98–108.

Muindi, J. R. F., Sinha, B. K., Gianni, L., Myers, C. E., 1984. Hydroxyl radical production and DNA damage induced by anthracycline-iron complex. *FEBS Letters*, *172*, 226–230.

Muraoka, S., & Miura, T., 2003. Inactivation of mitochondrial succinate dehydrogenase by adriamycin activated by horseradish peroxidase and hydrogen peroxide. *Chemico-Biological Interactions*, *145*, 67–75.

Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K., Young, R. C., 1977. Adriamycin: The Role of Lipid Peroxidation in Cardiac Toxicity and Tumor Response. *Science*, *197*, 165–167.

Naaktgeboren, W. R., Binyam, D., Stuiver, M. M., Aaronson, N. K., Teske, A. J., van Harten, W. H., Groen, W. G., May, A. M., 2021. Efficacy of Physical Exercise to Offset Anthracycline-Induced Cardiotoxicity: A Systematic Review and Meta-Analysis of Clinical and Preclinical Studies. *Journal of the American Heart Association*, *10*. https://doi.org/10.1161/JAHA.121.021580

Naaktgeboren, W. R., Stuiver, M. M., van Harten, W. H., Aaronson, N. K., Scott, J. M., Sonke, G., van der Wall, E., Velthuis, M., Leiner, T., Teske, A. J., May, A. M., Groen, W. G., 2023. Effects of

exercise during chemotherapy for breast cancer on long-term cardiovascular toxicity. *Open Heart*, *10*, e002464.

Narikawa, M., Umemura, M., Tanaka, R., Hikichi, M., Nagasako, A., Fujita, T., Yokoyama, U., Ishigami, T., Kimura, K., Tamura, K., Ishikawa, Y., 2019. Doxorubicin induces trans-differentiation and MMP1 expression in cardiac fibroblasts via cell death-independent pathways. *PLOS ONE*, *14*, e0221940.

Natarajan, V., Ha, S., Delgado, A., Jacobson, R., Alhalhooly, L., Choi, Y., Kim, J., 2022. Acquired αSMA Expression in Pericytes Coincides with Aberrant Vascular Structure and Function in Pancreatic Ductal Adenocarcinoma. *Cancers*, *14*, 2448.

Nicolay, K., Aue, W. P., Seelig, J., van Echteld, C. J. A., Ruigrok, T. J. C., de Kruijff, B., 1987. Effects of the anti-cancer drug adriamycin on the energy metabolism of rat heart as measured by in vivo 31P-NMR and implications for adriamycin-induced cardiotoxicity. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *929*, 5–13.

Nousiainen, T., Jantunen, E., Vanninen, E., Hartikainen, J., 2002. Early decline in left ventricular ejection fraction predicts doxorubicin cardiotoxicity in lymphoma patients. *British Journal of Cancer*, *86*, 1697–1700.

Octavia, Y., Tocchetti, C. G., Gabrielson, K. L., Janssens, S., Crijns, H. J., Moens, A. L., 2012. Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies. *Journal of Molecular and Cellular Cardiology*, *52*, 1213–1225.

Ohhara, H., Kanaide, H., Nakamura, M., 1981. A protective effect of coenzyme Q10 on the adriamycin-induced cardiotoxicity in the isolated perfused rat heart. *Journal of Molecular and Cellular Cardiology*, *13*, 741–752.

Pak, K., Seok, J. W., Kim, H. Y., Nguyen, T. L., Kim, K., Kim, S. J., Kim, I.-J., Hopper, J., 2020. Prognostic value of metabolic tumor volume and total lesion glycolysis in breast cancer: a meta-analysis. *Nuclear Medicine Communications*, *41*, 824–829.

Pan, J.-A., Zhang, H., Lin, H., Gao, L., Zhang, H.-I., Zhang, J.-F., Wang, C.-Q., Gu, J., 2021. Irisin ameliorates doxorubicin-induced cardiac perivascular fibrosis through inhibiting endothelial-to-mesenchymal transition by regulating ROS accumulation and autophagy disorder in endothelial cells. *Redox Biology*, *46*, 102120.

Parihar, A. S., Dehdashti, F., Wahl, R. L., 2023. FDG PET/CT–based Response Assessment in Malignancies. *RadioGraphics*, *43*. https://doi.org/10.1148/rg.220122

Parry, T. L., & Hayward, R., 2015. Exercise training does not affect anthracycline antitumor efficacy while attenuating cardiac dysfunction. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *309*, 675.

Pedersen, B. K. K., Pedersen, L., Pedersen, K. S. S., Idorn, M., Olofsson, G. H. H., Lauenborg, B., Nookaew, I., Hansen, R. H. H., Johannesen, H. H. H., Becker, J. C. C., Dethlefsen, C., Nielsen, J., Gehl, J., thor Straten P., Hojman, P., 2016. Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. *Cell Metabolism*, *23*, 554–562.

Phungphong, S., Kijtawornrat, A., Kampaengsri, T., Wattanapermpool, J., Bupha-Intr, T., 2020. Comparison of exercise training and estrogen supplementation on mast cell-mediated doxorubicin-induced cardiotoxicity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *318*, R829–R842.

Piek, A., de Boer, R. A., Silljé, H. H. W., 2016. The fibrosis-cell death axis in heart failure. *Heart Failure Reviews*, *21*, 199–211.

Pillai, V. B., Kanwal, A., Fang, Y. H., Sharp, W. W., Samant, S., Arbiser, J., Gupta, M. P., 2017. Honokiol, an activator of Sirtuin-3 (SIRT3) preserves mitochondria and protects the heart from doxorubicin-induced cardiomyopathy in mice. *Oncotarget*, *8*, 34082–34098.

Pitkäniemi, J., Malila, N., Heikkinen, S., Seppä, K., 2024. Cancer in Finland 2022. *Finnish Cancer Registry,* Retrieved from https://syoparekisteri.fi/assets/themes/ssy3/factsheets/cancer_in_finland_2022.html

Podyacheva, E. Y., Kushnareva, E. A., Karpov, A. A., Toropova, Y. G., 2021. Analysis of Models of Doxorubicin-Induced Cardiomyopathy in Rats and Mice. A Modern View From the Perspective of the Pathophysiologist and the Clinician. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.670479

Pointon, A. V., Walker, T. M., Phillips, K. M., Luo, J., Riley, J., Zhang, S.-D., Parry, J. D., Lyon, J. J., Marczylo, E. L., Gant, T. W., 2010. Doxorubicin In Vivo Rapidly Alters Expression and Translation of Myocardial Electron Transport Chain Genes, Leads to ATP Loss and Caspase 3 Activation. *PLoS ONE*, *5*, e12733.

Powers, S. K., Sollanek, K. J., Wiggs, M. P., Demirel, H. A., Smuder, A. J., 2014. Exercise-induced improvements in myocardial antioxidant capacity: the antioxidant players and cardioprotection. *Free Radical Research*, *48*, 43–51.

Pugazhendhi, A., Edison, T. N. J. I., Velmurugan, B. K., Jacob, J. A., Karuppusamy, I., 2018. Toxicity of Doxorubicin (Dox) to different experimental organ systems. *Life Sciences*, *200*, 26–30.

Quiros, P. M., Goyal, A., Jha, P., Auwerx, J., 2017. Analysis of mtDNA/nDNA Ratio in Mice. *Current Protocols in Mouse Biology*, *7*, 47–54.

Rainio, O., Han, C., Teuho, J., Nesterov, S. V., Oikonen, V., Piirola, S., Laitinen, T., Tättäläinen, M., Knuuti, J., Klén, R., 2023. Carimas: An Extensive Medical Imaging Data Processing Tool for Research. *Journal of Digital Imaging*, *36*, 1885–1893.

Raja-aho, S., Kanerva, M., Eeva, T., Lehikoinen, E., Suorsa, P., Gao, K., Vosloo, D., Nikinmaa, M., 2012. Seasonal variation in the regulation of redox state and some biotransformation enzyme activities in the barn swallow (Hirundo rustica L.). *Physiological and Biochemical Zoology: PBZ*, *85*, 148–158.

Rajagopalan, S., Politi, P. M., Sinha, B. K., Myers, C. E., 1988. Adriamycin-induced free radical formation in the perfused rat heart: implications for cardiotoxicity. *Cancer Research*, *48*, 4766–4769.

Räsänen, M., Degerman, J., Nissinen, T. A., Miinalainen, I., Kerkelä, R., Siltanen, A., Backman, J. T., Mervaala, E., Hulmi, J. J., Kivelä, R., Alitalo, K., 2016. VEGF-B gene therapy inhibits doxorubicin-induced cardiotoxicity by endothelial protection. *Proceedings of the National Academy of Sciences*, *113*, 13144–13149.

Refaie, M. M. M., El-Hussieny, M., Abdel-Hakeem, E. A., Fawzy, M. A., Mahmoud Abd El Rahman, E. S., Shehata, S., 2022. Phosphodiesterase inhibitor, Vinpocetine, guards against doxorubicin induced cardiotoxicity *via* modulation of HIF/VEGF and cGMP/cAMP/SIRT signaling pathways. *Human & Experimental Toxicology*, *41*. https://doi.org/10.1177/09603271221136209

Rundqvist, H., Veliça, P., Barbieri, L., Gameiro, P. A., Bargiela, D., Gojkovic, M., Mijwel, S., Reitzner, S. M., Wulliman, D., Ahlstedt, E., Ule, J., Östman, A., Johnson, R. S., 2020. Cytotoxic T-cells mediate exercise-induced reductions in tumor growth. *ELife*, *9*. https://doi.org/10.7554/eLife.59996

Saks, V. A., Kongas, O., Vendelin, M., Kay, L., 2000. Role of the creatine/phosphocreatine system in the regulation of mitochondrial respiration. *Acta Physiologica Scandinavica*, *168*, 635–641.

Sanchez-Alvarez, R., Martinez-Outschoorn, U. E., Lamb, R., Hulit, J., Howell, A., Gandara, R., Sartini, M., Rubin, E., Lisanti, M. P., Sotgia, F., 2013. Mitochondrial dysfunction in breast cancer cells prevents tumor growth: understanding chemoprevention with metformin. *Cell Cycle (Georgetown, Tex.)*, *12*, 172–182.

Sarocchi, M., Bauckneht, M., Arboscello, E., Capitanio, S., Marini, C., Morbelli, S., Miglino, M., Congiu, A., G., Ghigliotti, G., Balbi, M., Brunelli, C., Sambuceti, G., Ameri, P., Spallarossa, P., 2018. An increase in myocardial 18-fluorodeoxyglucose uptake is associated with left ventricular ejection fraction decline in Hodgkin lymphoma patients treated with anthracycline. *Journal of Translational Medicine*, *16*, 295.

Schadler, K. L., Thomas, N. J., Galie, P. A., Bhang, D. H., Roby, K. C., Addai, P., Till, J. E., Sturgeon, K., Zaslavsky, A., Chen, C. S., Ryeom, S., 2016. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. *Oncotarget*, *7*, 65429–65440.

Seet-Lee, C., Yee, J., Morahan, H., Ross, L. S., Edwards, K. M., 2022. The effect of aerobic exercise on tumour blood delivery: a systematic review and meta-analysis. *Supportive Care in Cancer*, *30*, 8637–8653.

Sequeira, C. M., Martins, M. A., Alves, R., Nascimento, A. L. R., Botti, G. C. R. M., Rocha, V. N., Matsuura, C., 2021. Aerobic exercise training attenuates doxorubicin-induced ultrastructural changes in rat ventricular myocytes. *Life Sciences*, *264*, 118698.

Sethi, V., Kurtom, S., Tarique, M., Lavania, S., Malchiodi, Z., Hellmund, L., Zhang, L., Sharma, U., Giri, B., Garg, B., Ferrantella, A., Vickers, S. M., Banerjee, S., Dawra, R., Roy, S., Ramakrishnan, S., Saluja, A., Dudeja, V., 2018. Gut Microbiota Promotes Tumor Growth in Mice by Modulating Immune Response. *Gastroenterology*, *155*, 33-37.e6.

Sharma, R. I., Welch, A. E., Schweiger, L., Craib, S., Smith, T. A. D., 2011. [18F]Fluoro-2-Deoxy-D-Glucose Incorporation by MCF-7 Breast Tumour Cells In Vitro Is Modulated by Treatment with Tamoxifen, Doxorubicin, and Docetaxel: Relationship to Chemotherapy-Induced Changes in ATP Content, Hexokinase Activity, and Glucose Transport. *International Journal of Molecular Imaging*, *2011*, 1–8.

Sheinboim, D., Parikh, S., Manich, P., Markus, I., Dahan, S., Parikh, R., Stubbs, E., Cohen, G., Zemser-Werner, V., Bell, R. E., Ruiz, S. A., Percik, R., Brenner, R., Leibou, S., Vaknine, H., Arad, G., Gerber, Y., Keinan-Boker, L., Shimony, T., Bikovski, L., Goldstein, N., Constantini, K., Labes, S., Mordechai, S., Doron, H., Lonescu, A., Ziv, T., Nizri, E., Choshen, G., Eldar-Finkelman, H., Tabach, Y., Helman, A., Ben-Eliyahu, S., Erez, N., Perlson, E., Geiger, T., Ben-Zvi, D., Khaled, M., Gepner, Y., Levy, C., 2022. An Exercise-Induced Metabolic Shield in Distant Organs Blocks Cancer Progression and Metastatic Dissemination. *Cancer Research*, *82*, 4164–4178.

Shi, J., Li, J., Li, J., Li, R., Wu, X., Gao, F., Zou, L., Mak, W. W.-S., Fu, C., Zhang, J., Leung, G. P.-H., 2021. Synergistic breast cancer suppression efficacy of doxorubicin by combination with glycyrrhetinic acid as an angiogenesis inhibitor. *Phytomedicine*, *81*, 153408.

Shimochi, S., Ihalainen, J., Parikka, V., Kudomi, N., Tolvanen, T., Hietanen, A., Kokkomäki, E., Johansson, S., Tsuji, M., Kanaya, S., Yatkin, E., Grönroos, T. J., Iida, H., 2024. Small animal PET with spontaneous inhalation of ^{15} O-labelled oxygen gases: Longitudinal assessment of cerebral oxygen metabolism in a rat model of neonatal hypoxic-ischaemic encephalopathy. *Journal of Cerebral Blood Flow & Metabolism*, *44*, 1024–1038.

Siu, P. M., Donley, D. A., Bryner, R. W., Alway, S. E., 2003. Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. *Journal of Applied Physiology*, *94*, 555–560.

Slart, R. H. J. A., Martinez-Lucio, T. S., Boersma, H. H., Borra, R. H., Cornelissen, B., Dierckx, R. A. J. O., Dobrolinska, M., Doorduin, J., Erba, P. A., Glaudemans, A. W. J. M., Giacobbo, B. L., Luurtsema, G., Noordzij, W., van Sluis, J., Tsoumpas, C., Lammertsma, A. A., 2024. [15O]H2O PET: Potential or Essential for Molecular Imaging? *Seminars in Nuclear Medicine*, *54*, 761–773.

Smeda, M., Przyborowski, K., Proniewski, B., Zakrzewska, A., Kaczor, D., Stojak, M., Buczek, E., Nieckarz, Z., Zoladz, J. A., Wietrzyk, J., Chlopicki, S., 2017. Breast cancer pulmonary metastasis is increased in mice undertaking spontaneous physical training in the running wheel; a call for revising beneficial effects of exercise on cancer progression. In *Am J Cancer Res* (Vol. 7). Retrieved from www.ajcr.us/

Souza, F. R., Campos, É. C., Lopes, L. T. P., Rodrigues, C. M., Gonçalves, D. L. N., Beletti, M. E., Mantovani, M. M., Duarte, P. R. A., Resende, E. S., 2022. Physical Training Improves Cardiac Structure and Function of Rats After Doxorubicin-Induced Cardiomyopathy. *International Journal of Cardiovascular Sciences*. https://doi.org/10.36660/ijcs.20210095

Spaepen, K., Stroobants, S., Dupont, P., Van Steenweghen, S., Thomas, J., Vandenberghe, P., Vanuytsel, L., Bormans, G., Balzarini, J., De Wolf-Peeters, C., Mortelmans, L., Verhoef, G., 2001. Prognostic Value of Positron Emission Tomography (PET) With Fluorine-18 Fluorodeoxyglucose ([^{18} F]FDG) After First-Line Chemotherapy in Non-Hodgkin's Lymphoma: Is [^{18} F]FDG-PET a

Valid Alternative to Conventional Diagnostic Methods? *Journal of Clinical Oncology*, *19*, 414–419.

Speck, R. M., Courneya, K. S., Mâsse, L. C., Duval, S., Schmitz, K. H., 2010. An update of controlled physical activity trials in cancer survivors: a systematic review and meta-analysis. *Journal of Cancer Survivorship*, *4*, 87–100.

Steinberg, J. S., Cohen, A. J., Wasserman, A. G., Cohen, P., Ross, A. M., 1987. Acute arrhythmogenicity of doxorubicin administration. *Cancer*, *60*, 1213–1218.

Strain, T., Flaxman, S., Guthold, R., Semenova, E., Cowan, M., Riley, L. M., … Zoma, L. R., 2024. National, regional, and global trends in insufficient physical activity among adults from 2000 to 2022: a pooled analysis of 507 population-based surveys with 5·7 million participants. *The Lancet Global Health*, *12*, e1232–e1243.

Sturgeon, K. M., Ky, B., Libonati, J. R., Schmitz, K. H., 2014a. The effects of exercise on cardiovascular outcomes before, during, and after treatment for breast cancer. *Breast Cancer Research and Treatment*, *143*, 219–226.

Sturgeon, K., Schadler, K., Muthukumaran, G., Ding, D., Bajulaiye, A., Thomas, N. J., Ferrari, V., Ryeom, S., Libonati, J. R., 2014b. Concomitant low-dose doxorubicin treatment and exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *307*, R685–R692.

Sun, X., Chen, H., Gao, R., Huang, Y., Qu, Y., Yang, H., Wei, X., Hu, S., Zhang, J., Wang, P., Zou, Y., Hu, K., Ge, J., Sun, A., 2023. Mitochondrial transplantation ameliorates doxorubicin-induced cardiac dysfunction via activating glutamine metabolism. *IScience*, *26*, 107790.

Suthivanich, P., Boonhoh, W., Sumneang, N., Punsawad, C., Cheng, Z., Phungphong, S., 2024. Aerobic Exercise Attenuates Doxorubicin-Induced Cardiomyopathy by Suppressing NLRP3 Inflammasome Activation in a Rat Model. *International Journal of Molecular Sciences*, *25*, 9692.

Svingen, B. A., & Powis, G., 1981. Pulse radiolysis studies of antitumor quinones: Radical lifetimes, reactivity with oxygen, and one-electron reduction potentials. *Archives of Biochemistry and Biophysics*, *209*, 119–126.

Swain, S. M., Whaley, F. S., Gerber, M. C., Weisberg, S., York, M., Spicer, D., Jones, S. E., Wadler, S., Desai, A., Vogel, C., Speyer, J., Mittelman, A., Reddy, S., Pendergrass, K., Velez-Garcia, E., Ewer, M. S., Bianchine, J. R., Gams, R. A., 1997. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. *Journal of Clinical Oncology*, *15*, 1318–1332.

Tadic, M., Genger, M., Baudisch, A., Kelle, S., Cuspidi, C., Belyavskiy, E., Burkhardt, F., Venneri, L., Attanasio, P., Pieske, B., 2018. Left Ventricular Strain in Chemotherapy-Naive and Radiotherapy-Naive Patients With Cancer. *Canadian Journal of Cardiology*, *34*, 281–287.

Tadokoro, T., Ikeda, M., Ide, T., Deguchi, H., Ikeda, S., Okabe, K., … Tsutsui, H. (2020). Mitochondria-dependent ferroptosis plays a pivotal role in doxorubicin cardiotoxicity. *JCI Insight*, *5*. https://doi.org/10.1172/jci.insight.132747

Tao, R.-H., Kobayashi, M., Yang, Y., Kleinerman, E. S., 2021. Exercise Inhibits Doxorubicin-Induced Damage to Cardiac Vessels and Activation of Hippo/YAP-Mediated Apoptosis. *Cancers*, *13*, 2740.

Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D., Liu, L. F., 1984. Adriamycin-Induced DNA Damage Mediated by Mammalian DNA Topoisomerase II. *Science*, *226*, 466–468.

Timour, Q., Nony, P., Lang, J., Lakhal, M., Trillet, V., Faucon, G., 1988. Doxorubicin concentrations in plasma and myocardium and their respective roles in cardiotoxicity. *Cardiovascular Drugs and Therapy*, *1*, 559–560.

Toda, N., Sato, T., Muraoka, M., Lin, D., Saito, M., Li, G., … Yamauchi, M. (2023). Doxorubicin induces cardiomyocyte death owing to the accumulation of dysfunctional mitochondria by inhibiting the autophagy fusion process. *Free Radical Biology and Medicine*, *195*, 47–57.

Todorovic, D., Stojanovic, M., Medic, A., Gopcevic, K., Mutavdzin, S., Stankovic, S., Djuric, D., 2021. Four Weeks of Aerobic Training Affects Cardiac Tissue Matrix Metalloproteinase, Lactate

Dehydrogenase and Malate Dehydrogenase Enzymes Activities, and Hepatorenal Biomarkers in Experimental Hyperhomocysteinemia in Rats. *International Journal of Molecular Sciences*, *22*. https://doi.org/10.3390/ijms22136792

Tokarska-Schlattner, M., Dolder, M., Gerber, I., Speer, O., Wallimann, T., Schlattner, U., 2007. Reduced creatine-stimulated respiration in doxorubicin challenged mitochondria: Particular sensitivity of the heart. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1767*, 1276–1284.

Tokarska-Schlattner, M., Zaugg, M., da Silva, R., Lucchinetti, E., Schaub, M. C., Wallimann, T., Schlattner, U., 2005. Acute toxicity of doxorubicin on isolated perfused heart: response of kinases regulating energy supply. *American Journal of Physiology-Heart and Circulatory Physiology*, *289*, H37–H47.

Upshaw, J. N., Finkelman, B., Hubbard, R. A., Smith, A. M., Narayan, H. K., Arndt, L., Domchek, S., DeMichele, A., Fox, K., Shah, P., Clark, A., Bradbury, A., Matro, J., Adusumalli, S., Carver, J. R., Ky, B., 2020a. Comprehensive Assessment of Changes in Left Ventricular Diastolic Function With Contemporary Breast Cancer Therapy. *JACC: Cardiovascular Imaging*, *13*, 198–210.

Upshaw, J. N., Hubbard, R. A., Hu, J., Brown, J. C., Smith, A. M., Demissei, B., Schmitz, K. H., Ky, B., 2020b. Physical activity during and after breast cancer therapy and associations of baseline physical activity with changes in cardiac function by echocardiography. *Cancer Medicine*, *9*, 6122–6131.

Uzzan, B., Nicolas, P., Cucherat, M., Perret, G.-Y., 2004. Microvessel Density as a Prognostic Factor in Women with Breast Cancer. *Cancer Research*, *64*, 2941–2955.

Vásquez-Vivar, J., Martasek, P., Hogg, N., Masters, B. S. S., Pritchard, K. A., Kalyanaraman, B., 1997. Endothelial Nitric Oxide Synthase-Dependent Superoxide Generation from Adriamycin. *Biochemistry*, *36*, 11293–11297.

Vathiotis, I. A., Moutafi, M. K., Divakar, P., Aung, T. N., Qing, T., Fernandez, A., Yaghoobi, V., El-Abed, S., Wang, Y., Guillaume, S., Nuciforo, P., Huober, J., Di Cosimo, S., Kim, S.-B., Harbeck, N., Gomez, H., Shafi, S., Syrigos, K. N., Fountzilas, G., Sotiriou, C., Pusztai, L., Warren, S., Rimm, D. L., 2021. Alpha-smooth Muscle Actin Expression in the Stroma Predicts Resistance to Trastuzumab in Patients with Early-stage HER2-positive Breast Cancer. *Clinical Cancer Research*, *27*, 6156–6163.

Ventura-Clapier, R., Mettauer, B., Bigard, X., 2007. Beneficial effects of endurance training on cardiac and skeletal muscle energy metabolism in heart failure. *Cardiovascular Research*, *73*, 10–18.

Villman, K., Ståhl, E., Liljegren, G., Tidefelt, U., Karlsson, M. G., 2002. Topoisomerase II-α Expression in Different Cell Cycle Phases in Fresh Human Breast Carcinomas. *Modern Pathology*, *15*, 486–491.

Vulczak, A., Souza, A. de O., Ferrari, G. D., Azzolini, A. E. C. S., Pereira-da-Silva, G., Alberici, L. C., 2020. Moderate Exercise Modulates Tumor Metabolism of Triple-Negative Breast Cancer. *Cells*, *9*, 628.

Vuori, K., & Kanerva, M., 2018. Catalase (CAT) activity assay for zooplankton samples. *Protocols.Io*. https://doi.org/10.17504/protocols.io.mwsc7ee

Wakefield, Z. R., Tanaka, M., Pampo, C., Lepler, S., Rice, L., Guingab-Cagmat, J., Garrett, T. J., Siemann, D. W., 2021. Normal tissue and tumor microenvironment adaptations to aerobic exercise enhance doxorubicin anti-tumor efficacy and ameliorate its cardiotoxicity in retired breeder mice. *Oncotarget*, *12*, 1737–1748.

Wang, F., Chandra, J., & Kleinerman, E. S., 2021. Exercise intervention decreases acute and late doxorubicin-induced cardiotoxicity. *Cancer Medicine*, *10*, 7572–7584.

Wang, F., Iskra, B., Kleinerman, E., Alvarez-Florez, C., Andrews, T., Shaw, A., Chandra, J., Schadler, K., Aune, G. J., 2018. Aerobic Exercise During Early Murine Doxorubicin Exposure Mitigates Cardiac Toxicity. *Journal of Pediatric Hematology/Oncology*, *40*, 208–215.

Wang, J., Liu, S., Meng, X., Zhao, X., Wang, T., Lei, Z., Lehmann, H. I., Li, G., Alcaide, P., Bei, Y., Xiao, J., 2024. Exercise Inhibits Doxorubicin-Induced Cardiotoxicity via Regulating B Cells. *Circulation Research*, *134*, 550–568.

Weber, R. E., Schulze, K. M., Kenney, N. J., Scheuermann, B. C., Kunkel, O. N., Ade, C. J., Musch, T. I., Behnke, B. J., Poole, D. C., 2025. Tumor bearing in untreated breast cancer decreases exercise tolerance without lowering maximal oxygen uptake in rats. *American Journal of Cancer Research*, *15*, 487–500.

Weigelt, B., Horlings, H., Kreike, B., Hayes, M., Hauptmann, M., Wessels, L. F. A., de Jong, D., Van de Vijver, M. J., Veer, L. J. V., Peterse, J., 2008. Refinement of breast cancer classification by molecular characterization of histological special types. *The Journal of Pathology*, *216*, 141–150.

Weiland, A., Roswall, P., Hatzihristidis, T. C., Pietras, K., Ostman, A., Strell, C., 2012. Fibroblast-dependent regulation of the stem cell properties of cancer cells. *Neoplasma*, *59*, 719–727.

Willis, M. S., Parry, T. L., Brown, D. I., Mota, R. I., Huang, W., Beak, J. Y., Sola, M., Zhou, C., Hicks, S. T., Caughey, M. C., D'Agostino, R. B., Jordan, J., Hundley, W. G., Jensen, B. C., 2019. Doxorubicin Exposure Causes Subacute Cardiac Atrophy Dependent on the Striated Muscle–Specific Ubiquitin Ligase MuRF1. *Circulation: Heart Failure*, *12*. https://doi.org/10.1161/CIRCHEARTFAILURE.118.005234

Wonders, K. Y., Hydock, D. S., Schneider, C. M., Hayward, R., 2008. Acute exercise protects against doxorubicin cardiotoxicity. *Integrative Cancer Therapies*, *7*, 147–154.

Xia, P., Chen, J., Liu, Y., Fletcher, M., Jensen, B. C., Cheng, Z., 2020. Doxorubicin induces cardiomyocyte apoptosis and atrophy through cyclin-dependent kinase 2–mediated activation of forkhead box O1. *Journal of Biological Chemistry*, *295*, 4265–4276.

Xu, L., Yang, M., Wei, A., Wei, Z., Qin, Y., Wang, K., Li, B., Chen, K., Liu, C., Li, C., Wang, T., 2024. Aerobic exercise-induced HIF-1α upregulation in heart failure: exploring potential impacts on MCT1 and MPC1 regulation. *Molecular Medicine*, *30*, 83.

Yang, H.-L., Hsieh, P.-L., Hung, C.-H., Cheng, H.-C., Chou, W.-C., Chu, P.-M., Chang, Y.-C., Tsai, K.-L., 2020. Early Moderate Intensity Aerobic Exercise Intervention Prevents Doxorubicin-caused Cardiac Dysfunction through Inhibition of Cardiac Fibrosis and Inflammation. *Cancers*, *12*, 1102.

Yang, L., Morielli, A. R., Heer, E., Kirkham, A. A., Cheung, W. Y., Usmani, N., Friedenreich, C. M., Courneya, K. S., 2021. Effects of Exercise on Cancer Treatment Efficacy: A Systematic Review of Preclinical and Clinical Studies. *Cancer Research*, *81*, 4889–4895.

Yao, C.-X., Li, W.-Y., Zhang, S.-F., Zhang, S.-F., Zhang, H.-F., Zang, M.-X., 2011. Effects of Doxorubicin and Fenofibrate on the Activities of NADH Oxidase and Citrate Synthase in Mice. *Basic & Clinical Pharmacology & Toxicology*, *109*. https://doi.org/10.1111/j.1742-7843.2011.00748.x

Ye, P., Li, W.-L., Bao, L.-T., Ke, W., 2022. Mulberrin Confers Protection against Doxorubicin-Induced Cardiotoxicity via Regulating AKT Signaling Pathways in Mice. *Oxidative Medicine and Cellular Longevity*, *2022*, 1–18.

Zeng, X., Cai, H., Yang, J., Qiu, H., Cheng, Y., Liu, M., 2019. Pharmacokinetics and cardiotoxicity of doxorubicin and its secondary alcohol metabolite in rats. *Biomedicine & Pharmacotherapy*, *116*, 108964.

Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L.-S., Lyu, Y. L., Liu, L. F., Yeh, E. T. H., 2012. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine*, *18*, 1639–1642.

Zhang, Y., Chen, Y., Zhang, M., Tang, Y., Xie, Y., Huang, X., Li, Y., 2014. Doxorubicin Induces Sarcoplasmic Reticulum Calcium Regulation Dysfunction via the Decrease of SERCA2 and Phospholamban Expressions in Rats. *Cell Biochemistry and Biophysics*, *70*, 1791–1798.

Zhou, M., Sun, X., Wang, C., Wang, F., Fang, C., Hu, Z., 2022. PFKM inhibits doxorubicin-induced cardiotoxicity by enhancing oxidative phosphorylation and glycolysis. *Scientific Reports*, *12*, 11684.

Zhou, S., Heller, L. J., Wallace, K. B., 2001. Interference with Calcium-Dependent Mitochondrial Bioenergetics in Cardiac Myocytes Isolated from Doxorubicin-Treated Rats. *Toxicology and Applied Pharmacology*, *175*, 60–67.

Zhu, Z., Jiang, W., Sells, J. L., Neil, E. S., McGinley, J. N., Thompson, H. J., 2008. Effect of Nonmotorized Wheel Running on Mammary Carcinogenesis: Circulating Biomarkers, Cellular

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