Impact of intra-infusion aerobic exercise on tumour vascularity in patients with cancer

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Statement of originality

This is to certify that the content of this thesis is my own work. This thesis has not been submitted for any other degree or purpose.

I certify that the intellectual content of this thesis is the product of my own work, and that all assistance received in preparing this thesis and all sources have been acknowledged.

Catherine Seet-Lee 26/08/2025

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Author attribution statement and publications

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This publication in Supportive Care in Cancer is included in Chapter 2 and the published article can be seen in the Appendix. I co-designed the systematic review and meta-analysis with Kate Edwards and Jasmine Yee. The analysis was performed by Heidi Morahan, and I interpreted the analysis. I wrote the drafts of the manuscript.

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This publication in the International Journal of Sports Medicine was written in response to a systematic review and meta-analysis published just prior to our published systematic review and meta-analysis. This systematic review came to different conclusions, and therefore we wrote a letter to the editor highlighting the discrepancies from our findings. I co-designed the letter with Kate Edwards, Heidi Morahan and Jasmine Edwards. I wrote the drafts of the manuscript. The published article can be seen in the Appendix.

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This publication has been submitted to the European Journal of Applied Physiology and at the time of this thesis submission, this paper is under review. This submission is included in Chapter 3 of this thesis.

In addition to the authorship attribution statements above, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

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Artificial intelligence statement

Generative AI tools were used to format references. No generative AI tools were used in any other part of the preparation of this thesis.

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Abstract

Exercise during chemotherapy treatment has been well established to reduce chemotherapy-related side effects such as fatigue as well as improving quality of life. However, most cancer patients do not meet exercise guidelines during treatment. Chemotherapy infusion may provide a window of time for patients to exercise in a supervised and safe environment. Patients may also benefit from physiological changes to chemotherapy delivery by altering blood flow to the tumour which has dysfunctional vasculature that may promote blood delivery with increased cardiac output from exercise.

The aims of this thesis are to: 1) evaluate the impact of aerobic exercise on tumour vascularity, 2) determine if acute exercise changes tumour blood flow and the relationship with exercise intensity, 3) evaluate any change in response to exercise when performed during chemotherapy infusion, and 4) investigate the effects of repeated intra-infusion aerobic exercise on chemotherapy symptoms and physical activity behaviour.

Chapter 2 is a published review of the preclinical evidence for chronic aerobic exercise on tumour vascularity, specifically hypoxia, vascularisation and blood flow, using a systematic search and meta-analysis. The findings in this chapter from primarily preclinical evidence, demonstrated mixed results in tumour vascularity, however no significant effects of chronic aerobic exercise on hypoxia, vascularisation or blood flow were found. Poor and heterogenous methodological design may explain the inconsistent findings, and therefore these findings but not be transferable to humans.

Chapter 3 provides the first clinical evidence for exercise effects on tumour blood flow. This chapter includes a cross-sectional study examining the effects of three exercise intensities on tumour blood flow in patients with liver metastases. After a 5-minute bout of acute aerobic exercise, there was a 152% increase in blood flow to the tumour after moderate intensity exercise, although no significant changes after low or high intensity exercise.

The fourth and fifth chapters assessed intra-infusion exercise. The fourth chapter demonstrated that intra-infusion exercise causes an exaggerated heart rate and blood pressure response compared to exercise off chemotherapy, and highlighted the importance of regular physiological monitoring with intra-infusion exercise. The findings in Chapter 4 are utilised in Chapter 5 which includes a randomised controlled trial that investigates the effects of intra-infusion exercise on chemotherapy side effects and physical activity behaviours in patients with stage I-III breast, colorectal or ovarian cancer. The study found that intra-infusion exercise across three chemotherapy cycles had high adherence and did not change chemotherapy side effects or exercise behaviour.

The findings from this thesis demonstrate that aerobic exercise can increase blood flow to tumours which may improve chemotherapy delivery. Intra-infusion exercise is a feasible and safe novel modality that may improve chemotherapy delivery when chemotherapy concentration is at its highest without increasing fatigue or other negative toxicities. However, intra-infusion exercise alone did not improve exercise behaviour outside of the infusion and therefore more regular support is likely needed to encourage exercise towards minimum physical activity guidelines which may promote tumour vascular adaptations that further facilitate chemotherapy delivery.

Presentations

Oral presentations

Sydney Catalyst Early Career Research Symposium, 2020 - *"The effect of aerobic exercise on tumour blood delivery: a systematic review of preclinical and clinical studies."*

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Glossary of acronyms

ACSM	American College of Sports Medicine
BFI	Brief Fatigue Inventory
BP	Blood pressure
BPM	Beats per minute
CAIX	Carbonic anhydrase IX
CI	Confidence interval
CIPN	Chemotherapy-induced peripheral neuropathy
CO	Cardiac output
COSA	Clinical Oncology Society of Australia
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic blood pressure
ECOG	Easter Cooperative Oncology Group
EDV	End diastolic velocity
EORTC QLQ-C30	European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire
ESAS-R	Revised Edmonton Symptom Assessment System
ESE	Exercise Self Efficacy
ESSA	Exercise and Sport Science Australia
FACIT-Fatigue	Functional Assessment of Chronic Illness Therapy – Fatigue
FACT/GOG-Ntx	Functional Assessment of Cancer Therapy/Gynaecologic Oncology Group-Neuropathy
HIF	Hypoxia-inducible factor
HR	Heart rate
HRR	Heart rate reserve
IPAQ	International Physical Activity Questionnaire
MAP	Mean arterial pressure
MVD	Microvessel density
PI	Principle investigator
PO_2	Partial pressure of oxygen
PSV	Peak systolic velocity

QOL	Quality of life
RI	Resistive index
RPE	Rate of perceived exertion
RPM	Revolutions per minute
SBP	Systolic blood pressure
SD	Standard deviation
SMD	Standard error of mean
SpO_2	Oxygen saturation
SV	Stroke volume
TPR	Total peripheral resistance
VEGF	Vascular endothelial growth factor

Chapter 1

Introduction

Cancer

Cancer is a leading cause of mortality and it was estimated that there were almost 19 million new cancer cases globally in 2022 (Ferlay et al, 2024). There are many known modifiable risk factors for cancer development, including reduced physical activity, obesity, tobacco smoking, alcohol and sun exposure (Matthews et al, 2019; Soerjomataram & Bray, 2021). Public health initiatives have aimed to reduce cancer incidence by reducing modifiable risk factors. Physical activity promotional efforts have been implemented to encourage people towards meeting minimum physical activity guidelines. However, despite public health initiatives, almost a quarter of the global population still do not meet minimum physical activity guidelines (Guthold et al, 2018).

Cancer has a societal and individual economic burden due to costly resources for diagnosis and treatment (Gordon & Rowell, 2015; Yabroff et al, 2021). In 2019, it was estimated that the economic burden of cancer screening, diagnosis and treatment was over $20 billion annually in the USA, and this figure does not include the personal burden to individuals and families (Yabroff et al, 2021). Cancer treatment is complex and often requires significant time to travel to, undergo and recover from which may result in reduced productivity at work and loss of income, combining to increase the risk of financial strain on individuals (Paul et al, 2016). Additionally, the long duration of treatment takes patients away from families and social activities resulting in reduced social wellbeing (Wang & Feng, 2022). Thus, with forecasted increases in cancer incidence and the enormous and far-reaching impacts of the disease, oncology research is important to reduce cancer burden to individuals and society.

Treatments for cancer are many and varied, often including chemotherapy, immunotherapy, targeted therapy, radiation and surgery. One of the most common treatments is intravenous chemotherapy, an approach of administering cytotoxic drugs intravenously to destroy abnormal cancer cells and reduce tumour growth (Nygren, 2001). However, due to the potency of chemotherapy treatment and non-specificity of drugs, healthy cells are also damaged and

patients undergoing treatment may experience a range of debilitating side effects which can impact on quality of life (QOL) (Pachman et al, 2012).

Chemotherapy side effects

Although side effects of chemotherapy are numerous, one of the most common is cancer-related fatigue: a fatigue that is disproportional to activity and is not relieved by rest. The underlying pathophysiology for cancer-related fatigue remains poorly understood although research suggests that the mechanisms that cause fatigue are multifaceted. These mechanisms can include the cancer itself, the cancer treatment itself, immune response dysregulation, inflammation, autonomic nervous system dysfunction, cognitive and behavioral symptoms, impaired physical function and cachexia (LaVoy, Fagundes & Dantzer, 2016; Saligan et al, 2015). Cancer-related fatigue occurs with most chemotherapy agents but with varying degrees of severity. The pattern of fatigue onset and severity differs between patients but often parallels the biologic activity of chemotherapy in the body (Schwartz et al, 2000; Wu, Dodd & Cho, 2008). For example, for a patient who has 1-week between chemotherapy infusions, fatigue severity may peak around days 2-3 when the chemotherapy is highly biologically active in the body and then fatigue slowly reduces by the end of the week as the chemotherapy is cleared from the body. However, due to the cumulative effect of repeated chemotherapy cycles, fatigue intensity remains elevated above pre-chemotherapy baseline levels and increases in intensity with each chemotherapy cycle (Kirkham et al, 2020). The consequences of fatigue can cause significant distress in patients as it can impact a patient's ability to work, to be involved in social activities and to maintain personal responsibilities (Hartvig et al, 2006).

Although fatigue is one of the most common chemotherapy side effects, many others have significant impact, notably cachexia, cardiotoxicity and chemotherapy-induced peripheral neuropathy (CIPN) that impact treatment completion and QOL. Cachexia (significant weight and muscle loss) occurs due to the cancer itself, as well as reduced appetite and physical activity associated with other treatment side effects. A significant clinical implication of cachexia is reduced treatment tolerance resulting in reduced treatment completion and increased risk of recurrence in some cancers (de Boer et al, 2017; Prado et al, 2011). Many chemotherapy agents are cardiotoxic and increase the risk of cardiovascular disease (Kinoshita et al, 2021). Anthracyclines and alkylating agents for example are known to be highly

cardiotoxic. The clinical presentations of cardiotoxicity can include changes in left ventricular ejection fraction, increased resting heart rate (HR) and shortness of breath (Kirkham et al, 2019). CIPN is another common side effect of some chemotherapy agents, such as taxanes and platinum-based agents, which clinically presents as numbness and tingling, pain, temperature sensitivity and reduced motor function in the peripheries.

The psychological toll for individuals with cancer is a complex experience, with distress caused by the diagnosis, treatment side effects and uncertainty. Distress can present as anxiety and depression which are seen in cancer patients at any stage of cancer treatment (Britzenhofe-Szoc et al, 2009; Jadoon et al, 2010). A cancer diagnosis can prompt feelings of uncertainty related to treatment outcomes and life outlook (Shaha et al, 2008). Treatment-related side effects can cause distress, and the functional limitations of high symptom burden can impact personal responsibility, social activities and work which can cause further distress (Cleeland, 2007; Erdogan Yuce, Doner & Muz, 2021; Hong et al, 2016). The functional limitations caused by physical treatment side effects can also contribute to feelings of isolation due to reduced involvement in social activities and reduced social support in some patients (Erdogan Yuce, Doner & Muz, 2021; Wang et al, 2024). Fear of recurrence is common in patients with cancer, with levels of fear of recurrence not different between cancer type or stage (Crist & Grunfeld, 2013).

The medical approach of treating side effects pharmacologically is also fraught as pharmacological treatments that reduce chemotherapy side effects often pose additional unfavourable side effects that can further impact on QOL (Pachman et al, 2012). Hence non-pharmacological treatments to reduce chemotherapy side effects and subsequently increase QOL are highly sought after.

Exercise and chemotherapy symptom burden

Exercise during cancer treatment is safe and beneficial for patients in both physical and psychological domains (Campbell et al, 2019). National bodies such as the Clinical Oncology Society of Australia (COSA), the American College of Sports Medicine (ACSM) and Exercise and Sport Science Australia (ESSA) recommend that all cancer patients participate in regular physical activity to enhance cancer care and improve survivorship (Campbell et al, 2019;

Cormie et al, 2018; Hayes et al, 2019). The current COSA guidelines for patients with cancer recommend avoiding inactivity and to work towards meeting 150-minutes moderate intensity aerobic exercise (40-60% heart rate reserve [HRR]) or 75-minutes of high intensity aerobic exercise (60-85% HRR) plus 2-3 resistance exercise sessions per week (Cormie et al, 2018).

Physical and psychological benefits

There is compelling evidence that exercise during cancer treatment reduces many chemotherapy side effects and subsequently improves health-related QOL (Cormie et al, 2017; Mishra et al, 2012). The ACSM Roundtable in 2019 demonstrated strong evidence that exercise can reduce fatigue, physical function, anxiety, depressive symptoms and health-related QOL (Campbell et al, 2019).

Cancer-related fatigue is significantly reduced by combined aerobic plus resistance exercise and has a greater effect on reducing cancer-related fatigue compared to pharmaceutical options (Brown et al, 2011; Hilfiker et al, 2018; Mustian et al, 2017). Regarding intensity, moderate-to-vigorous intensity aerobic and moderate-to-vigorous intensity resistance training produce the greatest reductions in fatigue (Brown et al, 2011; Campbell et al, 2019). However, the mechanisms by which exercise improves fatigue are still unclear. It has been suggested that increasing aerobic capacity and muscle strength can improve fatigue by reducing the relative intensity of activities of daily living. Additionally, exercise can reduce inflammation by increasing anti-inflammatory markers. Biologically, chronic inflammation (expressed in biomarkers such as C-reactive protein, elevated white blood cells, elevated T-cells, increased tumour necrosis factor [TNFa] and interleukin 6) can occur due to the cancer itself as well as in response to healthy cell death from chemotherapy treatment (Fontvieille et al, 2024; LaVoy, Fagundes & Dantzer, 2016). This chronic inflammation can contribute to fatigue (Fontvieille et al, 2024; LaVoy, Fagundes & Dantzer, 2016). Immune function dysregulation due to treatment may also contribute to fatigue, specifically reduced activity of natural killer cells and cytotoxic T cells (Fontvieille et al, 2024; LaVoy, Fagundes & Dantzer, 2016). Exercise reduces inflammation by increasing anti-inflammatory cytokines (such as increasing IL10), reducing inflammatory cytokines (such as TNFa) and modulating the immune system (by increasing natural killer cell and T cell activity) subsequently improving fatigue (Fontvieille et al, 2024).

Resistance exercise can mitigate cachexia by increasing muscle mass and has been suggested to thereby increase treatment tolerance and improve clinical outcomes (Catala-Vilaplana et al, 2025; Prado, Birdsell & Baracos, 2009). Combined aerobic and resistance exercise has been shown to provide a cardioprotective effect by increasing cardiovascular fitness to prevent chemotherapy-related declines in cardiac function (Foulkes et al, 2023). Combined aerobic plus resistance training as well as fine motor exercises and balance training can improve CIPN symptoms such as numbness, tingling and cold sensitivity by reducing inflammation and improving sensory pathways (Kleckner et al, 2018; Streckmann et al, 2024).

Exercise training has a dose-response relationship with improved depressive symptoms in patients undergoing treatment, specifically a higher volume of aerobic exercise elicits greater improvement in depressive symptoms (Brown et al, 2012; Campbell et al, 2019; Zhang et al, 2025). Aerobic training can also reduce anxiety symptoms, and the effects are augmented when exercise is performed in a supervised setting (Campbell et al, 2019).

Clinical benefits

In addition to improving side effects, exercise has important clinical benefits both indirectly through side effect management and directly. Patients who experience significant side effects may have a treatment dose reduction resulting in the optimal prescribed chemotherapy dose not received. Exercise can increase tolerance to chemotherapy treatment by reducing chemotherapy side effects and subsequently enabling patients to receive the full planned doses of chemotherapy (Courneya et al, 2007; van Waart et al, 2015). A retrospective study by Wonders et al (2023) demonstrated that patients who exercised throughout treatment had less frequent dose reductions and dose delays compared to patients who did not exercise suggesting that patients who exercised received more of the planned chemotherapy treatment. If exercise results in increasing the likelihood of receiving the full prescribed doses of treatment, the efficacy of treatment may be also increased; patients may have a more complete response to treatment, and reduced cancer-related mortality and recurrence risk (Catala-Vilaplana et al, 2025; Wonders, Schmitz & Harness, 2023). Towards this hypothesis, a systematic review showed that exercise during cancer treatment reduced cancer-specific mortality by 28-44% cancer and reduced recurrence by 21-35% (Cormie et al, 2017). However, this review included mostly observational studies with methodological limitations.

Recently the effects of exercise post active treatment on cancer recurrence and mortality have been explicitly demonstrated. Courneya and colleagues (2025) published a large randomised controlled trial of 889 colorectal patients who after completing adjuvant chemotherapy were randomised to 3-years structured exercise or health education intervention groups. Disease-free survival was the primary outcome. Recreational physical activity and cardiorespiratory fitness were assessed at baseline, 6-months, 1-year, 2-years and 3-years, and QOL was assessed every 6-months for 3-years and at years 4 and 5. The results demonstrated that participants in the exercise group had a greater cardiorespiratory fitness and physical function at 3-years compared to the health education group. Participants in the exercise group had extended disease-free survival after 1-year and continued to have a reduction in the relative risk of death by 37% at 5-years follow up. Additionally, exercise reduced the risk of a new primary cancer or recurrence by 28%.

Tumour microenvironment

To understand the mechanisms of how exercise might affect treatment of cancer with chemotherapy we must first recognise the role of the tumour microenvironment that influences treatment efficacy. Cancer is the collection of abnormal and mutated cells that divide rapidly and proliferate. The collection of these abnormal cells can form a solid mass which is known as a tumour. The tumour microenvironment is the cellular environment formed as the tumour grows which includes blood vessels, signaling molecules, immune cells and the extracellular matrix, all of which dictate the structural and functional features of the tumour and can influence the growth and metastatic potential of the tumour (Labani-Motlagh, Ashja-Mahdavi & Loskog, 2020).

<u>Blood vessels</u>

As cells in a tumour continue to proliferate, the solid tumour mass grows and a vascular network is formed that supplies oxygen and nutrients to the tumour. Characteristically, this vascular network is unevenly distributed throughout the tumour with areas of high blood vessel density and areas of low blood vessel density (Dewhirst & Secomb, 2017; Fukumura & Jain, 2007, Jain 1990; Wiggins et al, 2018). The blood vessels themselves are also immature and dysfunctional in many ways, due to the high rate of uncontrolled cell proliferation which

prevents organised capillary net formation (Bielenberg & Zetter, 2015; Petrova et al; 2018, Wiggins 2018,). They show an uneven distribution of smooth muscle and consequently do not have a normal vasoconstriction response capacity (McCullough et al, 2014; Siemann & Horsman, 2015). Vessels have high permeability due to irregular endothelial cell distribution, this allows plasma to leak into the tumour interstitial space, and can increase interstitial fluid pressure (Horsman & Vaupel, 2016; Siemann & Horsman, 2015; Wiggins et al, 2018). This pressure increase is often found in the tumour centre with less pressure around the tumour borders and in the surrounding host tissue (Wu et al, 2013). The increased interstitial fluid pressure in the tumour centre leads to the collapse and abnormal compression of vessels, exacerbating the uneven and dysfunctional blood vessel network (Horsman & Vaupel, 2016; Wu et al, 2013).

Hypoxia

The consequences of the abnormal vasculature are functional abnormalities. The combination of heterogenous vascular distribution, the dysfunctional blood vessel structure, poor vascular permeability and high interstitial pressure results in areas of reduced perfusion (Siemann & Horsman, 2015). Areas of tumour that are closer to perfused vessels will have greater oxygen diffusion, however, areas of tumour that are further from perfused vessels will have limited diffusion of oxygen from the blood, this is particularly found in the tumour centre (Schumacher et al, 2021; Wiggins et al, 2018). The resultant effect is hypoxic regions within the tumour.

In response to hypoxic conditions, hypoxia-inducible factors (HIFs) such as subtypes HIF1a and HIF2a are expressed. In healthy tissue, these proteins function to regulate glucose metabolism to maintain homeostasis (Semenza, 2012). However, in the tumour microenvironment, these proteins can also promote cancer progression, through accelerated growth and metastasis (Petrova et al, 2018; Wicks & Semenza, 2022). Increased HIF expression, particularly the subtype HIF1a, can increase gene expression of vascular endothelial growth factor (VEGF). VEGF stimulates angiogenesis and increases perfusion (La Gory & Giaccia, 2016; Tang et al, 2004,). However increasing angiogenesis only compounds the problem of a disorganised tumour microenvironment and accelerates tumour growth by increasing the availability of metabolites to the tumour (Bielenberg & Zetter, 2015; LaGory & Giaccia, 2016; Niu & Chen, 2010; Petrova et al, 2018; Wiggins et al, 2018,). Furthermore, angiogenesis plays a vital role in

increasing risk of metastasis. A more developed tumour vascular network can provide greater opportunity for tumour cells to enter the body's circulation and spread to distant organs (Bielenberg & Zetter, 2015). Indeed, the clinical argument for reducing VEGF expression in tumours is strong and there are currently anti-VEGF pharmacological therapies used in treatment to suppress tumour blood supply and subsequent nutrient supply to reduce tumour growth and potential metastasis (Niu & Chen, 2010; Schumacher et al, 2021).

The hypoxic microenvironment may additionally cause a therapeutic barrier for intravenous drug delivery to the tumour (LaGory & Giaccia, 2016). The areas of low blood vessel density and subsequent poorer diffusion, particularly in the tumour centre, result in reduced routes for blood flow and therefore intravenous drug treatments cannot reach the entire tumour (Siemann & Horsman, 2015). This may result in poorer treatment efficacy and poorer patient prognosis (Siemann & Horsman, 2015).

Exercise in healthy tissue

To discuss the potential effects of the physiological response to exercise in tumours, we must first describe the changes that occur during exercise both acutely and chronically in healthy tissue.

In healthy tissue, blood is supplied to tissues and organs based on oxygen and energy requirements and the need to remove waste including carbon dioxide and H^+ ions. As demands change, blood flow is changed through feedback control loops. The control of blood flow to tissues is achieved through changes in constriction and dilation of blood vessels, primarily in arterioles and capillary beds. Healthy blood vessels have smooth muscle layers that can dilate and constrict to alter blood flow volume into tissue. Vessel vasodilation is controlled by release of local vasodilator signalling molecules from skeletal muscle and endothelial cells to increase blood flow to active tissue and organs (Clifford & Hellsten, 2004). Vessel vasoconstriction is controlled through sympathetic nervous system activation and results in shunting blood away from inactive tissue (Buckwalter & Clifford, 2001).

During aerobic exercise, the work of muscle contractions requires significant increases in metabolic energy pathways. Oxidative metabolism requires oxygen delivered to the skeletal muscle and generates carbon dioxide that must be removed (through the lungs via the blood). (Brooks, 2005). The generation of protons (H^+) in metabolic pathways changes the pH of cells and blood and must be buffered to protect protein integrity. The response to these stimuli is facilitated by activation of the sympathetic nervous system and the release of vasodilators in active tissue. This controls the redistribution of blood flow shunting of blood away from inactive tissue and increasing flow in active muscle. However, redistribution of blood towards active tissue does not alone meet the significant increase in demand for oxygen and is paralleled by additional cardiovascular responses to increase overall blood flow.

Cardiac output (CO) is the total amount of blood pumped by the heart to the body and is the result of changes HR and stroke volume (SV) (CO = SV x HR). Aerobic exercise stimulates a sympathetic response and decreases parasympathetic activation which results in increased HR and cardiac contractility. When combined with increased blood being returned to the heart (affected principally by the action of the skeletal muscle pump), increased contractility and increased end-diastolic volume result in increased SV. CO is therefore increased, and the enhanced rate of blood flow through vessels, delivery of oxygen and fuel and wase removal, facilitates the maintenance of homeostasis in response to the increased metabolism demanded by exercise. Additionally, CO and total peripheral resistance (TPR) interact to alter mean arterial pressure (MAP) (MAP = CO x TPR). As local skeletal vessels vasodilate, there is decreased vascular resistance within the vessel and this contributes to decreases in total peripheral resistance. Therefore, the increase in MAP driven by CO increase is offset by TPR decreases, resulting in relatively lower increases in MAP.

With repeated bouts of exercise, training effects are observed in skeletal muscle. Peripherally, training effects in skeletal muscle include hypertrophy of muscle fibres and vascular remodelling, including increased activity of VEGF. Through repeated bouts of exercise and subsequent increase in VEGF expression, there is an increase in the number of capillaries to supply blood to active tissue. This provides a means to transport increased blood flow volume to tissue to meet oxygen requirements. There are other physiological and metabolic adaptations that occur with repeated exercise bouts including mitochondrial changes and fuel use changes, although these adaptions fall outside the scope of this thesis.

Cardiac remodeling is also seen with repeated bouts of exercise due to sustained increased volume and pressure of blood flow. Training effects of increased left ventricular wall thickness and ventricular volume are seen (Pluim et al, 2000). The resultant effect is increased SV and subsequently increased total blood flow capacity (Brooks, 2005).

Acute vs chronic changes in tumour vascularisation

It is possible that both acute and chronic exercise may modulate the dysfunctional tumour microenvironment. Through the same mechanisms and adaptations that occur with exercise in healthy tissue, such as increased angiogenesis and blood flow, exercise could potentially improve clinical outcomes through greater delivery of intravenous treatment (He et al, 2024). If exercise (alongside treatment) can modulate tumour vasculature through changes in microvessel number, structure and function, then perfusion can be increased (and subsequently hypoxia can be reduced) and provide a means to transport more of the intravenous treatment to the tumour (Wiggins et al, 2018).

Acute exercise

A single bout of exercise may acutely improve blood flow in tumours found in both active and inactive tissue (Jia et al, 2021). In active tissues during exercise, blood flow increases, likely facilitating greater blood flow to tumours located there. This may be exaggerated by an effect called vascular co-option which refers to tumours hijacking the pre-existing vasculature of the host tissue to increase vascularisation of the tumour without the need for angiogenesis (Cuypes et al, 2022; Donnem et al, 2013; Garcia et al, 2016; Ribatti & Pezzella, 2022). So, in highly vascularised host tissue, such as the lungs, vascular co-option may result in increased number of blood vessels leading to the tumour that provide a pathway for transportation of blood to the tumour with increased CO from acute exercise.

In healthy host tissue during exercise, sympathetic nervous system activation results in smooth muscle vasoconstriction in the absence of vasodilatory signals, resulting in reduced blood flow in inactive tissue. However, due to the dysfunctional tumour vasculature, blood flow to tumours in inactive host tissue may be increased by exercise facilitated by increased CO. Unlike in

healthy tissue, blood vessel dysfunctionality in tumours results in inability to vasoconstrict with sympathetic stimulation due to the uneven smooth muscle distribution. When increased overall CO during exercise is coupled with this lack of vasoconstriction response in tumours, the resultant outcome is a potential increase in total blood flow to the tumour despite host tissue that is less active during exercise.

Table 1.1 summarises the preclinical and clinical evidence for the effects of acute exercise on tumour blood flow, vascularisation and hypoxia.

TABLE 1.1: Preclinical and clinical evidence for the effects of acute exercise on tumour blood flow, vascularisation and hypoxia

Author, year	Participants per group	Cancer type	Familiarisation	Intervention	Outcome measure	Measurement technique	Result
Preclinical studies							
McCullough, 2014	N=6-7 Copenhagen rats	Prostate: orthotopically injected	Mode: Treadmill Duration: 5 days Time: 5 minutes/day	Mode: Treadmill Time: 5 minutes Intensity: 60% maximal aerobic capacity	Blood flow Vascular resistance	Microsphere technique[1]	Blood flow ↑ ~200% Vascular resistance ↓ ~65%
McCullough, 2014	N=7-8 Copenhagen rats	Prostate: orthotopically injected	Mode: Treadmill Duration: 5 days Time: 5 minutes/day	Mode: Treadmill Time: 5 minutes Intensity: 60% maximal aerobic capacity	Patent blood vessels	Hoeschst-33342[2]	Number of patent blood vessels ↑
McCullough, 2014	N=7 Copenhagen rats	Prostate: orthotopically injected	Mode: Treadmill Duration: 5 days Time: 5 minutes/day	Mode: Treadmill Time: 60 minutes Intensity: 60% maximal aerobic capacity	Hypoxia	EF5[3]	Hypoxia ↓ ~50%
McCullough, 2014	N=6-8 Copenhagen rats	Prostate: orthotopically injected		Exposed in vitro to norepinephrine[4]	Vessel function	Vessel diameter change	Vasoconstriction ↓

Garcia, 2016	N=4-6 Dunning rats	Prostate: orthotopically and ectopically injected into rear flank	Mode: Treadmill Duration: 5 days Time: 5 minutes/day Intensity: 15/min at 0° incline (50-60% maximal aerobic capacity)	Mode: Treadmill Time: 5 minutes Intensity: 15/min at 0° incline (50-60% maximal aerobic capacity)	Blood flow Vascular resistance	Microsphere technique	Blood flow ↑ ~180% in orthotopic tumour Blood flow ↓[6] in ectopic tumour Vascular resistance ↓ in orthotopic tumour
Garcia, 2016	N=6-14 Dunning rats	Prostate: orthotopically and ectopically injected into rear flank		Exposed in vitro to norepinephrine[4]	Vascular reactivity		Vasoconstriction ↓ in orthotopic and ectopic tumours
Elming, 2023	N=11 CDF1 mice	Breast: orthotopically injected	Mode: Treadmill Duration: 4-5 days Time: 15 minutes/day Intensity: 0-18m/min	Mode: Treadmill Time: 30 minutes Intensity: 6m/min (low), 12m/min (moderate) or 18m/min (high)	Hypoxia Perfused blood vessels	Pimonidazole hydrochloride[5] Hoeschst-3342[2]	Hypoxia ↓ 37% after high intensity Hypoxia ↔ after moderate or low intensity Perfused vessels ↔ at all intensities
Clinical studies							
Djurhuus, 2022	N=30 total in a 2:1 ratio for exercise:no exercise	Prostate		Mode: Cycling Time: 4 x 1 minute Intensity: 100% W_{peak}	Hypoxia Microvessel density	Pimonidazole hydrochloride[5] CD31[7]	Hypoxia ↔ Microvessel density ↔

[1]The microsphere technique involves injection of radiolabeled microspheres into the bloodstream via the aorta and caudal tail artery. The microspheres become lodged in tissue and the microspheres are quantified in harvested tissue after euthanasia

[2]Hoeschst-3342 is a nucleic acid stain that can quantify perfused vessels

[3]EF5 is an imaging marker that binds to hypoxic cells

[4]Norepinephrine produces a physical stimulus to increase intraluminal pressure

[5]Pimonidazole hydrochloride is an immunohistochemical staining to quantify tumour hypoxia

[6]Host tissue blood flow in the prostate was unchanged and host tissue blood flow in the skin (which was the host tissue for ectopic tumours) was reduced

[7]CD31 is an immunohistochemical staining to quantify microvessel density

Preclinical evidence

There have been three published preclinical studies investigating the impact of acute aerobic exercise on tumour vasculature. McCullough and colleagues (2014) and Garcia and colleagues (2016) both found that acute moderate intensity exercise increases blood flow, reduces hypoxia and reduces vascular resistance in orthotopic tumours. However, the findings from Elming et al (2023) contrasted with the findings of McCullough et al (2014) and Garcia et al (2016); the authors found that there was no significant differences in hypoxia in mice that ran at low or moderate intensity compared to no exercise and there was no significant difference in the number of perfused vessels at any intensities. The authors noted that the differences in animal and tumour model assessed as well as differences in the measurement technique of hypoxia may explain the findings of no change in hypoxia with moderate intensity exercise compared to the findings of McCullough et al (2014) and Garcia et al (2016). It is also plausible that the reason for this finding is that as overall blood flow volume increases with exercise (which is facilitated through an increase in CO), blood flow speed will also increase resulting in reduced capillary transit time and consequently there may be less opportunity for oxygen to be diffused from the blood into the tumour resulting in increased hypoxia (Ostergaard et al, 2013). Garcia et al (2016) also found that blood flow to ectopic tumours located in inactive host tissue reduced with exercise which highlights that blood flow to tumours is influenced by the vasoactive properties of host tissue.

Clinical evidence

Although there are no clinical studies reporting changes in tumour perfusion with acute exercise, one clinical study has investigated the effects of an acute high intensity interval exercise bout on tumour hypoxia and microvessel density in prostate tumours (Djurhuus et al, 2022). In this randomised controlled trial, n=30 patients with prostate cancer and scheduled for a radical prostatectomy were randomised in a 2:1 allocation ratio to either a single bout of high intensity interval exercise or no exercise, The exercise group performed four high intensity interval cycles (1-minute at 100%W_{peak} and 3-minutes active recovery at 30% W_{peak}) on a stationary bike one day prior to surgery. The control group did not exercise. Hypoxia and microvessel density were measured in tumour tissue samples taken during the prostatectomy the day after exercise. Pimonidazole was administered orally prior to surgery in both groups to measure hypoxia in the tumour tissue sample and microvessel density was assessed by CD31 staining of the tumour tissue sample. The authors found that there was no difference in hypoxia

or microvessel density between groups. It is possible that acute blood flow changes occurred during exercise but that these changes were not sustained at time of assessment which occurred the day after exercise. Therefore, a clinical gap remains in the literature. Chapter 3 of this thesis aims to provide clinical evidence for the effects of acute aerobic exercise on tumour blood flow in humans.

Chronic exercise

Chronic exercise in healthy tissue increases VEGF expression to induce angiogenesis and the formation of new blood vessels. Given there is an overall increased VEGF expression with chronic exercise, it is possible that exercise may induce similar angiogenic changes in tumours to that of its host tissue. This may result in normalisation of the tumour microenvironment and in the setting of an acute exercise bout, when coupled with an increase in CO, could result in overall increased tumour perfusion. Preclinical studies have investigated the effect of chronic aerobic exercise on the tumour microenvironment which is examined in detail in Chapter 2 of this thesis. I specifically review the evidence for changes in tumour hypoxia, vascularisation and blood flow in response to exercise training. In addition, I have summarised significant additions to the literature in Table 1.2 which have been published since the completion of the systematic review.

TABLE 1.2: Additional preclinical and clinical evidence for the effects of chronic exercise on tumour blood flow, vascularisation and hypoxia

Author, year	Participants per group	Cancer type	Familiarisation	Intervention	Outcome measure	Measurement technique	Result
Preclinical studies							
Esteves, 2023	N=14 C57BL/6 mice	Prostate: ectopically injected into rear flank		Mode: Voluntary wheel running Duration: 28 days	Microvessel density Vessel regularity	CD31[1] Vessel perimeters	Microvessel density ↑ More regularly shaped vessels
Xiao, 2023	N=6 BALB/c nude mice	Liver: ectopically injected into thigh	Mode: Swim Time: 30 minutes/day Duration: 1 week	Mode: Swim Frequency: 5 days/week Time: 30 minutes/day Duration: 4 weeks	Hypoxia Microvessel density	Pimonidazole hydrocholoride[2] HIF-1a expression CD31[1]	Hypoxia ↓ Microvessel density ↔ Vascular maturity ↑
Yan, 2023	N=6 C57BL/6 mice	Melanoma		Mode: Swim Frequency: 5 days on, 1 day off Duration: 17 days Intensity: 6 minutes per day (low) or 12 minutes per day (moderate)	Hypoxia	Pimonidazole hydrocholoride[2]	Hypoxia ↓ at low and moderate intensity[3]

Gomes-Santos, 2024	N=8-10 FVB mice	Breast: Orthotopically injected	Mode: Treadmill	Mode: Treadmill Frequency: 5 days on, 1 day off Time: 30 minutes/day Duration: 11 days Intensity: 30% maximal aerobic capacity (low), 60% maximal aerobic capacity (moderate) or 90% maximal aerobic capacity (high)	Microvessel density Tumour growth	CD31[1]	Microvessel density ↑ at low and moderate intensity Microvessel density ↔ at high intensity
Voltarelli, 2024	N=10-12 BALB/c mice	Colorectal: ectopically injected into upper flank	Mode: Treadmill Frequency: 5 days/week Time: 1 hour/day Duration: 30 days Intensity: 60% maximal velocity	Mode: Treadmill Frequency: 5 days/week Time: 1 hour/day Duration: 9 days Intensity: 60% maximal velocity	Hypoxia	Pimonidazole hydrocholoride[2]	Hypoxia ↓
Clinical studies							
Van Blarigan, 2015	N=572	Prostate		Self-reported questionnaires of physical activity[4]	Vessel size Microvessel density	CD34[5]	Vessel size ↑ and more regularly shaped with men with increased walking pace[6]
Jones, 2013	N=20[7]	Breast undergoing neoadjuvant chemotherapy		Mode: Cycling Frequency: 3 days/week Time: 20-45 minutes/day Duration: 12 weeks Intensity: 55-100% $VO2_{peak}$	Microvessel density Hypoxia Blood flow	CD31[1 8] NB100-479[9] PET scan	Microvessel density ↔ Hypoxia ↔ Blood flow ↓ 38%

[1]CD31 is an immunohistochemical staining to quantify microvessel density

[2]Pimonidazole hydrochloride is an immunohistochemical staining to quantify tumour hypoxia

[3]Did not compare hypoxia between exercise groups

[4] Questionnaires included questions about average time per week participating in sport, daily number of flights of stairs climbed and walking pace

[5]CD34 is an immunohistochemical staining that marks endothelial cells

[6]Walking pace was a proxy for cardiovascular fitness. Those with a faster walking pace had presumed greater cardiovascular fitness

[7]Only 5 participants had data in the exercise group and no data was reported in the control group for blood flow. Tumour biopsy was available on 5 participants per group due to pathologic complete response to chemotherapy

[8] Microvessel density in a tumour biopsy was assessed after 9 weeks of the intervention

[9] NB100-479 is a rabbit polyclonal antibody that binds to HIF1a markers to quantify hypoxia

Preclinical evidence

The additional preclinical studies as shown in the above table suggest that chronic exercise may reduce hypoxia and improve the structure and function of tumour vasculature. When added to the pre-existing literature reviewed in Chapter 2, it is possible that exercise may impact hypoxia, vasculature and perfusion. However, variability in study methodology including different animal models, tumour types and exercise parameters may influence results when comparing studies.

Although there is some evidence for how exercise can alter tumour vasculature, animal models cannot be compared to humans. Exercise parameters in preclinical studies may have greater flexibility and may not reflect the strict exercise parameters performed in clinical populations. For example, it is difficult to quantify the intensity or specific duration of voluntary wheel running so extrapolating these preclinical results to a clinical population may be inaccurate. Environmental conditions may induce a stress response in animals which may impact on the immune response that contributes to the development of the tumour vasculature (Thaker et al, 2006). Among others, housing conditions including the number of animals per cage and voluntary wheel running vs forced treadmill running may cause stress in rodents which would not be seen in clinical populations (Bartolomucci, 2007). Additionally, measures of vessel perfusion and hypoxia are measured in animals after euthanasia which cannot be replicated in humans.

Clinical evidence

There have been few clinical studies investigating the effects of chronic exercise on the tumour vasculature. Van Blarigan et al (2015) found that men who regularly walked as a brisk pace (as a proxy for cardiovascular fitness) had more regularly shaped vessels in the prostate tumour that were larger in size in excised tumour tissue. Jones et al (2013) assessed tumour blood flow (using PET/CT), hypoxia (measured by HIF1a) and microvessel density (measured by CD31 staining after tumour excision) in breast cancer patients who performed aerobic exercise for 12-weeks. The study showed an increase in tumour blood flow and no difference in hypoxia or microvessel density. However due to the limited data, there may not have a large enough sample size to preclude meaningful conclusions from this study. Therefore, further clinical evidence is required to support the preclinical findings.

Tumour growth

The hypothesis of this thesis is that by increasing tumour angiogenesis through chronic exercise, there is a greater number of vessels that deliver more blood and reduce hypoxia, subsequently more intravenous chemotherapy can be delivered to the tumour to reduce tumour growth. However, it can also be argued that increasing angiogenesis through chronic exercise may promote tumour growth by reducing hypoxia and increasing the availability of metabolites through increased blood flow. Several systematic reviews have been published that review the literature on the impacts of exercise on tumour growth.

Ashcraft et al. (2016) performed a systematic review and meta-analysis that summarised the effects of aerobic exercise on tumour growth and progression (via metastases) in preclinical models. Fifty-three articles were included in the review and 33 articles assessed tumour growth in mice or rats. Tumour types included intestinal, liver, breast, prostate, renal, lung, sarcoma, lymphoma, leukemia and melanoma. Exercise types included forced treadmill running, voluntary wheel running and swimming. The authors found that exercise reduced tumour growth in 64% of the studies, exercise had no effect on 21% of the studies and exercise increased tumour growth in 9% of the studies. However, the authors noted that the considerable heterogeneity of methodology and data reporting precluded whether exercise influences tumour growth.

Figueira et al. (2018) reviewed the literature investigating the effects of exercise training on breast tumour outcomes such as growth, incidence and angiogenic tumour response in preclinical models. Twenty-eight articles were included in the review and 11 articles assessed tumour volume changes. Exercise modalities included voluntary exercise, free wheel running, motorised wheel running and forced treadmill running. The total duration of exercise ranged from 2 to 35 weeks. Of the 11 articles assessing tumour volume, eight studies demonstrated a reduction in tumour volume with exercise and three studies demonstrated an increase in tumour volume. The authors suggested that reduced tumour proliferation because of exercise may be a factor for reduced tumour volume. Low intensity exercise elicited the greatest reductions in tumour volume although the authors noted that the heterogeneity of exercise prescription may preclude determining the most effective exercise dose for significant tumour volume changes particularly when translating these results to a clinical population.

Eschke et al. (2019) performed a systematic review and meta-analysis examining the impact of aerobic exercise on tumour growth and tumour weight in mice and rats. Seventeen articles were included in the review. Tumour types included breast, liver, lung, melanoma and prostate. The exercise prescription ranged from 4 to 7 days per week for 2 to 35 weeks total either forced treadmill running, voluntary wheel running or swimming. The authors concluded that exercise reduced tumour growth and an exercise prescription of less than 10-weeks produced a greater tumour reduction than a duration of more than 10-weeks aerobic exercise. However, similar to Ashcroft et al, the authors acknowledged that the heterogeneity of study designs and the stress response of forced exercise in animals may affect tumour growth outcomes.

With the systematic reviews all favouring exercise reducing tumour growth, it is possible that performing exercise whilst receiving chemotherapy could augment the effects of chemotherapy and result in a greater tumour reduction response than with chemotherapy alone. Schadler et al (2016) had mice with pancreatic tumours run on a treadmill for five consecutive days for 45-minutes at 12m/min (moderate intensity). Mice were divided into chemotherapy alone, exercise alone or exercise plus chemotherapy. Although exercise alone had no significant effect on tumour growth, exercise plus chemotherapy inhibited tumour growth significantly compared to chemotherapy alone. The authors concluded that the mechanism for tumour growth inhibition with exercise was the normalisation of blood vessels including increased number of vessels and increased vessel length that provided greater means for drug delivery to the tumour.

Intra-infusion exercise

Despite the robust evidence of the benefits of exercise during treatment to manage side effects and the publication of exercise guidelines, less than 50% of patients with cancer meet these physical activity guidelines due to barriers such as side effects of chemotherapy (specifically fatigue), lack of support and exercise education, and lack of time (Clifford et al, 2018; Elshahat, Treanor & Donnelly, 2021; Galvao et al, 2015; LeMasters et al, 2014). Hence, there is a potential vicious cycle whereby physical inactivity due to physical barriers, such as side effects, may cause an increase in symptom severity which may result in an additional barrier to exercise adherence. Therefore, strategies are needed to overcome these barriers and to promote exercise participation and adherence in patients with cancer.

One strategy to overcome the barriers of time and lack of support is intra-infusion exercise, that is exercise performed concurrent to intravenous chemotherapy infusion. Patients who undergo chemotherapy can sit in the treatment chair for many hours, which provides an opportunity to overcome time and access barriers and to perform exercise supervised by a clinical exercise professional. Given that it is well established that exercise before and after treatment can improve side effects, it is possible that exercising during the infusion can also improve side effects whilst overcoming barriers to exercise. Supervised intra-infusion exercise could also overcome barriers of lack of support and exercise education by providing an opportunity for exercise support and education to encourage physical activity behavioural change outside of the infusion. Although beyond the scope of this thesis and the intra-infusion exercise intervention in its infancy, it should be recognised that there is significant complexity in behaviour change frameworks for successful exercise interventions. These frameworks should be utilised by clinical exercise professionals to guide patient-led discussions to establish goals, and identify perceived barriers and motivational factors that influence exercise behaviour. For example, successful behaviour change frameworks commonly used include social cognitive theory, transtheoretical model and theory of planned behaviour (Liu et al, 2022). These frameworks are likely to provide the best success in establishing long-term and autonomous exercise behaviour change outside of the infusion

Our team have previously published a pilot study investigating the safety and feasibility of intra-infusion exercise (Thomas et al, 2018). In this randomised crossover study, 10 participants with mixed primary cancer performed a single bout of 20-minutes of low intensity intra-infusion cycling using a foot pedal placed in front of the chemotherapy chair. There were no adverse events and no impact on chemotherapy symptoms measured using ESAS-r. Participants reported that intra-infusion exercise was no less comfortable and no more difficult than chemotherapy alone, and that boredom was significantly reduced. The conclusion of the study was that a bout of low intensity exercise for 20-minutes intra-infusion did not have any safe issues and was feasible in the chemotherapy infusion suite.

Since the publication of the pilot study, there have been two other published studies and one published case study that have assessed low intensity intra-infusion exercise. Kerrigan et al

(2022) conducted a pilot study investigating the safety and feasibility of low intensity intra-infusion exercise. Patients with stage I-III breast cancer who were involved in the ExCITE trial were invited to participate in the intra-infusion trial. Participants were allocated to n=5 intra-infusion exercise or n=9 usual chemotherapy infusion based on availability of staff to supervise the intra-infusion exercise. The intra-infusion group performed light-intensity cycling during infusion (30-40% HRR, ≤11 rate of perceived effort [RPE]) for 10-20 minutes. Both groups performed additional supervised exercise outside of the infusion as part of the ExCITE trial. Adverse events were recorded during the intervention and for 3-weeks following the intervention. There were fewer clinically meaningful adverse events, such as anaemia and fever, in the 3-weeks after each infusion with exercise compared to infusions without exercise (12% vs 53%, respectively). Recently, Dantas et al (2025) performed a randomised crossover trial investigating the effects of low intensity intra-infusion exercise on mood. N=24 women with any stage breast cancer performed 20-minutes of low intensity intra-infusion exercise (20-30% HRR, RPE 3-6/10). The authors reported that there was no effect on mood although patients experienced a high level of pleasure and satisfaction with intra-infusion cycling. McLaughlin et al. (2019) published a case study of a patient with stage 3 pancreatic cancer who performed six bouts of intra-infusion cycling every 2-weeks for 40-minutes at 60% HR maximum. The authors reported 100% adherence to the intra-infusion exercise and no adverse events. This study concluded that 40-minutes of moderate intensity intra-infusion exercise in a patient with pancreatic cancer is safe and feasible.

Although there have been no reported safety or feasibility issues with moderate intensity intra-infusion exercise, the physiological impacts of intra-infusion exercise on chemotherapy delivery and subsequently tumour response is not yet determined. In accordance with preclinical evidence for the effects of exercise on tumour vascularisation, it is possible that an increase in CO with an acute bout of exercise during infusion could further enhance tumour perfusion and acutely increase intravenous chemotherapy delivery at the time of infusion. This could have significant clinical implications by increasing chemotherapy efficacy and subsequently decreasing tumour growth. However, the most effective exercise dose for tumour perfusion changes remains unclear.

Thesis aims and outline

Intra-infusion exercise has the potential to be included as part of cancer care in the future if logistical and physiological benefits are demonstrated. However, there are gaps in understanding whether intra-infusion exercise impacts treatment-related side effects. Intra-infusion also has the potential to increase chemotherapy delivery and subsequent efficacy through changed tumour perfusion. However, there are gaps in the clinical translation of preclinical evidence for the effects of exercise on tumour vasculature. Therefore, further research is needed to determine whether exercise changes tumour blood perfusion in humans and what exercise dose produces the greatest perfusion effect.

The aims of this thesis are:

1) To examine the existing research that investigates the impact of chronic exercise on tumour vasculature (Chapter 2)

2) To determine if exercise intensity affects tumour blood flow (Chapter 3)

3) To determine the physiological response of exercise during chemotherapy (Chapter 4)

4) To determine if intra-infusion exercise affects chemotherapy side effects and physical activity behaviours (Chapter 5)

Chapter 6 will include a discussion and concluding remarks.

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Chapter 2

The effect of aerobic exercise on tumour blood delivery: a systematic review and meta-analysis

ABSTRACT

Background: Tumour blood vessels are structurally and functionally abnormal resulting in areas of hypoxia and heterogenous blood supply. Aerobic exercise may modulate tumour blood flow and normalise the tumour microenvironment to improve chemotherapy delivery. This systematic review and meta-analysis aimed to evaluate the effects of aerobic exercise on tumour hypoxia, vascularisation and blood flow.

Methods: Four online databases were searched. Preclinical and clinical randomised controlled trials examining the effects of aerobic exercise training on hypoxia, vascularisation or blood flow in solid tumours were included. Risk of bias was assessed and a meta-analysis performed.

Results: Seventeen preclinical studies and one clinical study met criteria. Eleven studies assessed hypoxia, 15 studies assessed vascularisation and seven evaluated blood flow. There was large variability in measurement methods, tumour type and exercise interventions. Overall risk of bias was unclear in clinical and preclinical studies largely due to poor reporting. There was no significant effect of exercise on hypoxia (SMD = -0.17; 95% CI = -0.62, 0.28; I^2 = 60%), vascularisation (SMD = 0.18; 95% CI = -0.28, 0.64; I^2 = 68%) or blood flow (SMD = 0.01; 95% CI = -0.59, 0.61; I^2 = 63%).

Conclusion: There is heterogeneity in methodology resulting in evidence that is inconsistent and inconclusive for the effect of exercise on hypoxia, vascularisation and blood flow. Most evidence of aerobic exercise effects on tumour blood flow is in animal models, with very limited evidence in humans.

KEYWORDS: exercise, tumor, hypoxia, vascularisation, blood flow

INTRODUCTION

Cancer incidence worldwide in 2016 was 17.2 million cases, with a 28% increase observed between 2006 and 2016 (Global Burden of Disease Cancer et al, 2019). There are many modifiable risk factors for cancer development including obesity and physical inactivity which combined contribute to 25% of cancer incidence (De Boer et al, 2017; Colditz & Wei 2012). Exercise during cancer treatment is recognised as an important adjunct to cancer treatment, with significant benefits on QOL and reduction of treatment side effects (Cave 2018, Cormie et al, 2017; Mishra et al, 2012; Tomlinson et al, 2014). It has been proposed that exercise may also induce adaptations to tumour vasculature and thus stimulate benefit to clinical outcomes of cancer treatment (Wiggins et al, 2018).

Unlike in healthy tissues, where blood vessels typically run in parallel, blood vessels in tumours have an unstructured distribution resulting in tumour centres that lack blood vessels. This heterogeneous distribution is compounded by functional abnormalities such as high permeability, resulting in heterogenous blood flow (Dewhirst & Secomb, 2017; Jain, 1990). These structural and functional features create a hypoxic environment, proposed to suppress immune function and increase metastasis, leading to poorer patient prognosis (Kumar 2014, Wiggins 2018).

Acute aerobic exercise increases total blood flow to healthy active tissue (e.g. contracting skeletal muscle) through the combination of increased CO, increased blood pressure (BP) and local vessel vasodilation, with vasoconstriction reducing or maintaining blood flow to inactive tissues (Brooks, 2005; Wiggins et al, 2018). Arterioles in tumours are poorly developed and lack functional smooth muscle, and subsequently have reduced myogenic vasoconstriction response at high pressures such as during aerobic exercise (McCullough et al, 2014; Siemann & Horsmann, 2015). This inability to vasoconstrict suggests that exercise-induced increased CO would drive an increase in tumour perfusion through increased blood flow (McCullough et al, 2014).

Repeated bouts of aerobic exercise cause vascular adaptations in healthy tissue including angiogenesis and decreased resistance (Brooks, 2005). These effects have been proposed to be paralleled in tumours, such that exercise training may cause adaptations that modulate tumour

blood flow through increased blood vessel density and improved organisation and vessel function (Siemann & Horsmann, 2015; Wiggins et al, 2018). If these adaptions occur, aerobic exercise training may normalise the tumour microenvironment and facilitate increased blood flow and reduced hypoxia, which may have benefits for reducing cancer progression. Given many cancer treatments such as chemotherapy are administered intravenously, exercise training effects of increased blood flow and improved vessel function have the additional potential for improving the delivery and therefore efficacy of such treatments (McCullough et al, 2014).

The purpose of this systematic review is to: i) summarise the effects of aerobic exercise training on tumour hypoxia, vascularisation and blood flow and ii) evaluate the methodological rigour of this literature.

METHODS

This systematic review and meta-analysis is reported in accordance with the Preferred Reporting items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. This review was registered with the PROSPERO Register of Systematic Reviews (CRD42020159201).

Eligibility criteria

Trials were included if they: 1) were a peer-reviewed journal full text article in English; 2) used a randomised or quasi-randomised study design with a control group; 3) involved humans or animals with a solid malignant tumour; 4) the intervention group performed repeated bouts of any type of aerobic exercise of ≥ 2 sessions; 5) the control group performed no structured or unstructured aerobic exercise; 6) measured hypoxia, vascularisation, blood flow or indicators thereof.

Search strategy and study selection

We searched Medline via OvidSP (1946-present), EMBASE via OvidSP (1947-present), Scopus (all years) and CINAHL Complete (all years) in September 2021 using the following search terms: “exercise” OR “physical activity” OR “exercise therapy” OR “aerobic exercise” AND

“neoplasm” OR “tumour” OR “tumor” OR “carcinoma” OR “cancer” OR “tumour microenvironment” OR “tumor microenvironment” OR “tumour vasculature” OR “tumor vasculature” AND “blood delivery” OR “blood flow” OR “vascularisation” OR “vascular function” OR “vascular remodeling” OR “hypoxia” OR “tumor hypoxia” OR “tumour hypoxia” OR “oxygenation”.

Two reviewers (CSL, JY) independently performed the initial screening by title and abstract based on the eligibility criteria. Full text versions of potential eligible studies were then assessed by two reviewers independently (CSL, JY). A third reviewer (KE) screened full text studies if there were disagreements between the researchers regarding eligibility.

Outcome measures and data extraction

Two reviewers (CSL, LR) independently extracted data and a third reviewer (KE) performed data extraction if there were disagreements between the researchers. Data was extracted for the three variables; hypoxia, vascularisation and blood flow. Hypoxia included any measure indicative of hypoxia as identified by the study author. Vascularisation included changes in vessel physiology and microvessel density. Blood flow included changes in perfusion. Data extracted was recorded as the change in mean values from baseline for each group, and standard error of the mean (SMD), standard deviation (SD) or confidence interval (CI) was also recorded. Study characteristics extracted included study details and design, recruitment source and method, participant results, experimental protocol, adherence and funding.

Quality Assessment

The included studies were assessed for internal validity using SYRCLE’s Risk of Bias Tool (Hooijmans et al, 2014) or Cochrane Risk of Bias Tool (Higgins et al, 2011). The SYRCLE Risk of Bias Tool is used to determine the internal validity of animal studies. The tool contains 10 questions relating to 6 different domains of bias; selection bias, performance bias, detection bias, attrition bias, reporting bias and other biases. Each entry scored as “no” (indicating high risk), “yes” (indicating low risk) or “unclear” (indicating unclear risk). Each study was assessed in its entirety irrespective of if the study had multiple outcome measures. Baseline characteristics included age, sex, tumour type, site of tumour injection and timing of tumour

induction prior to randomisation. Studies had to explicitly report study characteristics to assess the risk of bias. Housing allocation was also assessed in Domain 10, specifically if animals were housed individually and analysed individually.

The Cochrane Risk of Bias Tool is used to determine the internal validity of human studies (Higgins et al, 2011). The tool contains 5 questions that each covers a domains of bias; selection bias, performance bias, detection bias, attrition bias and reporting bias. Within each domain are signaling questions that draw relevant information from the study to determine risk of bias. The responses to the signaling questions are formulated in an algorithm to determine a judgement of "low risk", "some concern" or "high risk". The Risk of Bias Tool was used for each outcome measure if the study included multiple outcome measures.

Two researchers (CSL, LR) independently performed these assessments. A third reviewer (JY) performed assessment if there were disagreements between the researchers.

STATISTICAL ANALYSIS

Meta-analysis was performed for hypoxia, vascularisation and blood flow. Data from the outcome measures and indicators thereof were extracted as mean and SD. Data that was presented as SEM or CI were converted to SD. Data were included if they were quantitative and physiologically plausible. Relative values and fold changes were excluded if raw data was unable to be obtained. Negative values, such as for changes in hypoxia-inducible factor 1-alpha (HIF1α), were excluded as this is not physiologically feasible. For studies that had a range in sample size, the lower end of the sample size was used to avoid overpowering the study. The raw data can be found in the Supplemental Materials (Supplementary table 2.1).

The meta-analysis was performed in RStudio (R version 4.0.3) using a random effects model. Heterogeneity was assessed by I^2 and a subgroup analysis was performed on hypoxia, vascularisation and blood flow for animal, species, exercise mode, tumour location and study duration. The meta-analysis was controlled for the inclusion of multiple datasets that were

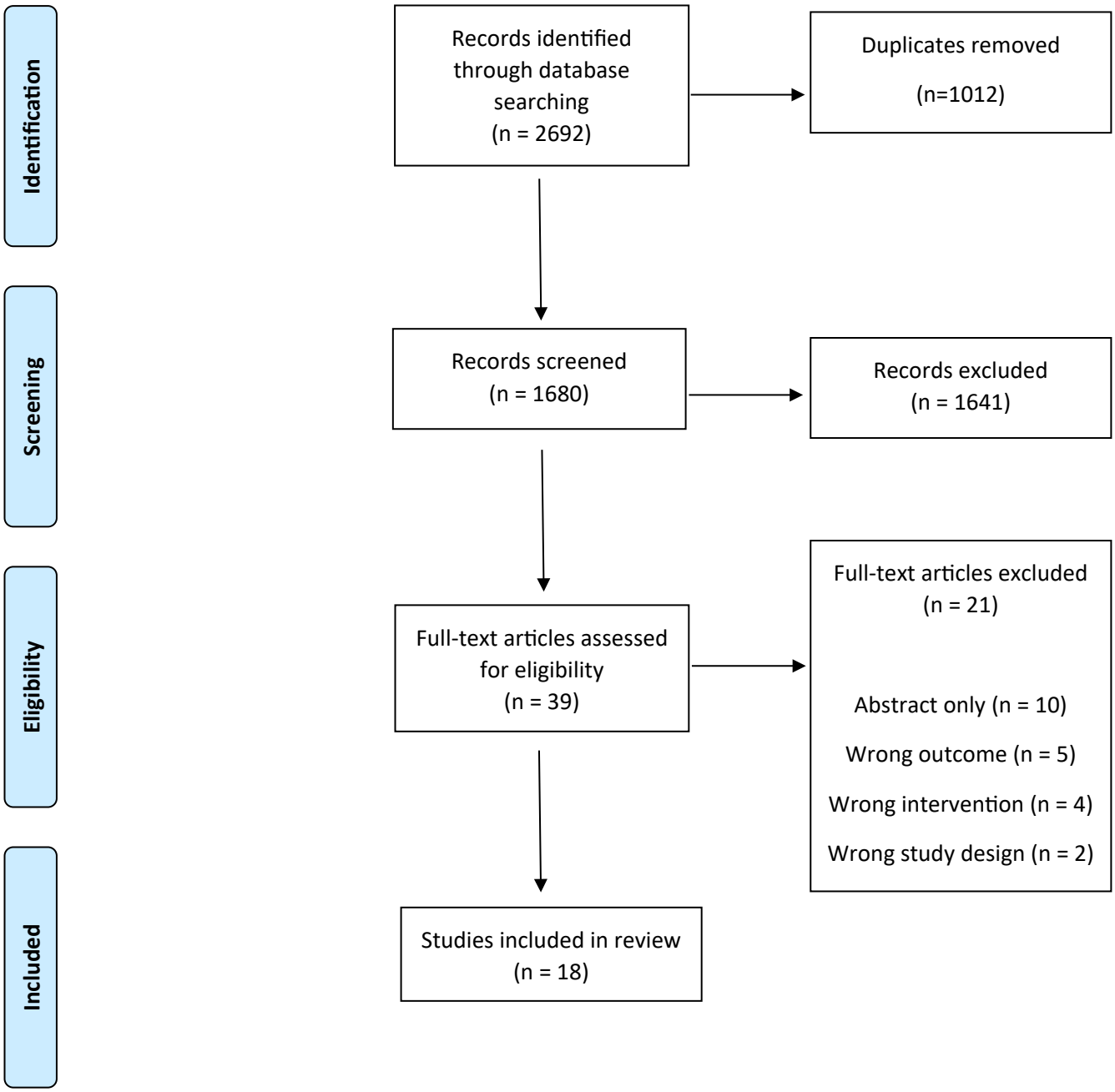
compared to the same control group within a single study using the following equation; $N_{corrected\ control} = N_{control}$/number of experimental groups (Vesterinen et al, 2014).

RESULTS

Study selection

The initial database searches yielded 2692 studies (Figure 2.1). After duplicates were removed, a total of 1680 studies were screened by title and abstract. Thirty-nine full text articles proceeded to further review. A total of 18 studies were deemed as suitable for inclusion in this review.

FIGURE 2.1: PRISMA flowchart



Study and participant characteristics

Details of study and participant characteristics of the included studies are presented in Table 2.1 and 2.2. Of the 18 studies included, 17 studies were preclinical and one was clinical. Only one study was published prior to 2010. Multiple studies assessed more than one outcome measure; 11 assessed hypoxia, 15 assessed vascularisation and seven evaluated blood flow. Of the 17 preclinical studies, 14 used mice and four used rats. Study duration ranged from five to 245 days. Exercise in the preclinical studies included both voluntary (n=6) and forced exercise (n=11). The six studies that used voluntary exercise adopted wheel running, performed daily or every second day. The remaining 11 studies used treadmill running of which nine studies were five times per week, two studies were daily and one study was every second day. One study used two different exercise frequencies in different groups. The one clinical study explored cycle training sessions three times per week at 55-100% VO_{2peak} in women with breast cancer.

TABLE 2.1: Study characteristics.

Variable	Preclinical trials (n=17)	Clinical trials (n=1)
Publication year		
2000-2009	1 (6)	0 (0)
2010-2021	16 (94)	1 (100)
Sample size		
≤20	7 (41)	1 (100)
21-49	7 (41)	0 (0)
≥50	3 (18)	0 (0)
Study duration		
<3 weeks	7 (41)	0 (0)
3-8 weeks	8 (47)	0 (0)
>8 weeks	2 (12)	1 (100)
Animal species ^		
Mice	14 (82)	-
Rat	4 (24)	-
Animal breed/strain		
BALB/c mice	5 (29)	-
Nude mice ^	3 (18)	-
C57BL/6 mice	3 (18)	-
Sprague-Dawley rat	2 (12)	-
Aythmic mice	2 (12)	-
ApoE-/- mice	1 (6)	-
Copenhagen rats ^	1 (6)	-
American Cancer Institute rats	1 (6)	-
Cancer type		
Breast *	9 (53)	1 (100)
Prostate	3 (18)	0 (0)
Pancreatic *	2 (12)	0 (0)
Melanoma *	2 (12)	0 (0)
Ewing Sarcoma *	1 (6)	0 (0)
Liver	1 (6)	0 (0)
Lymphatic	1 (6)	0 (0)

Exercise mode		
Type		
Treadmill running	11 (65)	0 (0)
Wheel running	6 (35)	0 (0)
Cycling	0 (0)	1 (100)
Frequency [#]		
1-4x/week	1 (6)	1 (100)
5-7x/week	17 (100)	0 (0)
Outcome measure [+]		
Hypoxia	10 (59)	1 (100)
Vascularisation	14 (82)	1 (100)
Blood flow	6 (35)	1 (100)

Data are presented as n (%).

[^] Some studies included more than one animal species and breed

* Some studies included more than one cancer type

[+] Some studies included more than one outcome measure

[#] Some studies included 2 groups that performed different exercise frequencies

TABLE 2.2: Summary of studies

Author, year	Participants	Housing condition	Cancer type/model	Intervention	Outcome measure: method of measure	Author reported result	Other comments
Preclinical studies							
Betof et al., 2015	N=22-24 female immunocompetent BALB/c mice	Housing groups: Control group were housed individually; experimental group not reported Temperature: not reported Humidity: not reported Light/dark cycle: mice exercised in the dark cycle Wheel diameter: 11.5cm	Breast: syngeneic 4T1 murine breast cancer cells orthotopically transplanted in the dorsal mammary fat pad	Mode: voluntary wheel running Frequency: continuous access to 11.5cm diameter wheel Duration: 18 days Speed/distance: not reported	Hypoxia: EF5 Vascularisation: MVD by CD31, vascular maturity Blood flow: Perfusion by magnetic resonance imaging (MRI)	Hypoxia: ↓ Vascularisation: ↑ MVD ↑ vessel maturity Blood flow: ↑	
Buss et al., 2018	N=30-43 female ApoE+/- mice	Housing groups: control group were housed in pairs or threes; experimental group were housed in pairs Temperature: ~22°C	Breast: syngeneic EO771 murine medullary breast adenocarcinoma implanted orthotopically into the 4th mammary fat pad	Mode: voluntary wheel running Frequency: continuous wheel access Duration: until tumours reached 600m^3 (~17 days)	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Hypoxia: ↔ Vascularisation: ↔ Blood flow: ↔	Mice were euthanised early if impact on welfare occurred due to ulceration of the tumor (n =8) or suspicion of internal tumors (n=5). One mouse was euthanised before

		Humidity: not reported Light/dark cycle: housed in 12:12 hour light/dark cycle Wheel diameter: not reported		Distance: 10km/day per pair			the tumor reached measurable size due to malocclusion.
Buss et al., 2018	N=30-43 female ApoE+/- mice	Housing groups: control group were housed in pairs or threes; experimental group were housed in pairs Temperature: ~22°C Humidity: not reported Light/dark cycle: housed in 12:12 hour light/dark cycle Wheel diameter: not reported	Breast: syngeneic EO771 murine medullary breast adenocarcinoma implanted orthotopically into the 4th mammary fat pad	Mode: voluntary wheel running Frequency: wheel access every 2nd day Duration: until tumours reached 600m^3 (~17 days) Distance: 8km/day per pair	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Hypoxia: ↔ Vascularisation: ↔ Blood flow: ↔	Mice were euthanised early if impact on welfare occurred due to ulceration of the tumor (n =8) or suspicion of internal tumors (n=5). One mouse was euthanised before the tumor reached measurable size due to malocclusion.
Buss et al., 2020	N=48 C57BL/6 female mice	Housing groups: housed in pairs Temperature: not reported Humidity: not reported	Melanoma and breast: B16-F10 melanoma cells or EO771 breast cells injected subcutaneously into the flank or	Mode: voluntary wheel running Frequency: continuous access to wheel Duration: until melanoma	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Hypoxia: ↔ Vascularisation: ↔ Blood flow: ↔	

		Light/dark cycle: housed in 12:12 hour light/dark cycle Wheel diameter: not reported	mammary fat pad	tumours reached 1000m^3 (median 17 days) or breast tumours reached 600m^3 (median 21 days) Distance: 8km/day			
Dufresne et al., 2020	N=17 athymic male Nude mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: housed in 12:12 hour light/dark cycle Stimulation for exercise: not reported	Prostate: human prostate cancer PPC-1 cells injected subcutaneously into the dorsal	Mode: treadmill running Frequency: 5x/wk Duration: 25-60min Speed: 18m/min Slope: 10%	Vascularisation: MVD by CD31	Vascularisation: ↔	
Faustino-Rocha et al., 2016	N=21 female Sprague-Dawley rats	Housing groups: not reported Temperature: 23±2°C Humidity: 50±10% Light/dark cycle: housed in 12:12 hour light/dark cycled; exercised	Breast: mammary tumors were induced by a single intraperitoneal administration of the carcinogen agent MNU at a dose of 50mg/kg	Mode: treadmill running Frequency: 60 mins/day for 5x/week Duration: 35 weeks Speed: not reported Slope: not reported	Vascularisation: MVD assessed visually	Vascularisation: ↑	One animal from the MNU exercised group did not adapt to the exercise training and was excluded from the study. During the experiment nine animals died: four animals from the MNU sedentary group (MI = 27%),

		in 12 hour dark cycle Stimulation for exercise: not reported					four animals from the MNU exercised group (MI = 29%) and one animal from the control sedentary (MI = 10%)
Faustino-Rocha et al., 2017	N=21 female Sprague-Dawley rats	Housing groups: not reported Temperature: 23±2°C Humidity: 50±10% Light/dark cycle: housed in 12:12 hour light/dark cycled; exercised in 12 hour dark cycle Stimulation for exercise: not reported	Breast: mammary tumors were induced by a single intraperitoneal administration of the carcinogen agent MNU at a dose of 50mg/kg	Mode: treadmill running Frequency: 60 mins/day for 5x/week Duration: 35 weeks Speed: not reported Slope: not reported	Blood flow: Doppler power ultrasound	Blood flow: ↔	One animal from the MNU exercised group did not adapt to the exercise training and was excluded from the study. During the experiment nine animals died: four animals from the MNU sedentary group (MI = 27%), four animals from the MNU exercised group (MI = 29%) and one animal from the control sedentary (MI = 10%) Due to their small size (mammary tumors<1.0 cm were not analysed), only 15 of 28 mammary tumors (54%) from the MNU sedentary group and 11 of 23

							(48%) from the MNU exercised group were evaluated by contrast-enhanced US.
Florez-Bedoya et al., 2019	N=10-14 male Nude mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Pancreatic: patient-derived xenograft of pancreatic ductal adenocarcinoma tumour tissue implanted subcutaneously into the flank	Mode: treadmill running Frequency: 45 mins/day for 5 days/week Duration: 4 weeks Speed: 12m/min Slope: not reported	Vascularisation: MVD by CD31, functional vessels by lectin perfusion	Vascularisation: ↑ MVD ↔ functional vessels	
Isanejad et al., 2016	N=16 female BALB/c mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: housed in 12:12 hour light/dark cycle; exercised at the end of dark cycle Stimulation for exercise: gentle tap on the tail or hindquarters	Breast: mouse mammary tumour cells MC4-L2 injected into the flank	Mode: treadmill running Frequency: 10-14 mins/day for 5 days/week Duration: 5 weeks Speed: 16-18m/min that increased each week Slope: 0% Stimulus: gentle tap by investigator on	Hypoxia: HIF1α Vascularisation: MVD by CD31	Hypoxia: ↓ Vascularisation: ↓	

				the tail or hindquarters			
Jones et al., 2010	N=50 female Aythmic mice	Housing groups: housed individually Temperature: not reported Humidity: not reported Light/dark cycle: exercised in dark cycle Wheel diameter: 11.5cm	Breast: human mammary adenocarcinoma cell line MDA-MB-231 injected orthotopically into the right dorsal mammary fat pad	Mode: voluntary wheel running Frequency: continuous access to a 11.5cm diameter wheel Duration: until tumours reached 1500m^3 (44±3 days) Distance: ~4 to ~6km/day	Hypoxia: HIF1α and CAIX Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Hypoxia: HIF1α ↔ CAIX ↔ Vascularisation: ↔ Blood flow: ↑	Histological analysis was only performed on tumors obtained from the 10 animals recording the highest mean exercise running distance and 10 random control animals.
Jones et al., 2012	N=38 male C57BL/6 mice	Housing groups: housed individually Temperature: 21°C Humidity: 35-45% Light/dark cycle: housed in 12:12 hour light/dark cycle Wheel diameter: 11.5cm	Prostate: transgenic adenocarcinoma of mouse prostate (TRAMP) C-1 cells injected orthotopically into the prostate	Mode: voluntary wheel running Frequency: continuous access to a 11.5cm diameter wheel Duration: Four mice per group were serially killed on days 14, 31, and 36; the remaining 38 mice (exercise, n=18; control,	Hypoxia: HIF1α Vascularisation: MVD by CD31 Blood flow: Perfusion by magnetic resonance imaging (MRI)	Hypoxia: ↑ Vascularisation: ↑ Blood flow: ↑	

				n=20) were killed on day 53. Distance: ~4 to ~6km/day			
McCullough et al., 2013	N=27 male Copenhagen and Nude rats	Housing groups: not reported Temperature: 23°C Humidity: not reported Light/dark cycle: housed in 12:12 light/dark cycle Stimulation for exercise: not reported	Prostate: Dunning R-3327 rat prostate adenocarcinoma cell line in both animal species	Mode: treadmill running Frequency: 60 mins/day for 5 days/week Duration: 7 weeks (Copenhagen rats) 5 weeks (Nude rats) Speed: 15m/min Slope: 15 degrees	Hypoxia: EF5 and PO_2 Vascularisation: Patent blood vessels	Hypoxia: EF5 ↓ PO_2 ↓ Vascularisation: ↔	The duration of intervention in Nude rats were shortened by 2 weeks to avoid the potential of tumor size constraints.
Morrell et al., 2019	N=10-20 male Nude mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Ewing Sarcoma: A673 and TC71 human Ewing Sarcoma cells injected into the backs of mice	Mode: treadmill running Frequency: 45 mins/day for 5 consecutive days/week Duration: 2 weeks Speed: 12m/min Slope: not reported	Hypoxia: HIF1α and CAIX Vascularisation: MVD by CD31, vessel morphology	Hypoxia: A673 tumours - HIF1α ↔ - CAIX↓ TC71 tumours - HIF1α ↔ - CAIX ↔ Vascularisation: ↔ MVD ↓ vessel permeability	

Rafiei et al., 2021	N=16 female BALB/c mice	Housing groups: not reported Temperature: 22±3°C Humidity: 40-60% Light/dark cycle: housed in 12:12 hour cycle Wheel diameter: not reported	Breast: MC4-L2 cancer cells injected subcutaneously	Mode: treadmill running Frequency: 30 minutes in the first 2 weeks and increasing by 5 minutes every fortnight Duration: 8 weeks Speed: 14m/min increasing to 20m/min in the last 2 weeks Slope: not reported	Hypoxia: HIF1α	Hypoxia: ↓	
Saran et al., 2018	N=18 American Cancer Institute rats (sex not stated)	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Liver: MH-3924A cells were implanted into the liver	Mode: treadmill running Frequency: 770m for 5 days/week Duration: 6 weeks pre tumour implantation, 4 weeks post tumour implantation Speed: not reported	Vascularisation: MVD by CD31	Vascularisation: ↓	

Schadler et al., 2016	N=10-12 male and female wild-type mice from C57B1/6J	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Melanoma and pancreatic: B16F10 Melanoma and PDAC-4662 pancreatic ductal adenocarcinoma tumors were injected subcutaneously into the flanks of mice	Mode: treadmill running Frequency: 45 mins/day for 5 consecutive days/week Duration: 3 weeks Speed: 12m/min in mice with PDAC4662 10m/min in mice with B16F10 Slope: not reported	Vascularisation: MVD by CD31, functional vessels by lectin, vessel length	Vascularisation: ↔ MVD ↑ vessel function ↑ vessel length	
Wakefield et al., 2021	N=14 female BALB/c mice	Housing groups: housed individually Temperature: not reported Humidity: not reported Light/dark cycle: not reported Wheel diameter: not reported	Breast: EMT6 murine mammary cells were implanted into the 4th mammary	Mode: voluntary wheel running Frequency: continuous access to wheel Duration: until tumours reached 200mm^3 (15±4 days) plus an additional 7 days Distance: 9-14km/day	Hypoxia: HIF1α and HIF2α	Hypoxia: ↓	
Zielinski et al., 2004	N=137 female BALB/cByJ mice	Housing groups: housed individually	Lymphoma: EL-4 lymphoid cells subcutaneously injected in the	Mode: treadmill running Frequency: 3 hours or until	Vascularisation: MVD by CD31	Vascularisation: ↓	

		Temperature: 23°C Humidity: not reported Light/dark cycle: housed in 12:12 hour reverse light cycle; exercised during dark cycle Stimulation for exercise: not reported	back behind the neck	volitional fatigue (mean time to fatigue 135±25 minutes) for 7 days/week Duration: 5-14 days Speed: 20-40m/min Slope: not reported			
Clinical studies							
Jones et al., 2013	N=20 females	Not applicable	Breast	Mode: cycling Frequency: 45 mins/day for 3 days/week Duration: 12 weeks Intensity: 55-100% VO_{2peak}	Hypoxia: HIF1α Vascularisation: MVD by CD31, cell proliferation Blood flow: PET scan	Hypoxia: ↔ Vascularisation: ↔ MVD ↔ vessel structure Blood flow: ↓	Results for hypoxia and vascularisation were only available for 5 participants per group. Blood flow data was limited due to technical difficulties

All results are $p<0.05$. HIF1α = hypoxia-inducible factor 1-alpha; HIF2a = hypoxia-inducible factor 2-alpha; CAIX = carbonic anhydrase IX; MVD = microvessel density; PO_2 = partial pressure of oxygen; MNU = two N-methyl-N-nitrosourea

Risk of bias

Preclinical: SYRCLE Risk of Bias

Risk of bias across the preclinical studies is reported in Figure 2.2. Of concern, more than half of the included studies (n=11) had high risk of attrition bias due to not reporting missing data appropriately. However, all 17 studies were free of selective outcome reporting as all available results were presented as described in the methods. Nine studies reported clear baseline characteristics and therefore were assessed as having a low risk although the remaining eight studies did not clearly report all baseline characteristics and were assessed as having an unclear risk. Most other outcomes were assessed as having an unclear risk due to poor reporting of study methodology.

FIGURE 2.2: SYRCLE Risk of Bias for preclinical studies

	Q1: Selection bias	Q2: Selection bias	Q3: Selection bias	Q4: Performance bias	Q5: Performance bias	Q6: Detection bias	Q7: Detection bias	Q8: Attrition bias	Q9: Reporting bias	Q10: Unit of analysis errors
Betof et al. 2015	?	?	?	?	?	?	?	-	+	?
Buss et al. 2018	?	+	?	?	?	?	+	-	+	-
Buss et al. 2020	?	+	?	?	?	?	+	-	+	+
Dufresne et al. 2010	?	+	?	?	?	?	?	+	+	?
Faustino-Rocha et al. 2016	?	?	?	?	?	?	?	-	+	?
Faustino-Rocha et al. 2017	?	?	?	?	?	?	?	-	+	?
Florez-Bedoya et al. 2019	?	?	?	?	?	?	?	-	+	?
Isanejad et al. 2016	?	+	?	?	?	?	?	+	+	?
Jones et al. 2010	?	+	?	?	?	?	+	-	+	+
Jones et al. 2012	?	+	?	?	?	?	?	-	+	+
McCullough et al. 2013	?	+	?	?	?	?	?	+	+	?
Morrell et al. 2019	?	+	?	?	?	?	?	-	+	?
Saran et al. 2018	?	?	?	?	?	?	+	+	+	?
Schadler et al. 2016	?	?	?	?	?	?	?	+	+	?
Rafiei et al. 2021	?	?	?	?	?	?	?	+	+	?
Wakefield et al.	?	+	?	?	?	?	?	-	+	+

2021										
Zielinski et al. 2004	?	?	?	?	?	?	+	-	+	?

- High risk

? Uncertain risk

+ Low risk

Clinical: Cochrane Risk of Bias

Overall, there was a high risk of bias for the clinical study, primarily a result of missing outcome data for hypoxia and vascularisation (Figure 2.3). There were additional concerns regarding randomisation as the study did not detail the concealment procedure. Blinding of participants was not possible due to the pragmatic nature of the exercise intervention although there was still low risk of bias for deviations from the intended intervention. Although the overall risk of bias was high, there was low risk of bias in the measurement and reporting of all outcomes.

FIGURE 2.3: Cochrane Risk of Bias for clinical studies

	1: Randomisation process	2: Deviations from the intended intervention	3: Missing outcome data	4: Measurement of the outcome	5: Selection of reported results	Overall risk of bias
Hypoxia	?	+	-	+	+	-
Vascularisation	?	+	-	+	+	
Blood flow	?	+	+	+	+	

- High risk

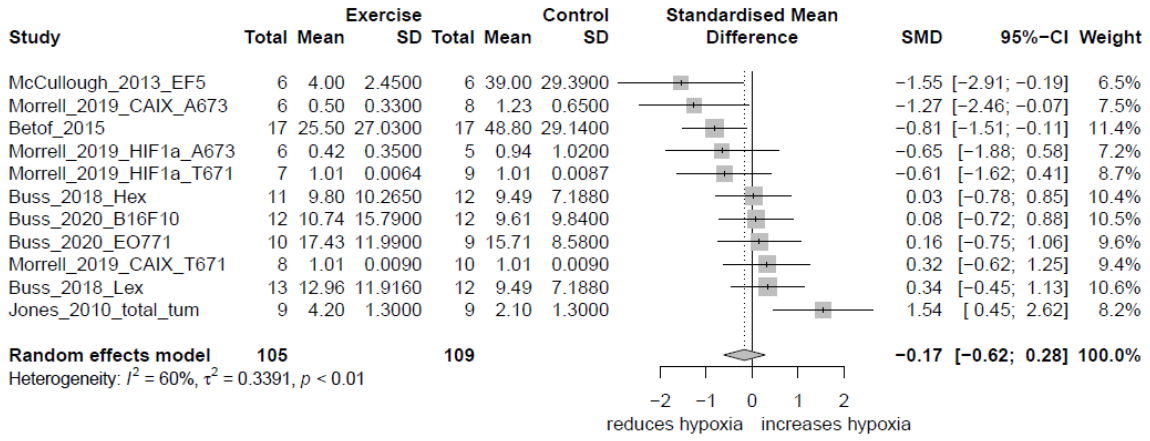
? Uncertain risk

+ Low risk

Hypoxia

Measures of hypoxia included HIF1α (n=6), EF5 (n=2), pimonidazole (n=2), carbonic anhydrase IX (CAIX) (n=2) and partial pressure of oxygen (PO_2) (n=1). Some studies assessed multiple measures of hypoxia. Of the 11 studies which assessed hypoxia, six studies reported a decrease in hypoxia (Betof et al, 2015; Isanejad et al, 2016; McCullough et al, 2013; Morrell et al, 2019; Rafiei et al, 2021; Wakefield et al, 2021), one study reported an increase (Jones et al, 2012) and five studies reported no change (Buss & Dachs, 2018; Buss et al, 2020; Jones et al 2010; Jones et al, 2013; Morrell et al, 2019). One study (Morrell et al, 2019) utilised four different data sets inclusive of two different tumour types and two different measurement methods, finding a decrease and no change in hypoxia within the one paper. Six studies were included in the meta-analysis for hypoxia (Buss & Dachs, 2018; Buss et al, 2020; Betof et al, 2015; Jones et al, 2010; McCullough et al, 2013; Morrell et al, 2019;), with three studies having multiple measures analysed (Buss & Dachs, 2018; Buss et al, 2020; Morrell et al, 2019). Four studies were excluded from the meta-analysis due to implausible physiological values, fold change values or no mean data (Isanejad et al, 2016; Jones et al, 2012; Wakefield et al, 2021). Overall, there was no significant effect of exercise on hypoxia (SMD = -0.17; 95% CI = -0.62, 0.28; I^2 = 60%) (Figure 2.4)

FIGURE 2.4: Meta-analysis of preclinical studies investigating the effect of exercise on hypoxia

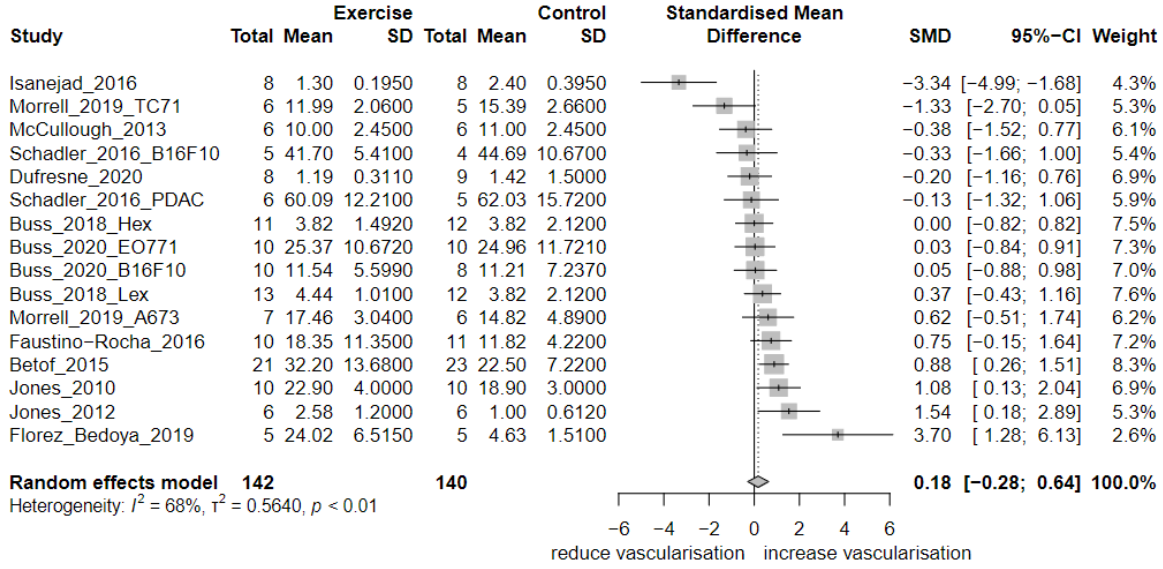


SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

Vascularisation

Measures of vascularisation included microvessel density (n=14), functional vessels (n=2), vessel length (n=1), patent vessels (n=1), and indicators for vessel function and structure (n=3). Four studies reported an increase in microvessel density (Betof et al, 2015; Faustino-Rocha et al, 2016; Florez Bedoya et al, 2019; Jones et al, 2012), three studies reported a decrease in microvessel density (Isanejad et al, 2016; Saran et al, 2018; Zielinski et al, 2004) and eight studies reported no change in microvessel density (Buss & Dachs, 2018; Buss et al, 2020; Jones et al, 2010; Faustino-Rocha et al, 2017; Jones et al, 2013; McCullough et al, 2013; Morrell et al, 2019; Schadler et al, 2016). Thirteen studies were included in the meta-analysis for vascularisation (Betof et al, 2015; Buss & Dachs 2018; Buss et al, 2020; Dufresne et al, 2020; Faustino-Rocha et al, 2016; Florez Bedoya et al, 2019; Isanejad et al, 2016; Jones et al, 2010; Jones et al, 2012; Jones et al, 2013; McCullough et al, 2013; Morrell et al, 2019; Schadler et al, 2016) with four studies having multiple measures (Buss & Dachs, 2018; Buss et al, 2020; Morrell et al, 2019; Schadler et al, 2016). One study was excluded due to no baseline data (Zielinski et al, 2004). One study (Saran et al, 2018) was excluded from the presented forest plot for vascularisation due to being an outlier resulting in reduced legibility of the remainder of the meta-analysis results (SMD = 0.07; 95% CI = -0.51, 0.64; I^2 = 79%) (full plot available in Supplemental figure 2.1). The exclusion made no difference in the overall effect size. Overall, there was no effect of exercise on vascularisation (SMD = 0.18; 95% CI = -0.28, 0.64; I^2 = 68%) (Figure 2.5)

FIGURE 2.5: Meta-analysis of preclinical studies investigating the effect of exercise on vascularisation

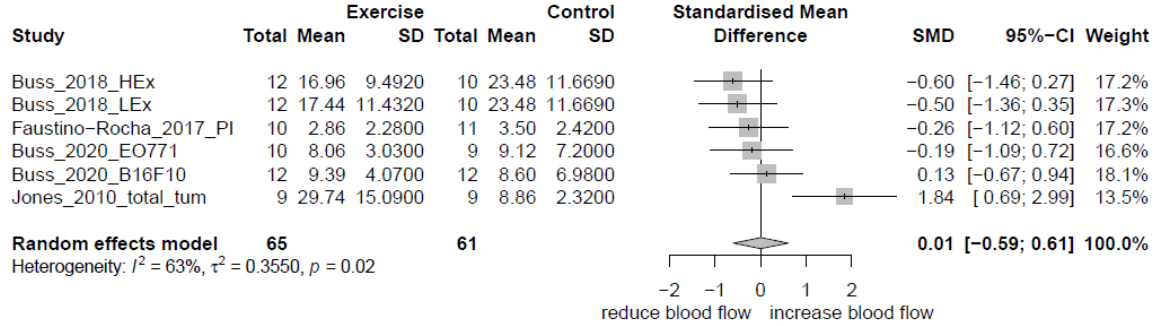


SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

Blood flow

Of the seven studies that evaluated blood flow, three studies reported an increase (Betof et al, 2015; Jones et al, 2010; Jones et al, 2012), one study reported a decrease (Jones 2013), and three studies reported no change (Buss & Dachs 2018; Buss et al, 2020; Faustino-Rocha et al, 2017). Four studies were included in the meta-analysis for blood flow (Buss & Dachs, 2018; Buss et al, 2020; Faustino-Rocha et al, 2017; Jones et al, 2010; Jones et al, 2013) with two studies having multiple measures including blood flow and perfusion (Buss & Dachs, 2018; Buss et al, 2020). One study was excluded from the meta-analysis due to no quantitative data reported and was another study excluded as there was no mean data (Betof et al, 2015; Jones et al, 2012). Overall, there was no significant effect of exercise on blood flow (SMD = 0.01; 95% CI = -0.59, 0.61; I^2 = 63%) (Figure 2.6)

FIGURE 2.6: Meta-analysis of preclinical studies investigating the effect of exercise on blood flow



SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

Sub-group analysis

Sub-group analysis for animal, species, exercise mode, tumour location and study duration showed no significant effect in all analyses. Sub-group analyses can be found in the Supplemental Materials (Supplemental figures 2.2 - 2.16).

DISCUSSION

The findings from this systematic review and meta-analysis demonstrate that among 17 preclinical studies and one clinical study, aerobic exercise training had no significant effect on tumour hypoxia, vascularisation or blood flow. To our knowledge, this is the only systematic review exploring the effects on all three of these related outcomes of hypoxia, vascularisation and blood flow. Concerningly, there was high risk for attrition bias in the preclinical studies and most other domains of bias were of unclear risk. Of the 18 studies included in this review, 17 studies were published within the last decade suggesting that there is growing interest in this area. Although the preclinical body of evidence is growing, the synthesis of data is limited by variability in animal type and study methodology. The measurement of the outcomes reported herein are possible in a human population as evidenced in Jones et al (2013). Therefore, future gains may be made by exploring these effects in humans for translation to clinical practice.

We anticipated that exercise would drive an association between our outcome measures; that exercise training would result in an increase in vascularisation that would cause increased blood flow and consequently decreased hypoxia, representing beneficial changes in the tumour microenvironment with the potential to reduce tumour growth and improve patient prognosis. This assumption has been based on the literature, exemplified by recent narrative reviews (Esteves, Monteiro & Duarte, 2020; Schumacher et al, 2021). However, the expected pattern of change was not found in this review, with studies reporting exercise-induced decreases and increases in all outcome measures, and combinations of decreases and increases that do not support our assumption. Although our findings contrast with recent narrative reviews, our conclusions are supported by the systematic inclusion of all available data.

In the period between registration and completion of this review, Esteves et al. (38) published a systematic review and meta-analysis that included one of the outcomes we assessed: vascularisation. The authors found an effect of exercise on vascularisation, in conflict with the findings from this review. We have described a number of concerns with methodology, data extraction and data analysis of their review elsewhere (Seet-Lee et al, 2022). The current comprehensive review and meta-analysis uses a more appropriate statistical analysis recommended for preclinical studies (Borenstein et al, 2009, Higgins et al, 2021; Hoojimans et al, 2014) and includes all available studies, providing a more accurate evaluation of current literature.

Contrary to our expectation, we found that there were inconsistent findings for all explored outcomes. We expected to see decreases in hypoxia, however, of the 11 studies which assessed hypoxia, one study (Jones et al, 2012) found an unexpected increase and five studies (Buss & Dachs, 2018; Buss et al, 2020; Jones et al, 2010; Jones et al, 2013; Morrell et al, 2019) found no change. We expected increased vascularisation, however, three of 15 studies (Isanejad et al, 2016; Saran et al, 2018; Zielinski et al, 2004) showed a reduction and eight studies (Buss & Dachs, 2018; Buss et al, 2020; Dufresne et al, 2020; Jones et al, 2010; Jones et al, 2013; McCullough et al, 2013; Morrell et al, 2019; Schadler et al, 2016) showed no change. Our expectation of increased blood delivery was also not met, three studies demonstrated no change (Buss & Dachs, 2018; Buss et al, 2020; Faustino-Rocha et al, 2017) and one study

demonstrated a decrease (Jones et al, 2013) in blood flow (Betof et al, 2015; Jones et al, 2010; Jones et al, 2012). These unexpected results in hypoxia, vascularisation and blood flow may be a result of two potential mechanisms; angiogenesis and/or decreased inflammatory macrophages. Aerobic exercise induces the formation of new blood vessels through an increase in VEGF which promotes angiogenesis (Jones et al, 2012). Although angiogenesis may increase microvessel density and subsequent blood flow to tumours, the dysfunction in tumour blood vessels (specifically leaky blood vessels and low permeability) may mean that this is not translated to changes in hypoxia if oxygen diffusion is poor (Dewhirst et al, 2017; Jain, 1990; Saran et al, 2018). Furthermore, tumour blood vessels are heterogenous in distribution, leading to inconsistent oxygen supply (Wiggins et al, 2018), which may mean areas of increased hypoxia within the tumour despite potential increases in overall blood flow through increased microvessel density. Therefore, a single hypoxia measurement may not represent the level of hypoxia elsewhere in the tumour. Separately, it has been suggested that aerobic exercise training may reduce the activation of inflammatory macrophages in tumours (Zielinski et al, 2004). These cells promote angiogenesis, therefore reduction in inflammatory macrophages may reduce microvessel density resulting in less blood delivery and increased hypoxia (Zielinski et al, 2004).

Variability in study methodology and quality can create significant bias in the results. Features of poor study methodology such as lack of reporting, heterogeneity of baseline characteristics, and external influences such as housing (in preclinical research), exercise outside of the intervention and significant missing data, can largely impact results both within studies and when comparing studies. The risk of bias results from this review should be used to guide future preclinical and clinical methodology to better compare outcome measures across studies. Housing was poorly reported which is important for social stress effects (Bartolomucci, 2007). Additionally, the impact of stress on animals, such as stimulation for forced exercise, is particularly significant in cancer outcomes whereby it has been postulated that the immune response plays a part in the development of tumour vasculature to promote microvessel density and tumour growth in mice (Ashcraft et al, 2016; Thaker et al, 2006). The characteristics of the exercise was poorly reported across studies. Six studies (Betof et al, 2015; Buss & Dachs, 2018, Buss et al, 2020; Jones et al, 2010; Jones et al, 2012; Wakefield et al, 2021) used voluntary wheel running, which suffers from lack of control of intensity and therefore dose. Seven papers included a familiarisation period of exercise for an average of 18 days (Betof et al, 2015; Dufresne et al, 2020; Faustino-Rocha et al, 2016; Faustino-Rocha et al, 2017; Isanejad et al,

2016; McCullough et al, 2013; Zielinski et al, 2004) whereby subjects would perform additional exercise after tumour induction but prior to the exercise intervention. Outcome measures that were assessed did not exclude the familiarisation period thus the additive exercise to the intervention may potentially overpower the results. In the current analysis, we saw heterogeneity in animal type and species (14 mice studies with five different species and four rat studies with three different species) making comparison of results between papers problematic and suitability for human models debateable (Mollard et al, 2011).

Whilst our findings do not favour exercise training in terms of tumour hypoxia, vascularisation and blood flow, several systematic reviews and meta-analyses have found beneficial effects of exercise training for tumour growth and development (Ashcraft et al, 2016; Eschke 2019). Given this contrast, we briefly examined tumour growth outcomes which were reported in 13 studies included in this review (Betof et al, 2015; Buss & Dachs, 2018; Buss et al, 2020; Dufresne et al, 2020; Faustino-Rocha et al, 2016; Florez Bedoya et al, 2019; Jones et al, 2010; Jones et al, 2012; Rafiei et al, 2021; Saran et al, 2018; Schadler et al, 2016; Wakefield et al, 2021; Zielinski et al, 2004). Of these, there were inconsistent results in tumour growth; one reported increased growth, four showed decreased growth and nine showed no change in tumour size. One study (Schadler et al, 2016) showed both a decrease in one tumour type and an increase in another tumour type. We observed similar inconsistent patterns of change in hypoxia, vascularisation and blood flow even with studies showing the same tumour growth changes. However, it should be noted that tumour growth was not a primary outcome of this review and the reported findings are descriptive only. As the studies included in this review did not mirror the results from the two published systematic reviews on tumour growth, we believe that the mechanisms of action for exercise on tumour growth are complex and not well described by this literature. It is possible that the mechanisms of exercise effects could involve interaction between hypoxia, vascularisation and blood flow, however, more consistent data, methodology and reporting is necessary to further explore these associations. It is also likely that other systemic or intra-tumoral factors, such as inflammation and immune responses, impact tumour growth (Ashcraft et al, 2016; Pedersen, Christensen & Hojman 2015).

Limitations

Despite using author-reported results, we found that there were two instances where there were differences in the significance of findings between author-reported results and the meta-analysis calculation. Our careful review and meta-analysis showed inconsistencies within a paper which were confirmed with the authors but may have been the cause for the different outcome in the analysis. Subsequently it is possible that the conclusions of this review would differ without the inclusion of a meta-analysis calculation. Another limitation is that although we conducted sub-group analyses, the results were limited due to poor reporting and heterogenous study design.

CONCLUSION

Among the studies included in this systematic review, aerobic exercise training did not have an effect on tumour hypoxia, vascularisation or blood flow. However, there was great methodological heterogeneity which may have contributed to the inconsistent findings. Future preclinical studies need improved study design and reporting to provide deeper insights into the complex interactions between hypoxia, vascularisation and blood flow. Given the established benefits of exercise in reducing cancer burden, understanding the mechanisms is an important step towards designing the most efficacious interventions and best practice for translation to clinical studies.

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY TABLE 2.1: Raw data from studies

Author, year	Participants	Cancer type	Intervention	Outcome measure: method of measure	Hypoxia	Vascularisation (MVD results only)	Blood flow
Preclinical studies							
Betof et al., 2015	Female immunocompetent BALB/c mice	Breast	Voluntary wheel running for 18 days	Hypoxia: EF5 Vascularisation: MVD by CD31, vascular maturity Blood flow: Perfusion by magnetic resonance imaging (MRI)	Control: n=17; mean = 48.80 (95% CI = 35.5-63.2) Exercise: n=17; mean = 25.5 (95% CI = 12.7-38.4)	Control: n=23; mean = 22.5 (95% CI = 19.6-25.5) Exercise: n=21; mean = 32.2 (95% CI = 26.3-38.0)	No quantitative data shown
Buss et al., 2018	Female ApoE+/- mice	Breast	Voluntary wheel running until tumours reached 600m^3 (~17 days)	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Control: n=12; mean = 9.49; SEM = 2.075 Exercise: n=13; mean = 12.96 (SEM = 3.305)	Control: n=12; mean = 3.82; SEM = 0.635 Exercise: n=11; mean = 3.82; SEM = 0.45	Control: n=10; mean = 23.48; SEM = 3.69 Exercise: n=12; mean = 16.965; SEM = 2.74

Buss et al., 2018	Female ApoE+/- mice	Breast	Voluntary wheel running every 2nd day until tumours reached 600m^3 (~17 days)	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Control: n=12; mean = 9.49; SEM = 2.075 Exercise: n=12; mean = 9.8; SEM = 3.095	Control: n=12; mean = 3.82; SEM = 0.635 Exercise: n=13; mean = 4.44; SEM = 0.305	Control: n=10; mean = 23.48; SEM = 3.69 Exercise: n=12; mean = 17.44; SEM = 3.3
Buss et al., 2020	C57BL/6 female mice	Melenoma and breast	Voluntary wheel running until melanoma tumours reached 1000m^3 (median 17 days) or breast tumours reached 600m^3 (median 21 days)	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	B16F10 tumours Control: n=12; mean = 9.61 (95% CI = 3.86-15.0) Exercise: n=12; mean = 10.745 (95% CI = 1.42-19.29) EO771 tumours Control: n=9; mean = 15.71 (95% CI = 10.15-21.36) Exercise: n=10; mean = 17.43 (95% CI = 10.15-25.01)	B16F10 tumours Control: n=8; mean = 11.21 (95% CI = 6.195-16.226) Exercise: n=10; mean = 11.545 (95% CI = 8.08-15.02) EO771 tumours Control: n=10; mean = 24.965 (95% CI = 17.7-32.23) Exercise: n=10; mean = 25.37 (95% CI = 18.755-31.985)	B16F10 tumours Control: n=12; mean = 8.595 (95% CI = 4.78-12.68) Exercise: n=12; mean = 9.39 (95% CI = 6.22-10.83) EO771 tumours Control: n=9; mean = 9.12 (95% CI = 4.41-13.82) Exercise: n=10; mean = 8.06 (95% CI = 6.1-9.85)
Dufresne et al., 2020	Athymic male Nude mice	Prostate	Treadmill running 5 times/week for 25-60 minutes at 18m/min with 10% slope for 2 weeks	Vascularisation: MVD by CD31		Control: n=9; mean = 1.42; SEM = 0.5 Exercise: n=8; mean = 1.185; SEM = 0.11	

Faustino-Rocha et al., 2016	Female Sprague-Dawley rats	Breast	Treadmill running 5x/week for 60 minutes for 35 weeks	Vascularisation: MVD assessed visually		Control: n=11; mean = 11.82; SEM = 1.09 Exercise: n=10; mean = 18.35; SEM = 2.93	
Faustino-Rocha et al., 2017	Female Sprague-Dawley rats	Breast	Treadmill running 5x/week for 60 minutes for 35 weeks	Blood flow: Doppler power ultrasound			PI Control: n=11; mean = 0.87; SEM = 0.1 Exercise: n=10; mean = 0.79; SEM = 0.08
Florez-Bedoya et al., 2019	Male Nude mice	Pancreatic	Treadmill running for 5 days/week for 45 minutes at 12m/min for 4 weeks	Vascularisation: MVD by CD31, functional vessels by lectin perfusion		Control: n=5; mean = 4.63; SD = 1.51 Exercise: n=5; mean = 24.025; SD = 6.515	
Isanejad et al., 2016	Female BALB/c mice	Breast	Treadmill running 10-14 mins/day for 5 days/week at 6-18m/min that increased each week for 5 weeks	Hypoxia: HIF1α Vascularisation: MVD by CD31	Control: n=8; mean fold change = 1; SD = 0 Exercise: n=9; mean fold change = 0.215; SD = 0.01	Control: n=8; mean fold change = 2.4; SD = 0.395 Exercise: n=8; mean fold change = 1.3; SD = 0.195	
Jones et al., 2010	Female Aythmic mice	Breast	Voluntary wheel running until tumours reached 1500m^3 (44±3 days)	Hypoxia: HIF1α and CAIX Vascularisation: MVD by CD31 Blood flow: Perfusion by Hoechst 33342 staining	Total tumour HIF1α Control: n=9-10; mean = 2.1; SD = 1.3 Exercise: n=9-10; mean = 4.2; SD = 1.3 CAIX No data shown	Control: n=10; mean = 18.9; SD = 3.0 Exercise: n=10; mean = 22.9; SD = 4.0	Control: n=9-10; mean = 3; SD = 0.45 Exercise: n=9-10; mean = 7.4; SD = 1.8

Jones et al., 2012	Male C57BL/6 mice	Prostate	Voluntary wheel running for 53 days	Hypoxia: HIF1α Vascularisation: MVD by CD31 Blood flow: Perfusion by magnetic resonance imaging (MRI)	Control: n=6-10; mean = -7.69; SEM = 4.62 Exercise: n=6-10; mean = 14.205; SEM = 4.44	Control: n=6-10; mean = 1; SEM = 0.25 Exercise: n=6-10; mean = 2.585; SEM = 0.49	Control: n=5-6; median = 1.83 Exercise: n=5-6; median = 2.125
McCullough et al., 2013	Male Copenhagen and Nude rats	Prostate	Treadmill running for 5 days/week for 60 minutes at 15m/min with 15 degrees slope for 7 weeks (Copenhagen rats) and 5 weeks (Nude rats)	Hypoxia: EF5 and PO_2 Vascularisation: Patent blood vessels	EF5 Control: n=6; mean = 39; SEM = 12 Exercise: n=6; mean = 4; SEM = 1 PO_2 Control: n=6; mean = 6; SEM = 0.3 Exercise: n=9; mean = 12.2; SEM = 1	Control: n=6; mean = 11; SEM = 1 Exercise: n=6; mean = 10; SEM = 1	
Morrell et al., 2019	Male Nude mice	Ewing Sarcoma	Treadmill running for 5 days/week for 45 minutes at 12m/min for 2 weeks	Hypoxia: HIF1α and CAIX Vascularisation: MVD by CD31, vessel morphology	A673 tumours HIF1α Control: n=5; mean = 0.9396; SEM = 0.4542 Exercise: n=6; mean = 0.4218; SEM = 0.1432 CAIX Control: n=8; mean = 1.231; SEM = 0.2314	A673 tumours Control: n=6; mean = 14.82; SEM = 1.995 Exercise: n=7; mean = 17.46; SEM = 1.15 TC71 tumours Control: n=5; mean = 15.39; SEM = 1.19 Exercise: n=6; mean = 11.99; SEM = 0.84	

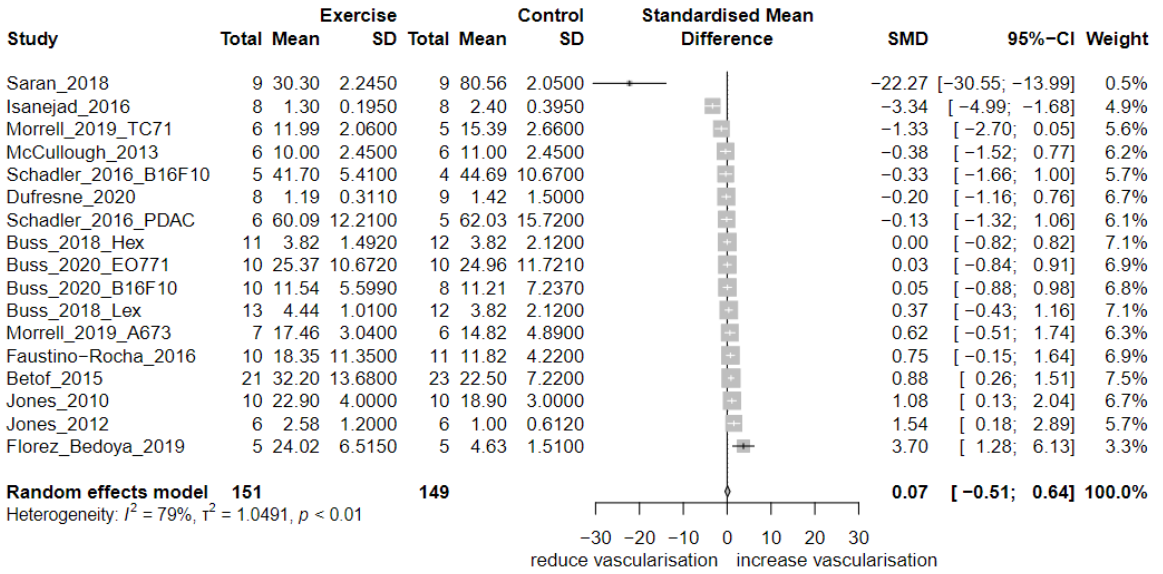
					Exercise: n=6; mean = 0.5; SEM = 0.1353 TC71 tumours HIF1α ↔ Control: n=9; mean = 1.011; SEM = 0.0029 Exercise: n=7; mean = 1.006; SEM = 0.0024 CAIX Control: n=10; mean = 1.01; SEM = 0.0028 Exercise: n=8; mean = 1.013; SEM = 0.0032		
Rafiei et al., 2021	Female BALB/c mice	Breast	Treadmill running for 5 days/week for 30-45 minutes at 14-20m/min for 8 weeks	Hypoxia: HIF1α	Control: n=8; mean fold change = 0.95; SD = 0.17 Exercise: n=8; mean fold change= 1.93; SD = 0.54		
Saran et al., 2018	American Cancer Institute rats	Liver	Treadmill running for 5 days/week for 770m for 6 weeks	Vascularisation: MVD by CD31		Control: n=9; mean = 80.565; SD = 2.05 Exercise: n=9; mean = 30.3; SD = 2.245	
Schadler et al., 2016	Male and female wild-type mice from C57B1/6J	Melanoma and pancreatic	Treadmill running for 5 days/week for 45 minutes at 12m/min (PDAC4662) or	Vascularisation: MVD by CD31, functional vessels		B16F10 tumours Control: n=4; mean = 44.685; SEM = 5.335	

			10m/min (B16F10) for 3 weeks	by lectin, vessel length		Exercise: n=5; mean = 41.705; SME = 2.42 PDAC tumours Control: n=5; mean = 62.03; SEM = 7.03 Exercise: n=6; mean = 60.095; SEM = 4.985	
Wakefield et al., 2021	Female BALB/c mice	Breast	Voluntary wheel running until tumours reached 200mm^3 (15±4 days) plus an additional 7 days	Hypoxia: HIF1α and HIF2α	HIF1a Control: n=7; median = 0.94 Exercise: n=7; median = 0.64 HIF2a Control: n=7; median = 0.98 Exercise: n=7; median = 0.83		
Zielinski et al., 2004	Female BALB/cByJ mice	Lymphoma	Treadmill running for 7 days/week for 3 hours or until volitional fatigue at 20-40m/min for 5-14 days	Vascularisation: MVD by CD31		Unable to calculate due to no baseline data shown	
Clinical study							
Jones et al., 2013	Females	Breast	Cycling for 3 days/week for 45 mins/day at 55-100% VO_{2peak} for 12 weeks	Hypoxia: HIF1α Vascularisation: MVD by CD31, cell proliferation	Data not shown	Data not shown	Data not shown; tumour blood flow reduced by 38%

				Blood flow: PET scan			

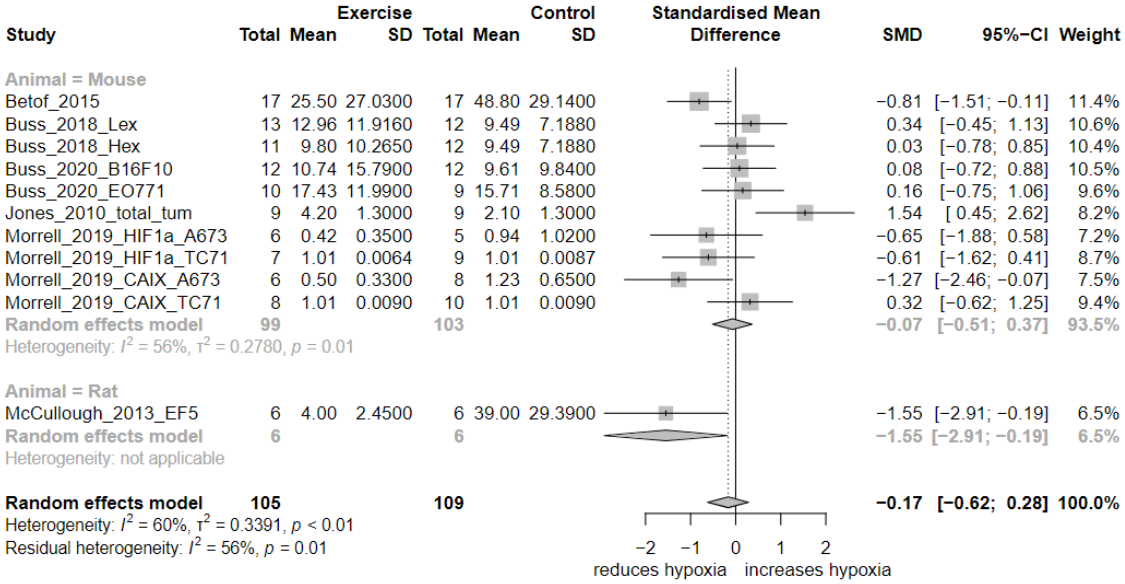
SD = standard deviation; SEM = standard error of the mean; 95% CI = 95% confidence interval; HIF1α = hypoxia-inducible factor 1-alpha; HIF2a = hypoxia-inducible factor 2-alpha; CAIX = carbonic anhydrase IX; MVD = microvessel density; PO_2 = partial pressure of oxygen; MNU = two N-methyl-N-nitrosourea

SUPPLEMENTARY FIGURE 2.1: Meta-analysis of vascularisation including Saran et al paper



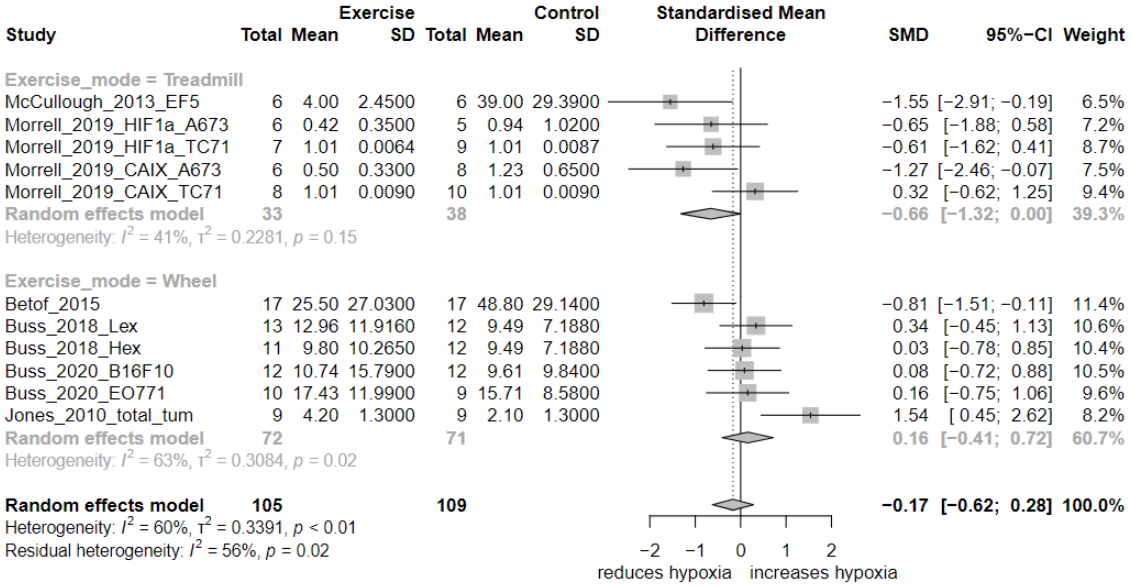
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.2: Sub-group analysis by animal for hypoxia



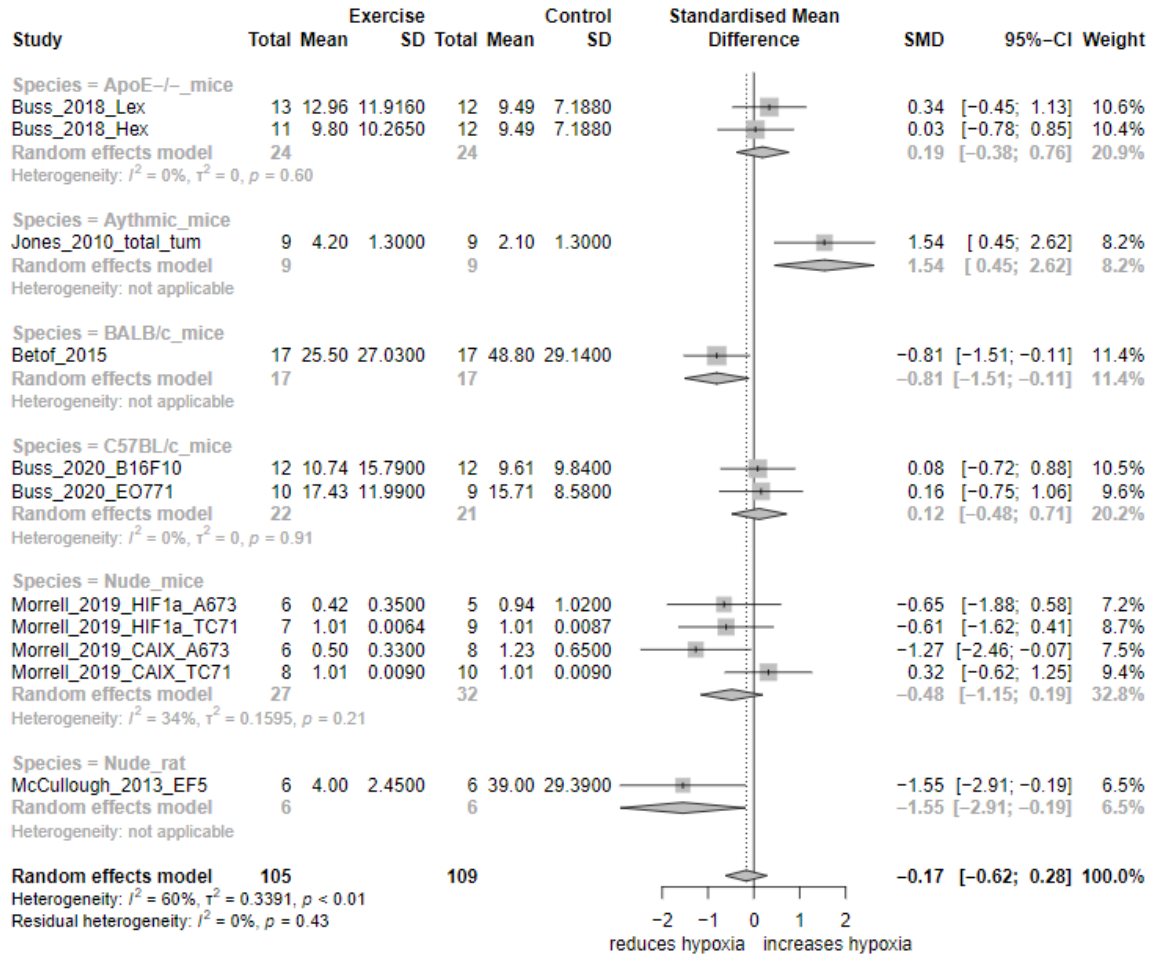
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.3: Sub-group analysis by exercise mode for hypoxia



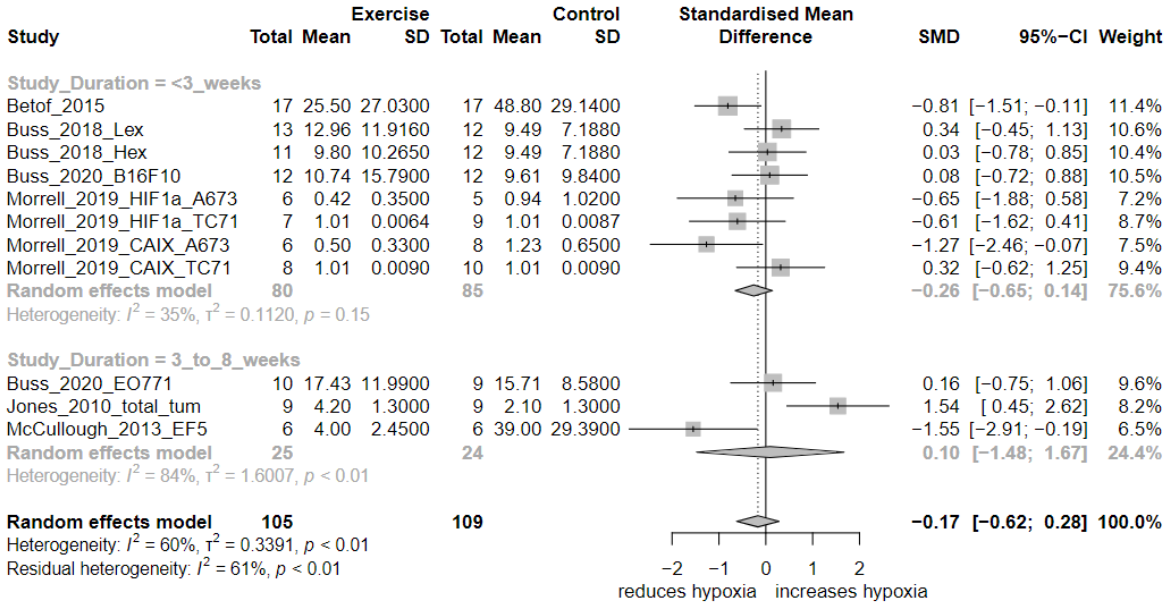
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.4: Sub-group analysis by animal species for hypoxia



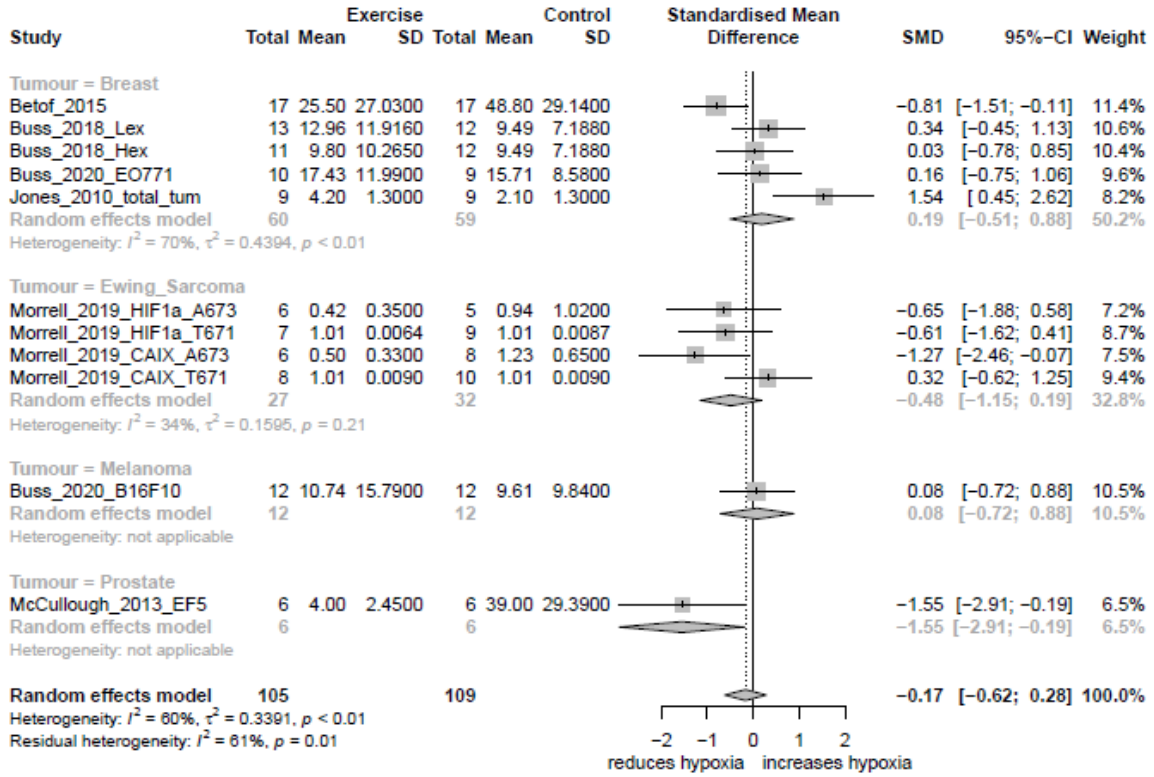
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.5: Sub-group analysis by study duration for hypoxia



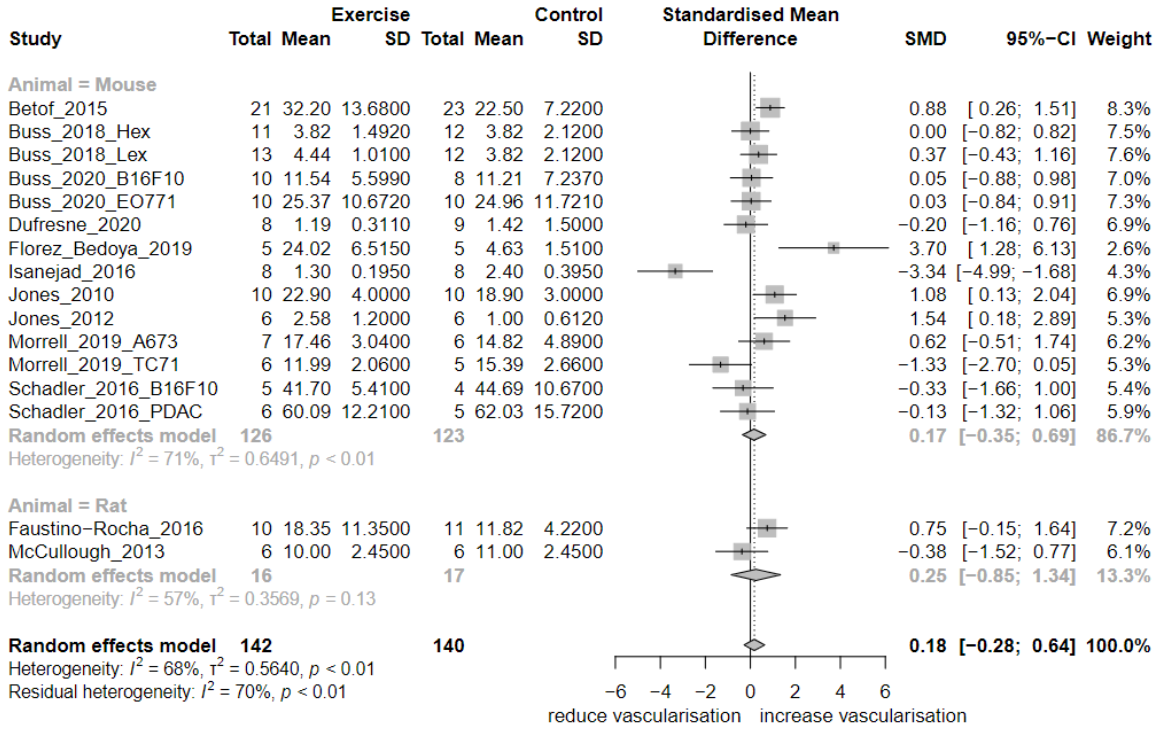
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.6: Sub-group analysis by tumour type for hypoxia



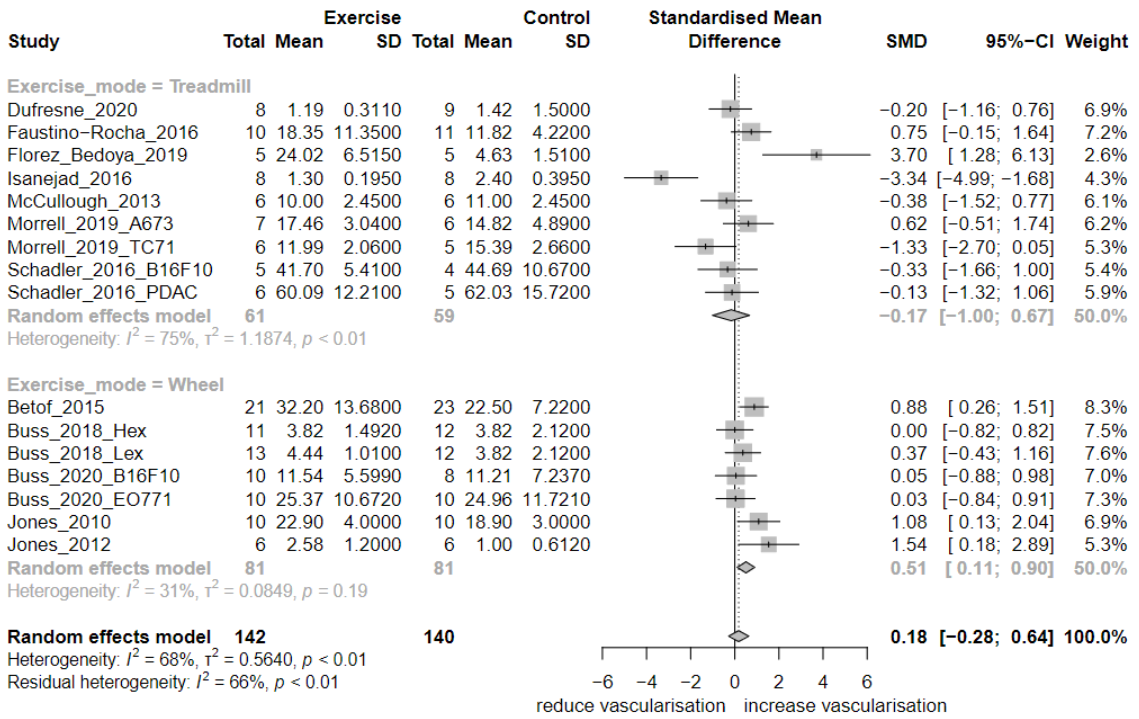
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.7: Sub-group analysis by animal for vascularisation



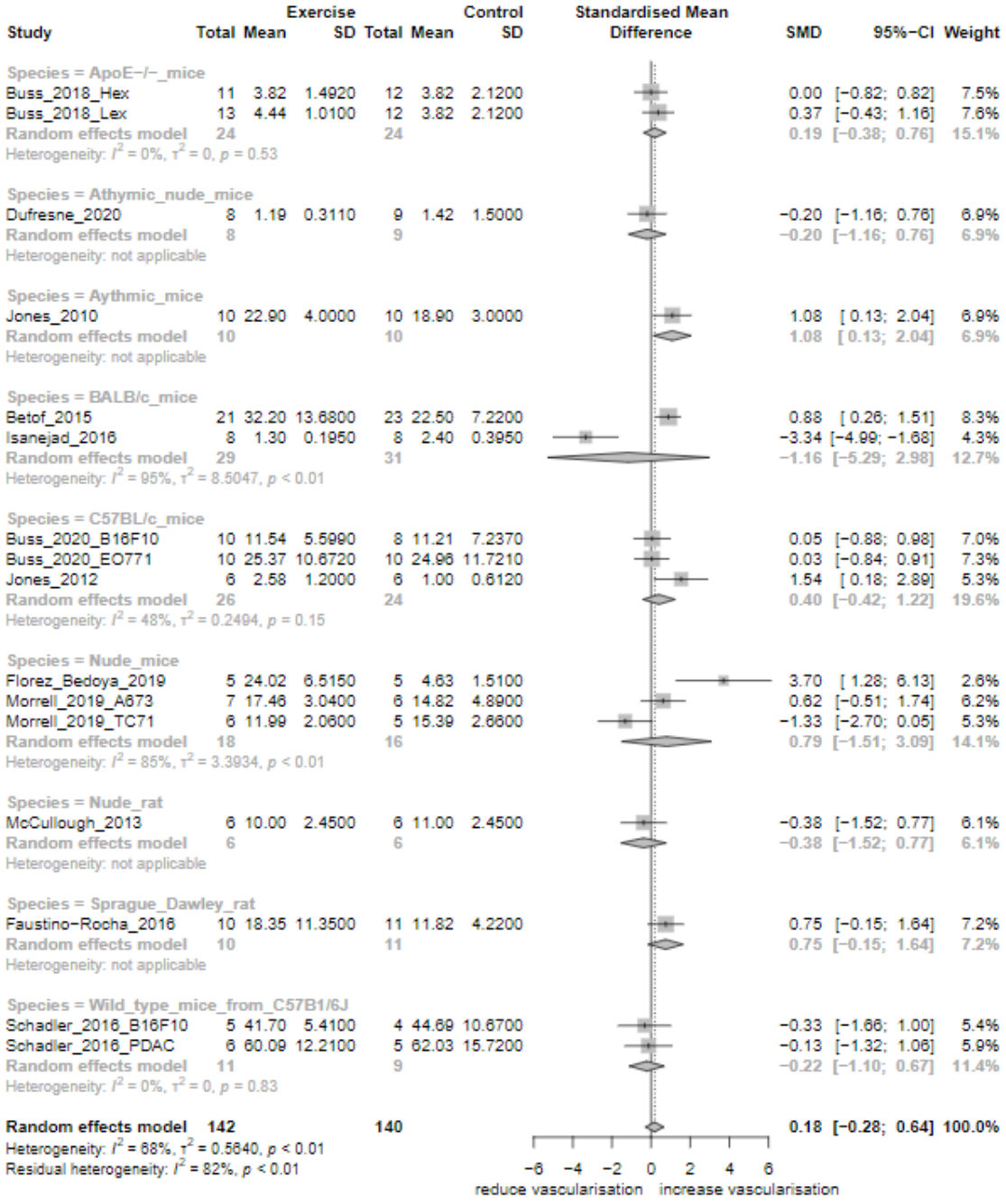
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.8: Sub-group analysis by exercise mode for vascularisation



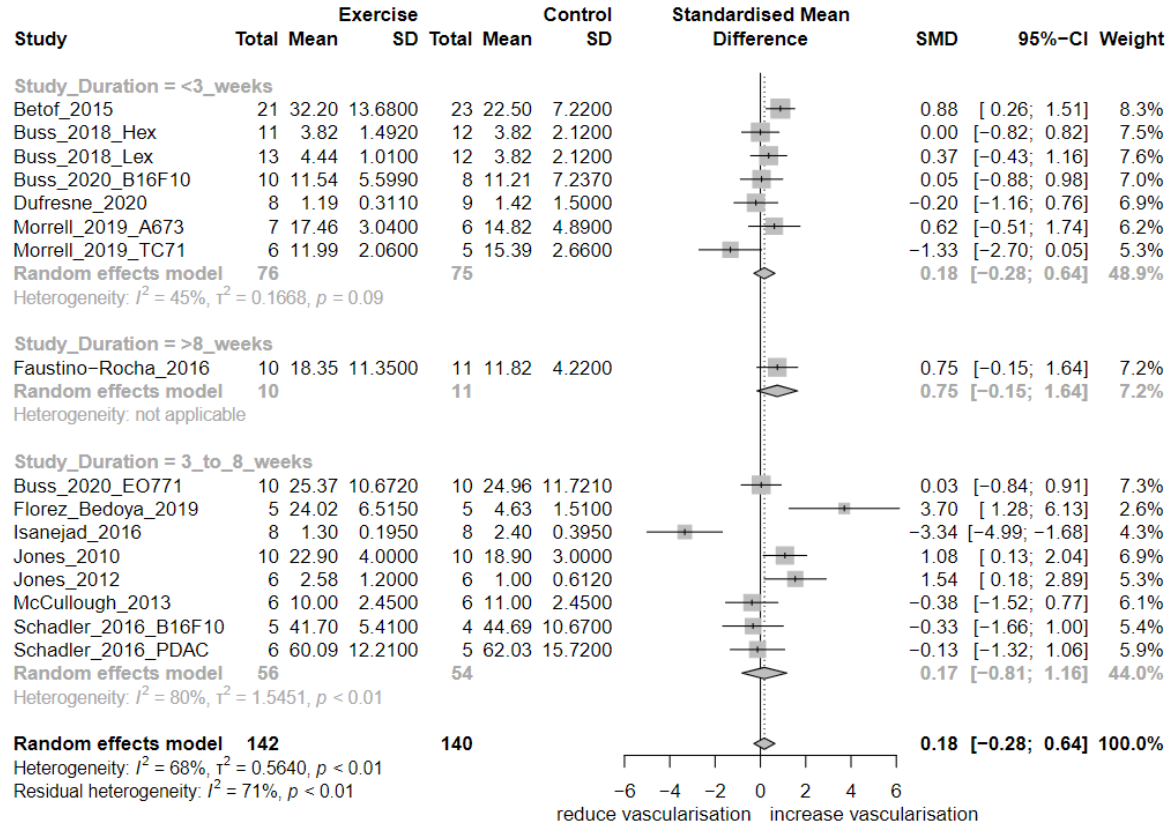
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.9: Sub-group analysis by animal species for vascularisation



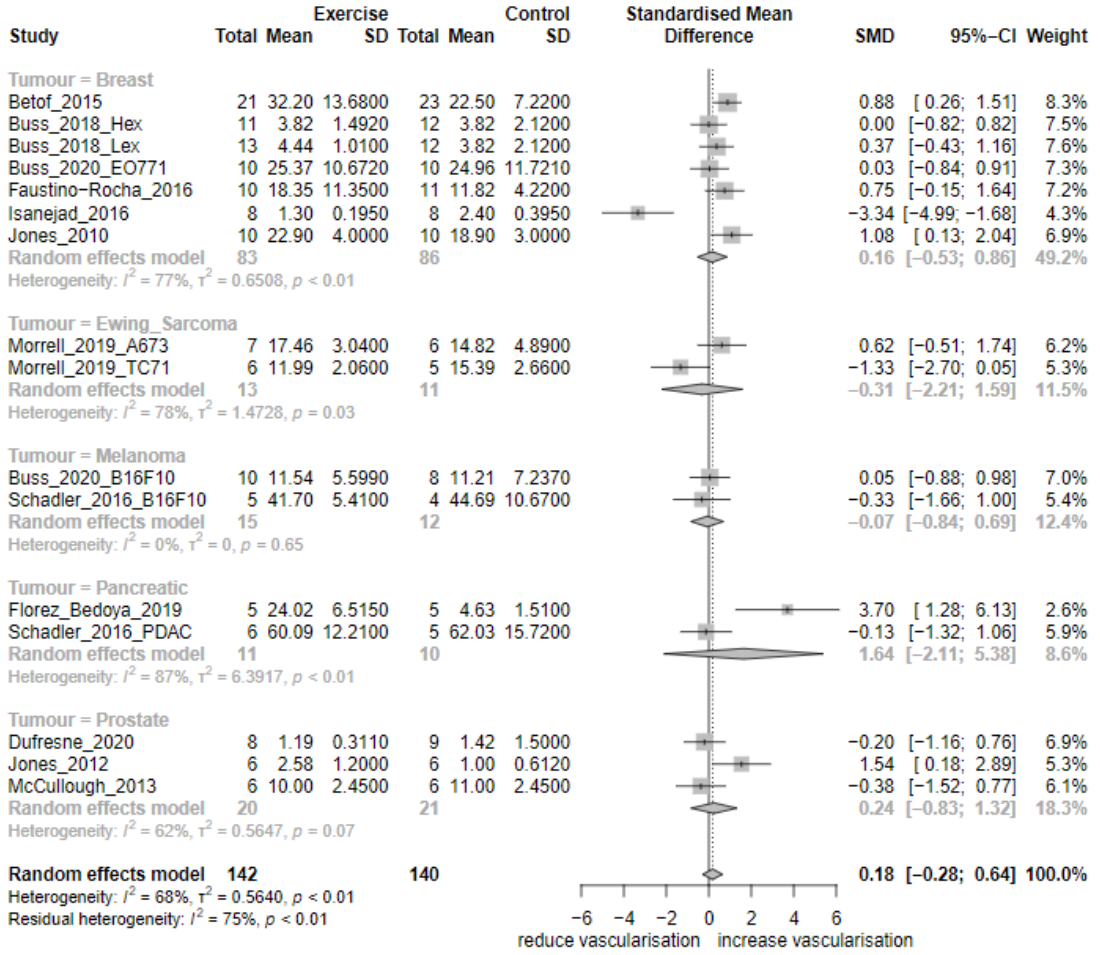
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.10: Sub-group analysis by study duration for vascularisation



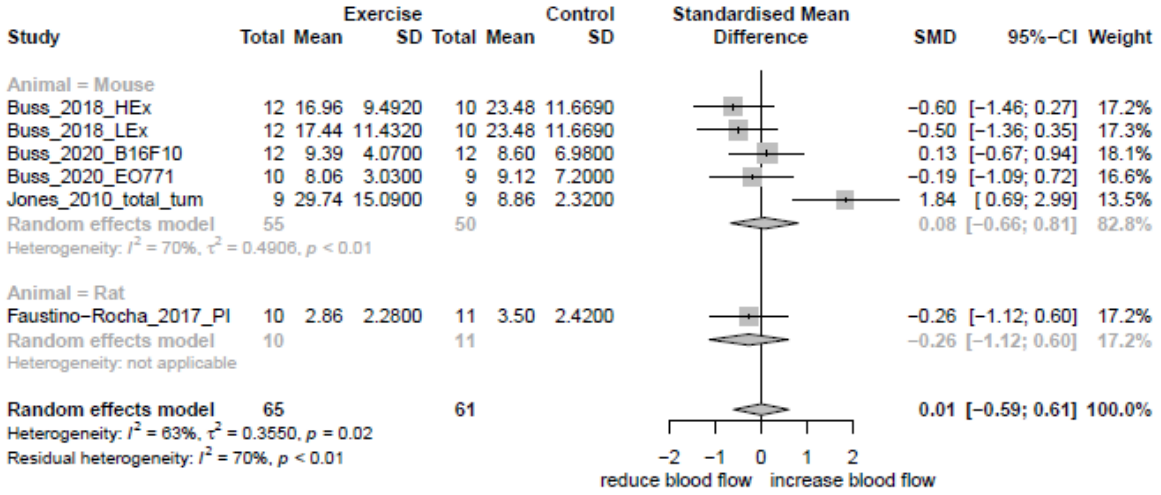
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.11: Sub-group analysis by tumour type for vascularisation



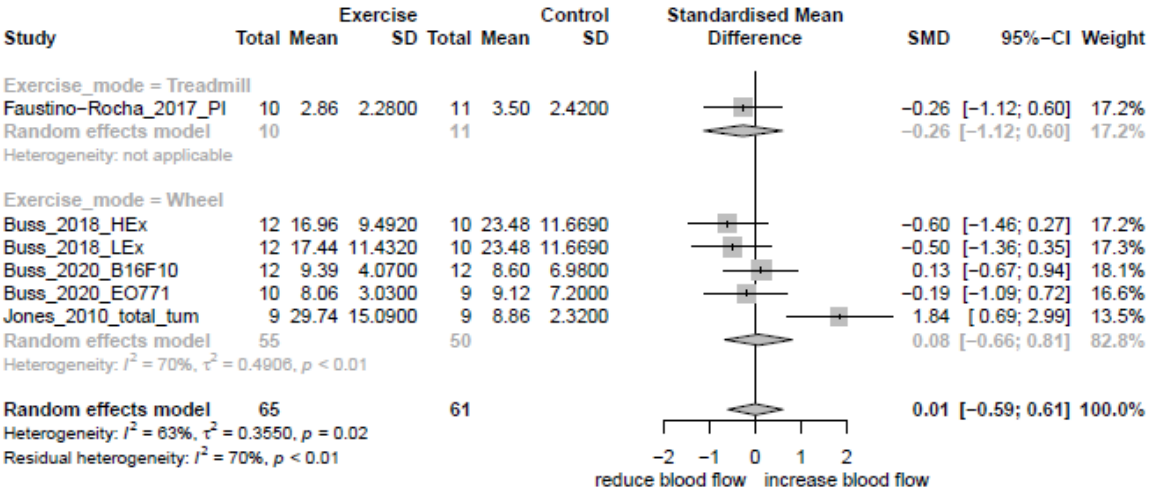
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.12: Sub-group analysis by animal for blood flow



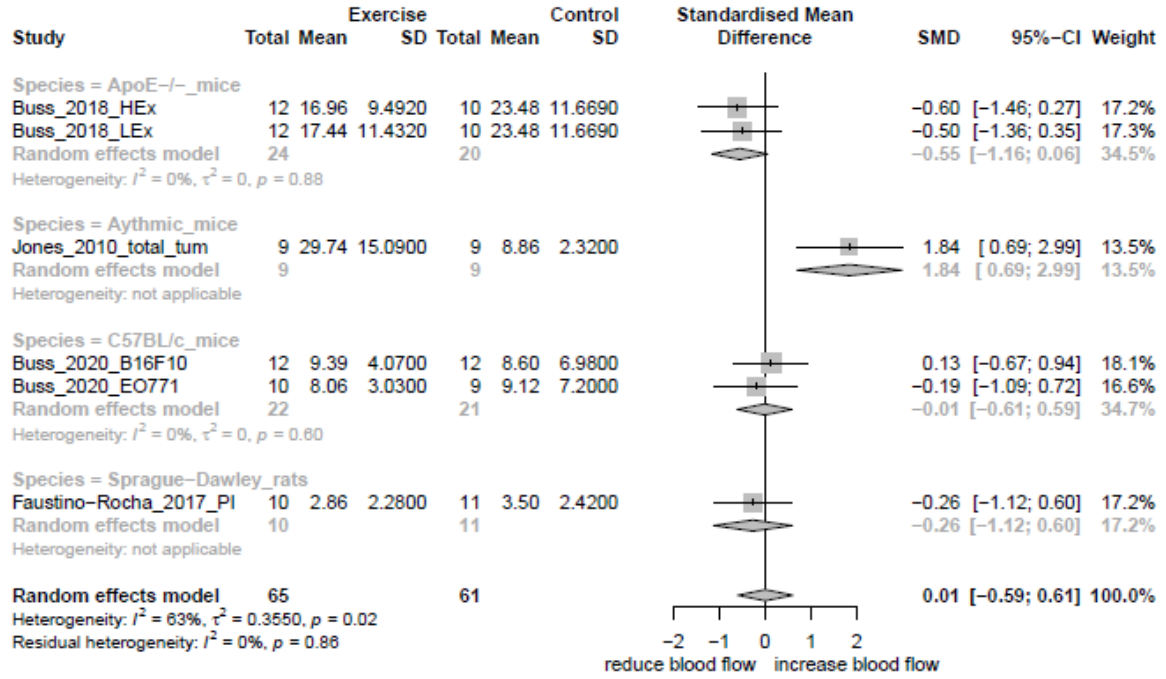
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.13: Sub-group analysis by exercise mode for blood flow



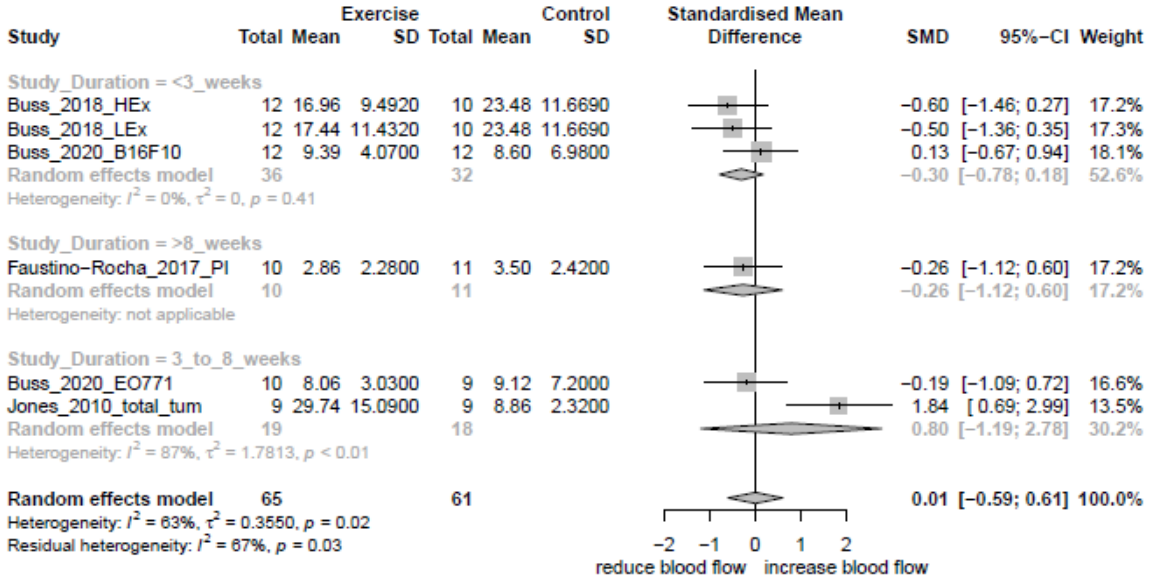
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.14: Sub-group analysis by animal species for blood flow



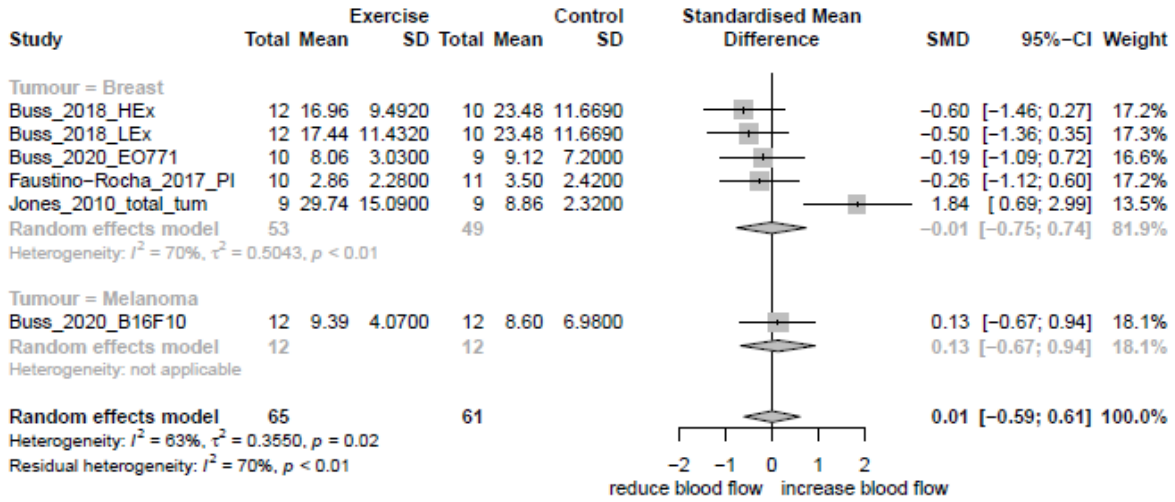
SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.15: Sub-group analysis by study duration for blood flow



SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

SUPPLEMENTARY FIGURE 2.16: Sub-group analysis by tumour type for blood flow



SD = standard deviation; SMD = standardised mean difference; 95% CI = 95% confidence interval (upper; lower limits)

Chapter 3

The effect of acute aerobic exercise intensity on blood flow to metastatic liver tumours using non-invasive imaging

ABSTRACT

Introduction: Tumours have heterogenous and dysfunctional vasculature that can cause a therapeutic barrier for intravenous chemotherapy delivery. As tumours lack a vasoconstrictive response, aerobic exercise may acutely increase tumour blood flow through increased cardiac output and potentially increase chemotherapy delivery. While preclinical studies have demonstrated increased tumour blood flow with moderate intensity aerobic exercise, no studies have assessed this effect in humans. This study examined the effect of acute aerobic exercise at varying intensities on tumour blood flow in people with liver metastases.

Methods: Participants with stage IV cancer with liver metastases performed an exercise test followed by three 5-minute bouts of light, moderate and high intensity cycling. Doppler ultrasound assessed blood flow to the hepatic artery (as a control) and liver tumour at baseline and after each exercise bout for 10-minutes.

Result: Eight participants completed the study, however, three were excluded from analysis due to a lack of sonographer confidence to identify tumour vessels for Doppler ultrasound, due to small size and poorly accessible locations. There was a 152% increase in blood flow (peak systolic velocity) to the tumour after moderate intensity exercise. There was also an increase in hepatic and tumour arterial resistance (resistive index) after high intensity exercise.

Conclusion: Moderate intensity exercise acutely increases blood flow to metastatic liver tumours. These findings support future work to examine whether aerobic exercise can improve clinical outcomes such as chemotherapy delivery and efficacy, particularly when delivered concurrent to infusion. Future research should incorporate larger sample sizes and examine other tumour types.

KEYWORDS: exercise, blood flow, tumour, ultrasound

INTRODUCTION

Intravenous chemotherapy is a common anti-cancer treatment administered to destroy cancer cells in tumours. Efficient chemotherapy relies on adequate doses of the chemotherapy agent to be delivered to the tumour, specifically the tumour centre, via the vascular system (Jain, 2013). Compared to healthy tissue, the tumour microenvironment has an abnormal structure and function (Wiggins et al, 2018). The tumour vascular network has a heterogenous vessel distribution leading to areas of both high and low blood vessel density. The vessels themselves are immature, lacking a normal vasoconstriction response (McCullough et al, 2014; Siemann et al, 2015), and have irregular endothelial cell distribution causing high permeability and increased interstitial pressure (Jain, 1990). In the tumour centre, these abnormalities result in collapsed vessels and reduced blood flow (Wu et al, 2013) leading to areas of low perfusion and hypoxic regions (Schumacher et al, 2021; Wiggins et al, 2018). This can cause a therapeutic barrier for chemotherapy delivery leading to less efficient chemotherapy response and poorer treatment outcomes.

Aerobic exercise is a promising intervention that may improve intravenous chemotherapy delivery to tumours and potentially modulate the dysfunctional tumour microenvironment (Wiggins et al, 2018). Aerobic exercise acutely increases CO and blood flow to tissues, and is mediated by vasoconstriction (shunting blood away from inactive tissue) and vasodilation (increasing vessel dilation to increase blood flow volume into active tissue) (Brooks 2005). In tumours, due to the abnormal vessel structure and lack of vasoconstriction response, an increase in CO with aerobic exercise may result in increased blood flow regardless of tissue activity signaling (Wiggins et al, 2018). With increased tumour blood flow, there is the possibility that aerobic exercise could increase intravenous chemotherapy delivery directly to the tumour.

Preclinical studies have demonstrated that acute moderate intensity aerobic exercise can increase tumour blood flow and induce favourable changes in tumour vasculature that promote tumour perfusion (Gomes-Santos et al, 2024; McCullough et al, 2013). However, there is currently no clinical evidence for the immediate acute effects of aerobic exercise on tumour blood flow in humans.

This study aims to address this gap and examine the effect of acute aerobic exercise at varying intensities on tumour blood flow in people with liver metastases, using non-invasive measurement techniques.

METHODS

Participants

Participants were recruited from Chris O'Brien Lifehouse, Australia (ANZCTR Trial ID ACTRN12619000846123). Participants were eligible if they met the following criteria: aged >18 years, diagnosed with stage IV cancer of any primary cancer, Eastern Cooperative Oncology Group (ECOG) 0-2 (Oken et al, 1982) and had at least 1 liver metastasis ≥1cm. Participants were excluded if they presented with an acute systemic infection or fever, had any condition that is a contraindication to exercise or were currently taking beta-blocker medication. Participants who were eligible were referred by their treating oncologist.

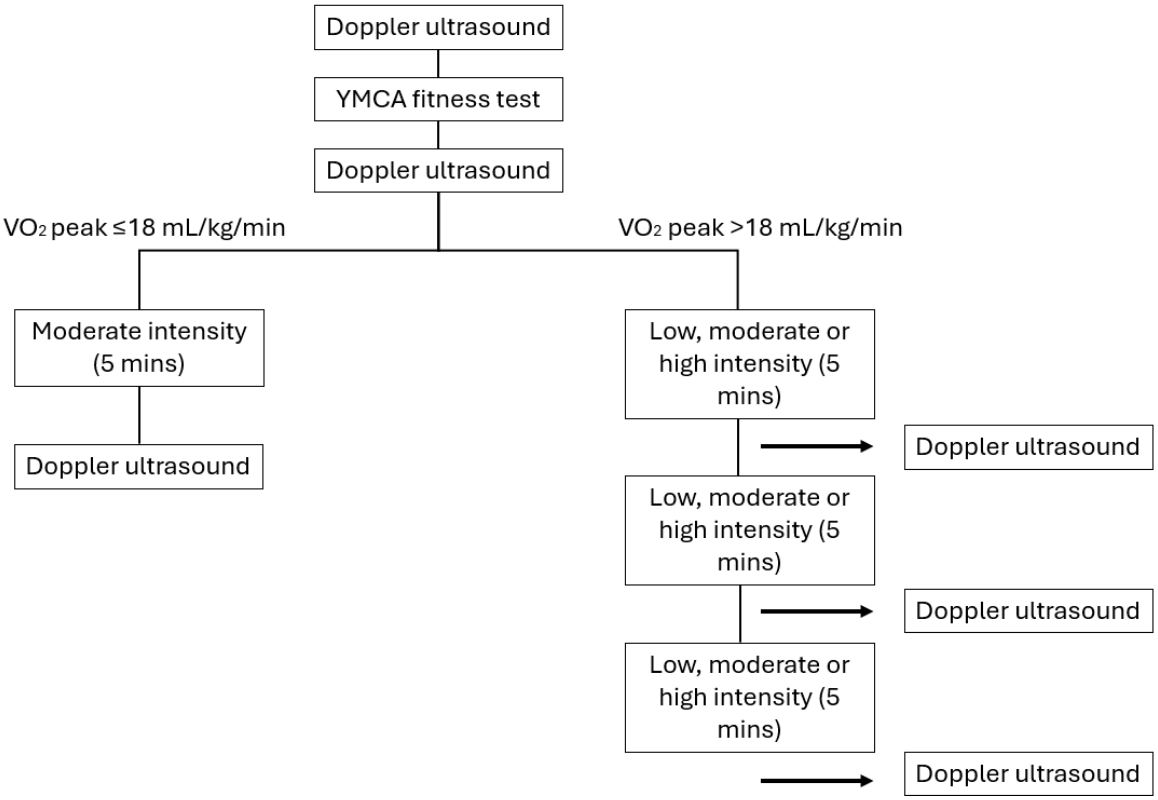
Study design

The study took place at the University of Sydney, Australia (ethics approval X19-0123 & 2019/ETH00544). After providing informed consent and completing demographic questionnaires, participants had their most accessible liver tumour identified by real-time ultrasound, and both this tumour vessel and their healthy hepatic artery (as a comparator) measured with Doppler, using a curved 1 - 5MHz transducer on a Sparq ultrasound machine (Philips Healthcare, Andover, Massachusetts, USA). Doppler ultrasound was chosen to measure blood flow as it is a non-invasive validated measurement of blood flow (Dodd 1994). Participants then undertook a YMCA submaximal cycle ergometer test on a recumbent bike (Lode Corival Recumbent) with expired gas analysis using a metabolic cart (Medgraphics Ultima CardiO2). After 10-minutes recovery, participants had ultrasound measures of their liver tumour vessel and hepatic artery.

Data from the YMCA test was used to calculate predicted VO_{2peak}. Participants with predicted VO_{2peak} ≤18 ml/kg/min performed a single 5-minute bout of moderate intensity cycling followed by 10-minutes of ultrasound measures. Participants with $VO_{2\,peak}$ > 18 ml/kg/min performed

three 5-minute bouts of light (27-37% VO_{2max}/20-30% HRR), moderate (46-56% VO_{2max}/40-50% HRR) and high intensity (64-74% VO_{2max}/60-70% HRR) cycling in a randomised order. This cut off for multiple intensity bout eligibility was based on a previous examination of fitness in individuals with liver metastases, which reported an average aerobic fitness (VO_{2peak}) of 18 ml/kg/min and demonstrated that this population were able to complete a high intensity exercise test to exhaustion (Dunne et al, 2016). At the completion of each exercise bout, the participant transferred immediately to a plinth for ultrasound measurements, alternating continuously between tumour vessel and hepatic artery, for 10-minutes. An overview of the study procedures can be seen in Figure 3.1.

FIGURE 3.1: Study schema



The order of intensities performed was randomised using a computer-generated random numbers list. Sealed opaque envelopes, prepared by the PI, containing the randomised exercise sequence were opened in front of the participant by the researcher on the day of testing.

Outcome measures

Participants completed baseline questionnaires including physical activity behaviour using the International Physical Activity Questionnaire (IPAQ) (Craig et al, 2003), QOL using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) (Aaronson et al, 1993) and fatigue using the Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue) (Webster, Cella & Yost, 2003). Demographic data (age, height, weight, and fat percentage via body impedance analysis (Tanita InnerScan)) and clinical information (including cancer diagnosis and medical history) was collected at baseline. During the exercise task and in recovery, HR and oxygen saturation (SpO2) was measured continuously and recorded every 2-minutes. BP was measured every 5-minutes. RPE (Borg, 1982) was measured every 5-minutes during the exercise bouts.

Doppler ultrasound was used to measure blood flow in the vessel of the single liver tumour and the hepatic artery, as previously identified immediately after exercise bout completion. Blood flow was unable to be measured during exercise due to movement artifacts. Peak systolic velocity (PSV), end diastolic velocity (EDV), and Resistive Index (RI); calculated as [PSV – EDV]/PSV) were recorded from the Doppler trace, with measures taken alternating between the tumour vessel and the hepatic artery (Supplementary Figures 1a and 1b). PSV refers to the velocity of blood flowing into a vessel during systole, EDV refers to the velocity of blood at the end of diastole, and RI refers to the resistance of blood flowing through a vessel (Baik 2010). The sonographer targeted the tumour vessel first (after each exercise segment) to detect maximum effect.

STATISTICAL ANALYSIS

SPSS (IBM SPSS Version 29.0.1.0 (171)) was used for the analysis. Wilcoxon signed rank test was used due to the small sample size, with PSV, EDV and RI baseline values compared to values taken in the first 3-minutes after exercise as the closest estimation of blood flow during exercise. All data are expressed as mean± SD. $P<0.05$ was considered significant.

RESULTS

Participants

A total of 11 patients were referred for recruitment to the study between December 2022 and October 2024. All patients met the eligibility criteria and eight patients (73%) consented to participate. Reasons for declining participation were all due to pain. Seven of the eight participants had VO_{2peak} >18 ml/kg/min. One participant had VO_{2peak} ≤18 ml/kg/min (15.14mL/kg/min), however, they completed the three exercise bouts due to a calculation error on the day of testing. No adverse events were recorded throughout the study for any of the participants. Three participants were excluded from analysis due a lack of sonographer confidence in identifying vessels due to the small size and/or difficult to access location of the tumour, resulting in a final analysed sample size of n=5. Demographics and baseline characteristics can be seen in Table 3.1. The participants in this study had lower fatigue scores than the general metastatic population (Vardy et al, 2014). In terms of QOL, relative to the average population with metastatic colorectal cancer the participants in this study had similar symptom scores but higher functioning scores (Conroy et al, 2010; Thomsen et al, 2017). Relative to a metastatic population, the participants in this study had a higher level of fitness suggesting greater physical activity levels than the general metastatic population (Yee et al, 2014).

TABLE 3.1: Demographics and baseline characteristics

	N=5 (%)
Age (years)	46±12.9
Sex	
Female	1 (20)
Male	4 (80)
BMI (kg/m^2)	22.8±1.7
Muscle (%)	51.7±21.2
Fat (%)	20.1±6.47
Cancer type	
Colorectal	3 (60)
Upper GI	1 (20)
Stomach	1 (20)
Current treatment	
Chemotherapy	5 (100)
Immunotherapy	3 (60)
VO_{2peak} (mL/kg/min)	28.7±9.9
IPAQ total score (met minutes/week)	3773.7±3187.2
EORTC-QLQ C30	
Global health status	75.0±23.57
Physical	97.33±5.963
Role	76.67±32.48
Emotional	81.67±20.75
Cognitive	90.0±14.91
Social	70.0±3.86
Fatigue	33.33±33.33
Nausea and vomiting	10.00±14.91
Pain	6.67±9.13
Dyspnoea	13.33±18.26
Insomnia	26.67±14.91
Appetite Loss	20.0±18.26
Constipation	26.67±27.89
Diarrhoea	6.67±14.91
Financial difficulties	13.33±18.26

FACIT-Fatigue score	33±13.2

Data presented as mean± SD

Physiological response to exercise

HR, systolic blood pressure (SBP) and perceived exertion rated on the Borg scale (RPE 6-20) increased as expected in a dose-response pattern with intensity (Table 3.2).

TABLE 3.2: Physiological measures

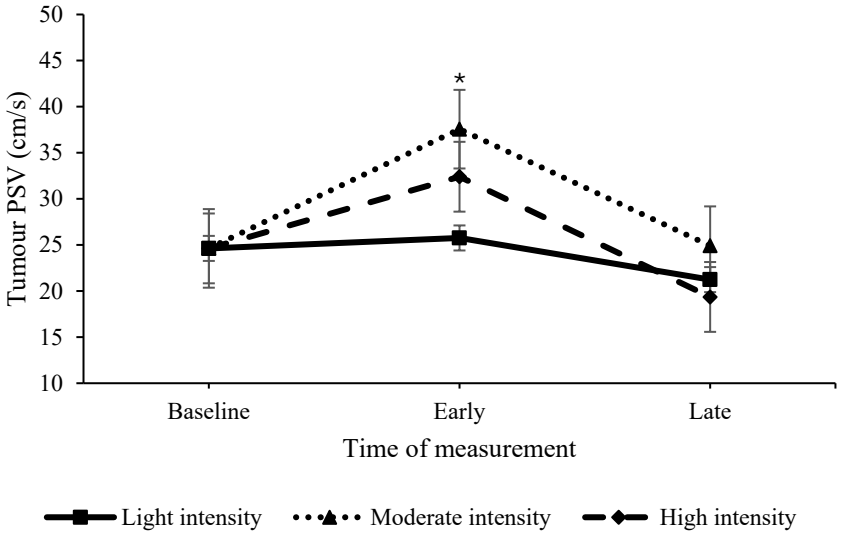
	Light intensity	Moderate intensity	High intensity
Heart rate (bpm)	103.6±12.7	120.12±10.8	132.0±10.4
Heart rate reserve (%)	27.8±5	44.7±5.8	56.4±3.5
Systolic blood pressure (mmHg)	128.6±12.4	147.2±16.7	165.2±26.0
Diastolic blood pressure (mmHg)	75.6±12.5	75.8±11.2	75.0±11.7
Rate of perceived exertion	8±1.2	10±1.6	13.5±1.5
Power output (watts)	37.4±22.0	78.6±34.7	117.6±53.0

Data presented as mean± SD

Blood flow measures

A significant increase of 152% compared to rest was observed in PSV in the tumour vessel after moderate intensity exercise ($z = 2.023$, $p = 0.043$) (Figure 3.2). There was no significant difference found for PSV in the tumour vessel after the light and high intensity bouts (Figure 3.2), and there was no significant change in EDV found for any exercise intensity in the tumour vessel (Figure 3.3).

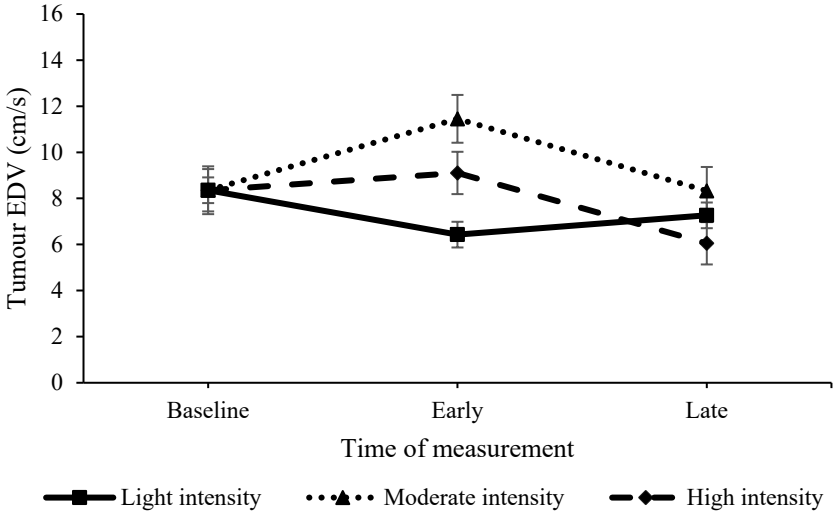
FIGURE 3.2: Tumour PSV changes



Early = within first 3-minutes of exercise cessation, late = 10-minutes after exercise cessation.

* $p<0.05$

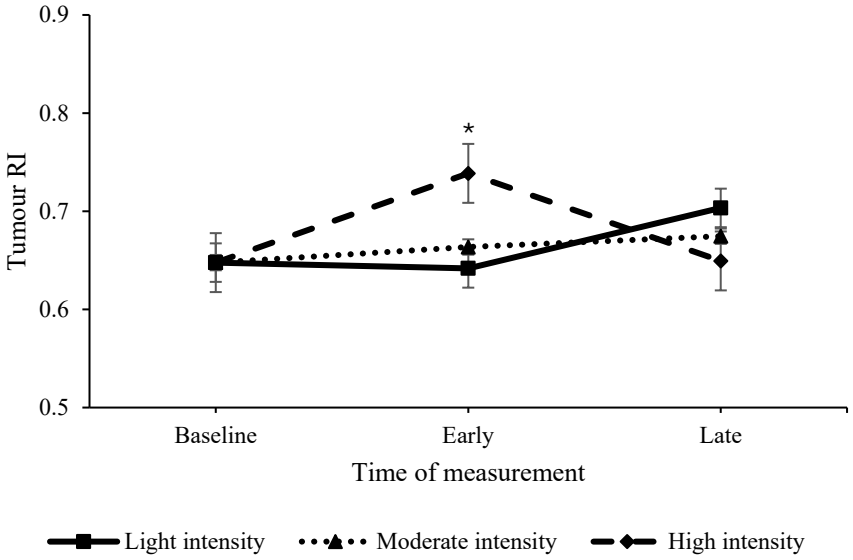
FIGURE 3.3: Tumour EDV changes



Early = within first 3-minutes of exercise cessation, late = 10-minutes after exercise cessation

The RI of the tumour vessel showed a significant increase of 114% (0.74±0.15) after the high intensity bout (z = 2.023, p = 0.043), but no difference after the light or moderate intensity bouts (Figure 3.4).

FIGURE 3.4: Tumour RI changes

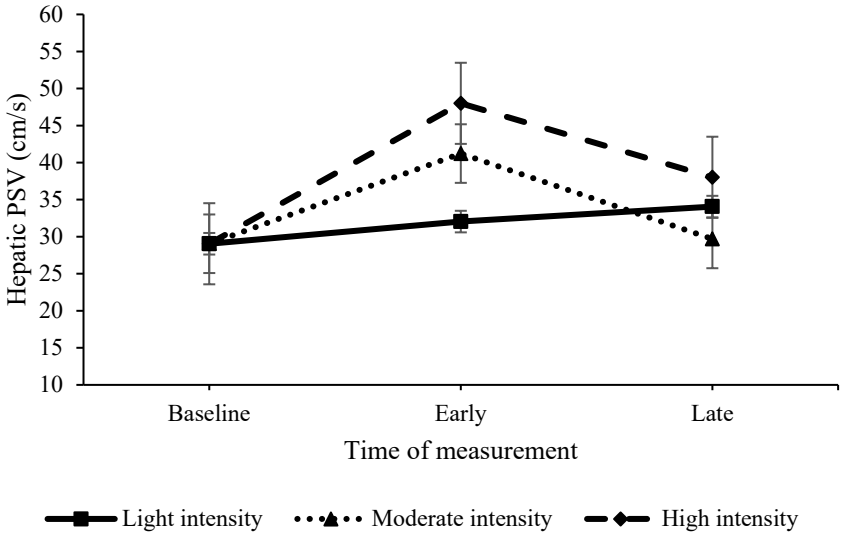


Early = within first 3-minutes of exercise cessation, late = 10-minutes after exercise cessation

* p<0.05

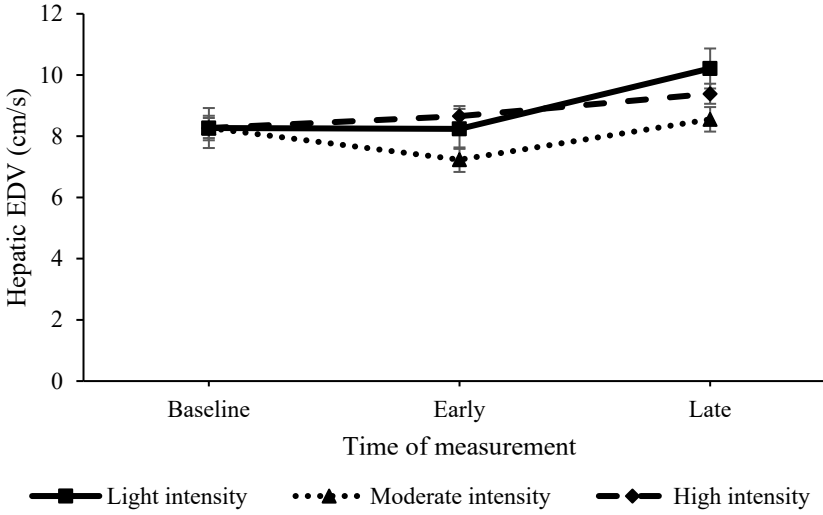
For the hepatic artery, there was no significance difference between baseline and post exercise in PSV or EDV at any intensities (Figures 3.5 and 3.6). For the hepatic artery RI measurements, similar to the RI changes in the tumour, there was a significant increase of 113% (0.81±0.15) after the high intensity bout (z = 2.023, p = 0.043), with no differences seen after the light and moderate intensity bouts (Figure 3.7).

FIGURE 3.5: Hepatic PSV changes



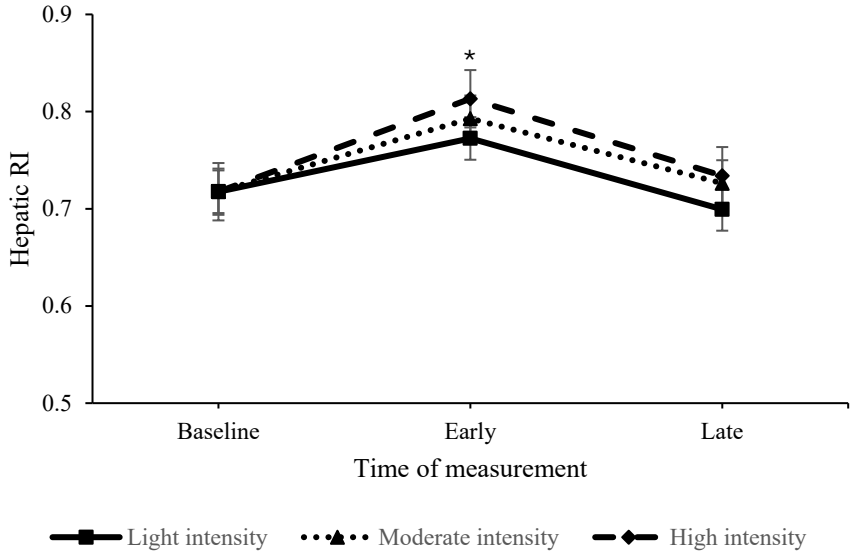
Early = within first 3-minutes of exercise cessation, late = 10-minutes after exercise cessation

FIGURE 3.6: Hepatic EDV changes



Early = within first 3-minutes of exercise cessation, late = 10-minutes after exercise cessation

FIGURE 3.7: Hepatic RI changes



Early = within first 3-minutes of exercise cessation, late = 10-minutes after exercise cessation

* $p<0.05$

DISCUSSION

This study demonstrates that acute moderate intensity aerobic exercise elicits increased blood flow to metastatic liver tumours in humans, using non-invasive measurement techniques. This result is consistent with the findings of published preclinical work. For example, Garcia et al (2016) measured blood flow in rats with prostate tumours that exercised for 5-minutes at moderate intensity. The authors found ~180% increase in blood flow during exercise compared to rest. Similarly, McCullough et al (2014) examined rats with prostate tumours that exercised for 5-minutes at moderate intensity and found a 200% increase in blood flow during exercise compared to rest. Our findings likely represent a conservative estimate of increases in blood flow as measures were taken after exercise rather than during exercise, which may explain the smaller magnitude of change observed. McCullough and colleagues (2014) also found an increase in the number of patent vessels and coupled with the increase in blood flow and found a ~50% reduction in hypoxia. Although we did not measure hypoxia or number of patent vessels, the similar blood flow findings at the same exercise intensity suggests that changes in patent blood vessels and hypoxia may also occur in a clinical population.

Although there is clinical evidence for the effects of chronic aerobic exercise on tumour blood flow (Florez Bedoya et al, 2019; Jones et al, 2013; Schumacher et al, 2025), there is only one

other clinical study to our knowledge that investigated the effects of acute aerobic exercise on the tumour microenvironment. Djurhuss et al (2022) investigated whether a single bout of high intensity interval exercise caused tumour microenvironment remodelling in men with prostate cancer. Participants cycled for 4 x 1-minute high intensity bouts (100% of peak power output) one day prior to a radical prostatectomy. Tumour microvessel density was assessed using tumour tissue sections taken during the prostatectomy but tumour blood flow was not measured. The authors found that there was no change in hypoxia or microvessel density compared to no exercise the day prior to surgery. It is possible that despite a lack of change in hypoxia and vessel density, acute blood flow changes occurred but were not sustained.

In the current study, we found that low and high intensity exercise did not elicit significant blood flow changes to the tumour. Given the lack of vasoconstrictive response in tumours, we expected to see a linear increase in tumour blood flow in parallel to the increase in CO with increasing exercise intensities. In the low intensity exercise bout, it is possible that the increase in CO did not sufficiently increase blood flow to the host tissue (liver) and therefore did not change tumour blood flow. In line with pre-clinical work, Suzuki et al (1981) demonstrated that liver blood flow increased with pharmacologically induced hypertension up to 145 mmHg, but then declined. Our results are consistent with this finding, with SBP during moderate exercise ~147mmHg, which may have been optimal for enhancing tumour blood flow. In contrast, during high intensity exercise, where SBP was ~162mmHg, we found no difference from baseline. Although care should be taken when translating pressure differences between humans and animals, the greater BP elicited during high intensity exercise may have induced a vasoconstrictive response in the hepatic artery that shunted blood away from the host tissue (liver) (Trefts & Wasserman, 2022) and resulted in the lower PSV in the tumour.

We observed a significant increase in tumour and hepatic RI with high intensity exercise with no-significant change in PSV or EDV. Given that hepatic RI increased likely due to vasoconstriction (Trefts & Wasserman, 2022), it is possible that there was also a vasoconstrictive response in the tumour elicited by the higher intensity. It may also relate to the heterogenous tumour blood vessel distribution and dysfunction interacting with changed cardiovascular variables. This finding may reflect changes in vessel function at higher pressures, but it should also be acknowledged that technical difficulty in obtaining accurate readings due to the movement artefacts of heavy respiration post-exercise could also

contribute to these findings. Further examination of vessel responses to higher blood pressures such as those elicited by higher intensity exercise may provide further elucidation.

Given there are few clinical studies, the implications of our results are significant. This study has demonstrated that moderate intensity exercise increases blood flow to metastatic liver tumours. If generalisable to tumours in other tissues, this effect might be harnessed to enhance drug delivery during treatment. Exercise performed simultaneous to chemotherapy infusion, termed intra-infusion exercise, has been proposed as a novel adjuvant to treatment using this mechanism. Pilot studies have demonstrated that low intensity intra-infusion exercise is safe and feasible (Kerrigan et al, 2022; McLaughlin, Christie & Campbell, 2019; Thomas et al, 2019). Combined with our findings, these studies support the need for future work to examine the clinical effect of intra-infusion exercise to determine if increased blood flow elicited by exercise subsequently affects treatment efficacy as hypothesised through increasing drug delivery to target tumours.

It is also important to consider the potential contribution of chronic exercise-induced adaptations. Repeated bouts of aerobic exercise (exercise training) can induce adaptions to the tumour vasculature such as increased blood vessel density, improved organisation and improved vessel function (Seet-Lee et al, 2022). These vasculature adaptations have been suggested to be stimulated by the cumulative effects of each acute bout. We provide initial evidence for the acute effect of exercise on tumour blood flow which may be used to further understand the chronic adaptations. Similarly, it is important to recognise that chronic exercise-induced adaptations may also augment acute blood flow delivery by increasing the number of normalised blood vessels (Seet-Lee et al, 2022). We saw that our cohort had higher mean VO_{2peak} relative to patients with metastatic liver cancer (Dunne et al, 2016) suggesting that the participants performed repeated bouts of aerobic exercise that may have induced tumour microenvironment adaptations. Due to the small sample size, we cannot assess the association between physical fitness and tumour blood flow, but we note that the participant with the highest predicted VO_{2peak} had the greatest PSV response after moderate intensity exercise. Future research with larger sample sizes should consider tumour vascular adaptations from chronic exercise that may influence acute blood flow delivery effects.

This study has several limitations. It included a very small sample size reflecting the difficulty in recruiting people with advanced metastatic cancer, well enough for oncologist referral to an exercise trial, to complete a single visit exercise trial with no likely individual benefit. Furthermore, it was not possible to measure blood flow with Doppler ultrasound whilst exercising due to movement artefact. Although we were able to obtain tumour vessel measures mostly within 1-minute post exercise, the sudden cessation of exercise along with the slight delay in measurement and the change in position from sitting to supine likely meant blood flow was lower than would have been observed during exercise.

CONCLUSION

In summary, this study provides initial clinical evidence that acute moderate intensity aerobic exercise increases blood flow to liver tumours. While replication in larger samples is essential, this study is a step toward the potential integration of aerobic exercise prescription into standard cancer treatment as a potential adjuvant strategy to improve chemotherapy delivery and efficacy.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 3.1: Mean PSV data

	Tumour			Hepatic		
	Light	Moderate	High	Light	Moderate	High
Baseline	24.63±5.66	24.63±5.66	24.63±5.66	29.05±6.69	29.05±6.69	29.05±6.69
Early	25.76±7.63	37.56±5.91^{+}	32.4±12.52	32.04±10.11	41.22±15.71	48.0±17.03
Late	21.24±6.67	24.92±4.56	19.36±5.08	34.06±7.70	29.7±6.25	38.02±13.54

N=5. Data is presented as mean±SD. $^{+}$p<0.05

SUPPLEMENTARY TABLE 3.2: Mean EDV data

	Tumour			Hepatic		
	Light	Moderate	High	Light	Moderate	High
Baseline	8.35±3.72	8.35±3.72	8.35±3.72	8.27±4.48	8.27±4.48	8.27±4.48
Early	6.43±1.15	11.45±3.80	9.1±7.76	8.24±3.12	7.23±0.96	8.66±2.54
Late	7.26±1.45	8.33±3.84	6.05±3.56	10.21±4.23	8.55±3.92	9.39±2.88

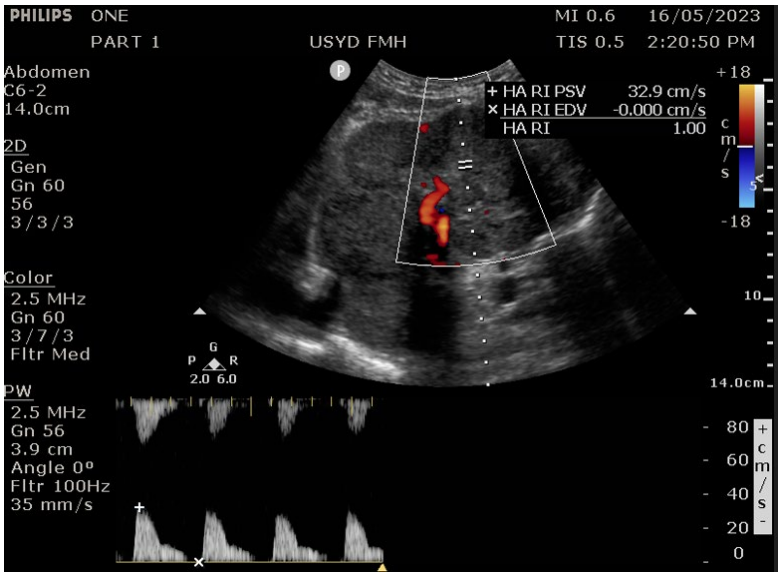
N=5. Data is presented as mean±SD. $^{+}$p<0.05

SUPPLEMENTARY TABLE 3.3: Mean RI data

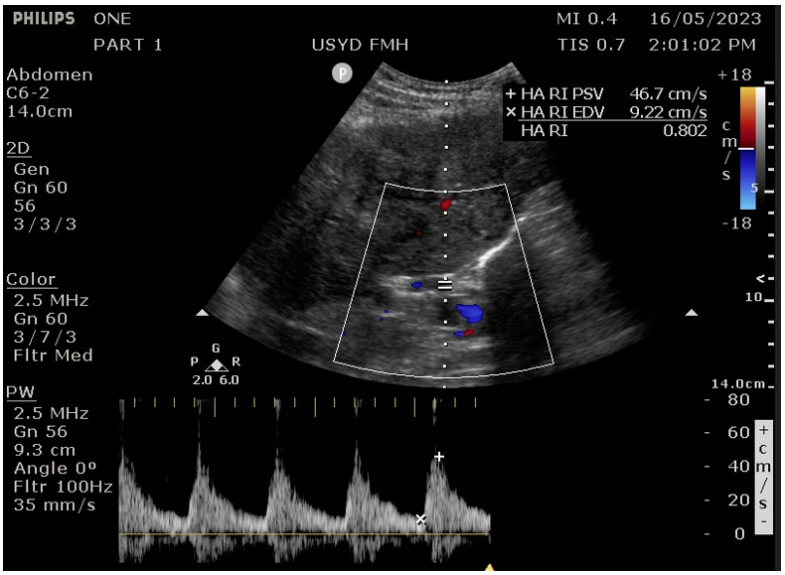
	Tumour			Hepatic		
	Light	Moderate	High	Light	Moderate	High
Baseline	0.65±0.14	0.65±0.14	0.65±0.14	0.72±0.14	0.72±0.14	0.72±0.14
Early	0.64±0.56	0.66±0.14	0.74±0.15^{+}	0.77±0.09	0.79±0.11	0.81±0.15^{+}
Late	0.70±0.18	0.67±0.13	0.65±0.21	0.70±0.12	0.73±0.12	0.73±0.16

N=5. Data is presented as mean±SD. $^{+}$ $p<0.05$

SUPPLEMENTARY FIGURE 3.1: Doppler trace of hepatic artery



SUPPLEMENTARY FIGURE 3.2: Doppler trace of liver tumour vessel



Chapter 4

Does concurrent chemotherapy infusion change physiological responses to exercise? A brief report

ABSTRACT

Introduction: Aerobic exercise performed whilst receiving intravenous chemotherapy infusion (intra-infusion exercise) may increase chemotherapy delivery to tumours via increased cardiac output and blood flow. However, it is unknown whether intra-infusion exercise alters the physiological responses to exercise. This crossover study aimed to evaluate the physiological responses to intra-infusion aerobic exercise compared to aerobic exercise alone.

Methods: Eight participants with stage I-III breast, colorectal or ovarian cancer performed intra-infusion exercise (on-chemotherapy) and an additional cycling session outside of chemotherapy infusion (off-chemotherapy) for 20-minutes on a stationary foot bike at moderate intensity (40-50% heart rate reserve, 12-14 rate of perceived effort (RPE)). Outcome measures included heart rate, blood pressure), oxygen saturation and RPE.

Results: Oxygen saturation remained >95% throughout both tasks. Heart rate and RPE were similar between conditions during the task. Blood pressure showed greater increases during exercise in the on-chemotherapy condition compared with off-chemotherapy (d=0.61-1.0). Power output and revolutions per minute showed moderate effects of condition with greater values observed in the off-chemotherapy condition.

Conclusion: Intra-infusion exercise exaggerates BP and HR response at the same power output compared to aerobic exercise alone, indicating the importance of monitoring for safety and use of HR rather than power output for exercise intensity in future studies.

KEYWORDS: chemotherapy, exercise, physiological response, heart rate, blood pressure

INTRODUCTION

Performing aerobic exercise whilst receiving chemotherapy infusion, termed intra-infusion exercise, may improve treatment efficacy by increasing blood flow to the tumour. Exercise-induced increases in BP and CO, may result in increased tumour blood flow due to dysfunctional tumour vasculature's inability to vasoconstrict (Wiggins et al, 2018). As the use of intra-infusion exercise is evolving, it is important to determine if hemodynamic responses to exercise are altered to guide the safety monitoring needs of this novel intervention.

During intravenous chemotherapy infusion, physiological responses may be affected by changes to blood volume through both fluid delivery and the chemotherapy agent itself. Chemotherapy has been noted to increase BP, potentially mediated by reduced nitric oxide generation, oxidative stress, endothelial dysfunction and vascular rarefaction (Cohen et al, 2023). Additionally, it is possible that any acute cardiotoxic effect of chemotherapy may be exacerbated by greater myocardial blood flow or related autonomic dysfunction which may combine to alter response to exercise (Ekholm et al, 2000).

Therefore, the aim of this crossover study was to evaluate the physiological responses to intra-infusion aerobic exercise compared to intensity-matched aerobic exercise alone.

METHODS

Participants in the EX-FUSION study conducted at Chris O'Brien Lifehouse in Australia (ACTRN12621000677808) who exercised whilst receiving chemotherapy infusion (on-chemotherapy) were invited to participant in an additional cycling intervention session outside of chemotherapy infusion (off-chemotherapy). Participants were eligible for the EX-FUSION study if they had stage I-III breast, colorectal or ovarian cancer and were receiving intravenous chemotherapy treatment >60-minutes duration (Table 4.1).

TABLE 4.1: Eligibility criteria for EX-FUSION study

Inclusion criteria	Exclusion criteria
• English speaking • Aged 18-75 years old • Histologically confirmed stage I-III breast, ovarian or colorectal cancer • Current or planned intravenous chemotherapy (minimum 4 cycles with duration >60 minutes) • Have undergone at least 1 cycle of intravenous chemotherapy • ECOG 0-2 • Medical clearance from oncologist • Willing and able to comply with all study requirements	• Presents with an acute systemic infection or fever • Has a known adverse reaction to infusion • Presents with any condition that is contraindicated to exercise • Presents with significant orthopaedic problems, peripheral neuropathy or pain that would limit lower body exercise • Currently taking beta-blocker medications • Currently receiving immunotherapy • Currently receiving anthracycline chemotherapy

Participants cycled on a foot bike (Monark 881E) whilst seated in their usual chemotherapy chair, for 20-minutes continuously at moderate intensity (40-50% HRR, rate of perceived exertion (RPE) 12-14), supervised by an exercise physiologist. HRR was calculated using HR_{max} - HR_{rest}, with HRmax calculated using 220 - age. Physiological measures were recorded for 10-minutes before (baseline) and after (recovery) the cycling intervention, as well as during (task) the cycling intervention. The order of the on-off cycling intervention was opportunistic depending on availability of the participant.

HR and SpO2 were recorded every minute using a finger pulse oximeter (Edan H100B Hand Held Pulse Oximeter). BP was recorded manually every 5-minutes (manual sphygmomanometer). During the task, power output (Watts) and speed of cycling (revolutions per minute (RPM)) were recorded and RPE every 2nd minute using the 6-20 RPE scale (Borg, 1982).

STATISTICAL ANALYSIS

Descriptive statistics were used for baseline characteristics. For all outcome measures (HR, BP, SpO2 and RPE), data were aligned by calculating 5-minute averages. Timepoints for baseline, task and recovery were analysed using the averages of data collected in the final 5-minutes. The change score from baseline to task was calculated and compared between on and off conditions with an effect size calculated for the difference between change scores. Cohen's d was calculated using G*Power (Faul et al, 2009) with 0.2 considered small, 0.5 considered medium and 0.8 considered large (Cohen, 1988), and only moderate and large effect sizes are discussed. Due to the small sample size, no probability testing was performed.

RESULTS

Participants

Eight participants completed the study (Table 4.2). Fifty percent of participants cycled off-chemotherapy prior to on-chemotherapy. The average time between on-off interventions was 13.5±9.7 days.

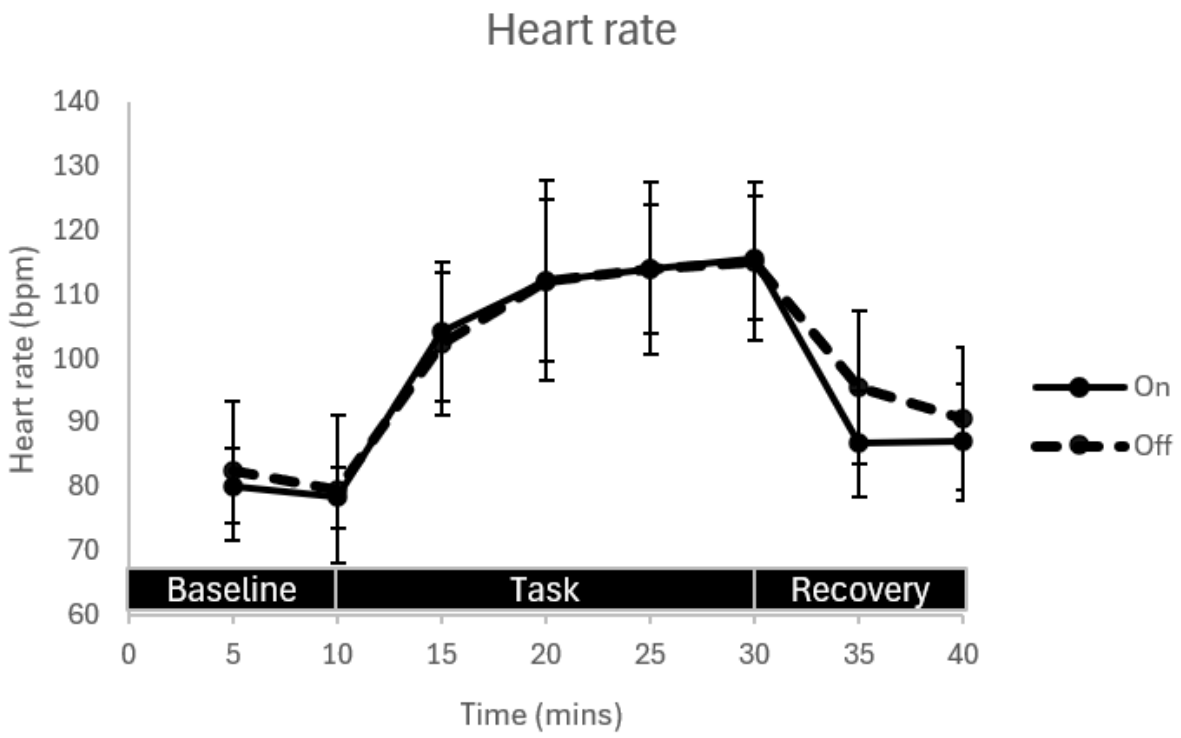
TABLE 4.2: Demographics and chemotherapy treatment

	N=8 (%)
Age (years)	53.4±13.7
Sex	
Female	7 (88)
Male	1 (12)
BMI (kg/m^2)	23.7±4.8
Cancer type	
Breast	4 (50)
Ovarian	3 (38)
Colorectal	1 (12)
Chemotherapy agent	
Paclitaxel	1 (12)
Carboplatin + paclitaxel	3 (38)
Docetaxel, carboplatin, trastuzumab + pertuzumab (TCHP)	1 (12)
Docetaxel + cyclophosphamide	2 (25)
Folinic acid, fluorauracil + oxiaplatin (FOLFOX)	1 (12)
Chemotherapy frequency	
Weekly	1 (12)
Fortnightly	1 (12)
3-weekly	6 (75)
Total number of chemotherapy cycles completed prior to EX-FUSION study enrolment	
2 cycles	3 (38)
3 cycles	1 (12)
6 cycles	3 (38)
8 cycles	1 (12)
Order of cycling	
On then off	4 (50)
Off then on	4 (50)

Heart rate and Rate of Perceived Effort

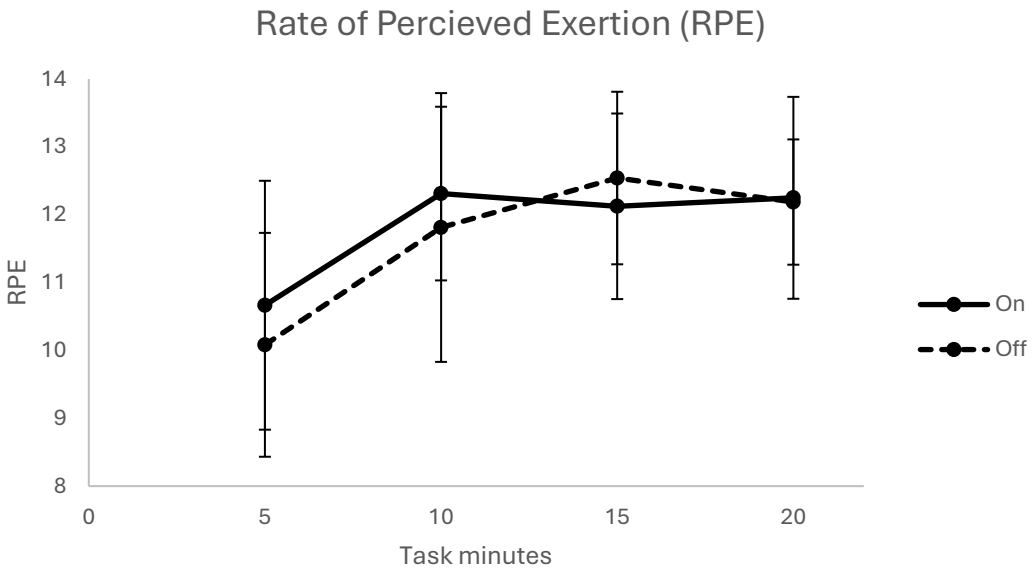
As expected, given the task was prescribed using these criteria, HR increased similarly from baseline to task in both conditions (Figure 4.1). RPE was similar between conditions (On=11.84±1.15, Off =11.66±1.19, Figure 4.2).

FIGURE 4.1: Heart rate



Data presented as mean±SD.

FIGURE 4.2: Rate of Perceived Exertion



Data presented as mean±SD.

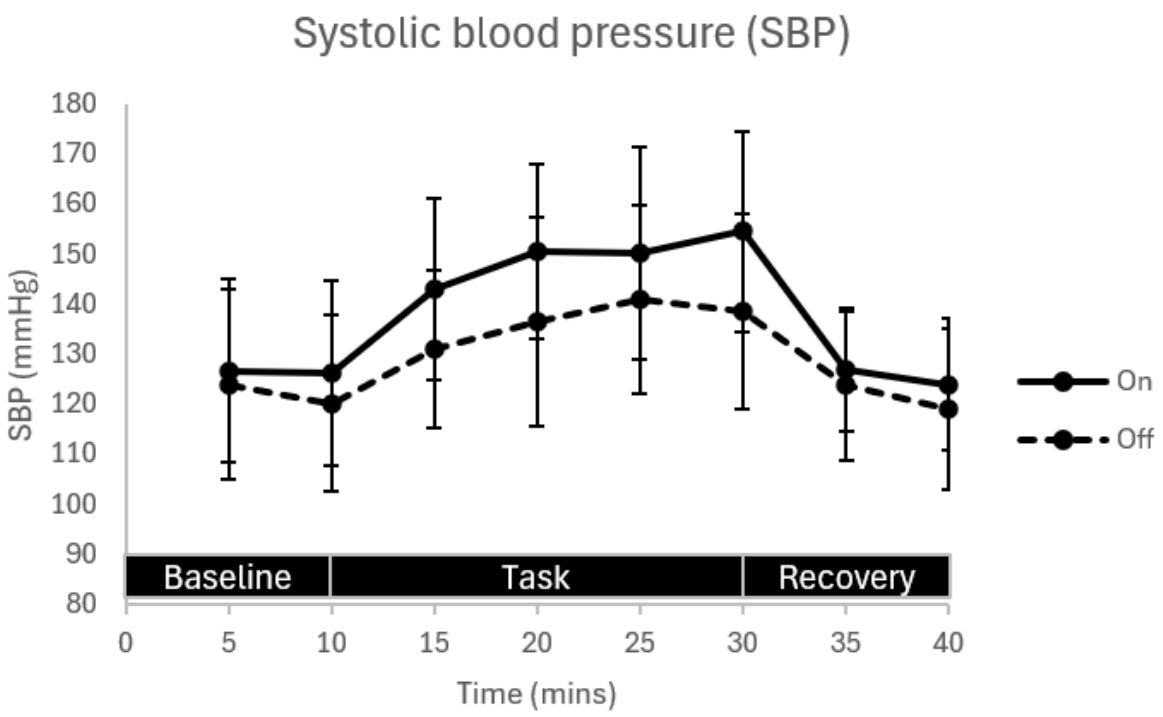
Oxygen saturation

SpO2 remained above 95% at all timepoints across both conditions and change from baseline to task was similar between conditions.

Blood pressure

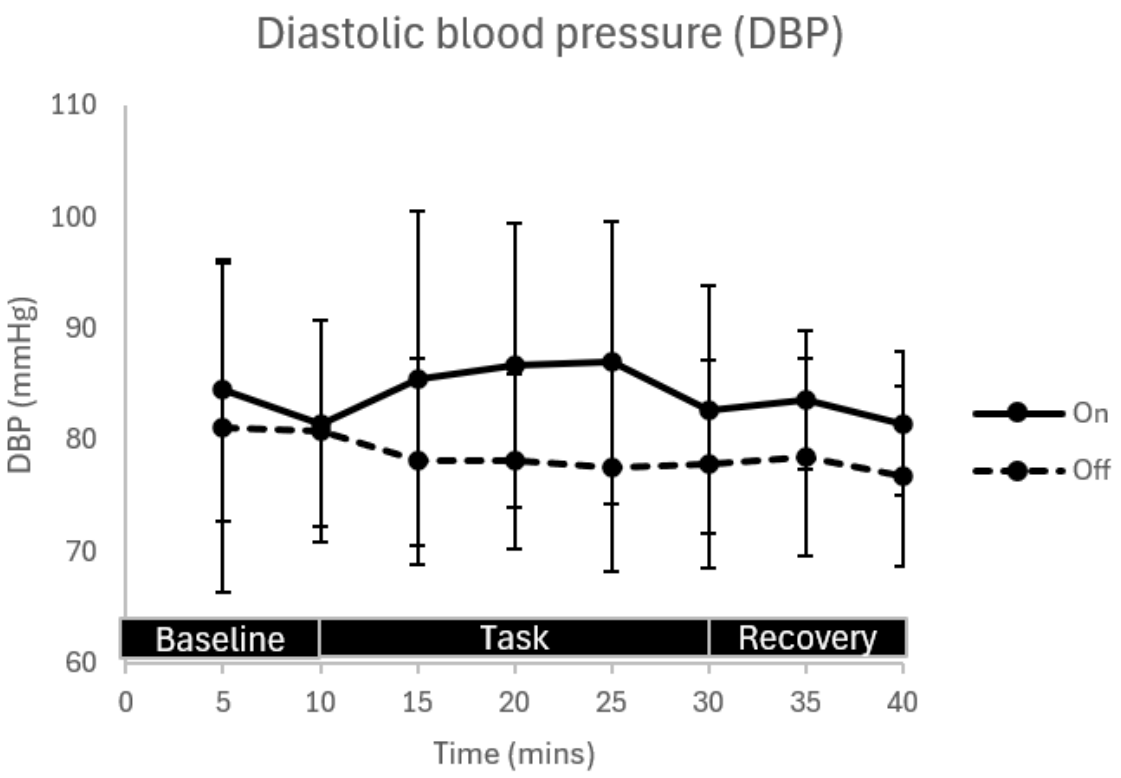
BP responses are illustrated in Figures 4.3 and 4.4. For both SBP and diastolic blood pressure (DBP) change from baseline to task showed moderate to large effect sizes between conditions (SBP d=0.61, DBP d=1.0) with greater changes during the on-chemotherapy condition.

FIGURE 4.3: Systolic blood pressure



Data presented as mean±SD.

FIGURE 4.4: Diastolic blood pressure



Data presented as mean±SD.

Power output

Power output and RPM between conditions showed a moderate effect sizes (Power output On=11.65±1.44 Watts, Off=12.54±1.27 Watts, d=0.65; RPM On=65±8.01, Off=70±7.08, d=0.75).

DISCUSSION

This is the first comparison of physiological responses to intra-infusion exercise compared to exercise alone.

SBP and DBP showed greater responses in the on-chemotherapy condition. From a clinical standpoint, these differences in BP are important as increases in BP present an increased risk of an occurrence of a cardiovascular event (Chrysant, 2020) and should be monitored. It can be hypothesised that the fluid volume from intravenous chemotherapy infusion increased total

blood volume and consequently increased BP. It is also possible that the chemotherapy agent itself contributed to the increased BP. Platinum-based chemotherapy agents, which were received by 63% of our cohort, can cause hypertension due to endothelial dysfunction (Cohen et al, 2023). It is also possible that chemotherapy causes adverse changes to blood vessel structure, specifically arterial stiffness, that impact on BP responses (Frye et al, 2018). However, these effects are chronic changes that likely do not explain the difference we observed in tasks ~ 14 days apart. Given our findings of elevated BP with intra-infusion exercise, future studies should consider the combination of intra-infusion exercise with agents known to have hypertensive effects specifically in patients who are already hypertensive. From an exercise safety standpoint, our findings highlight the need for regular BP monitoring with intra-infusion exercise, particularly for patients with a higher resting BP, to ensure that patients exercise safely within guidelines.

Our exercise tasks were matched on HR, yet we observed a moderate effect of condition on power output, with lower values during the on-chemotherapy condition. This suggests an exaggerated HR response relative to workload during intra-infusion exercise. Our limited data precludes us from determining the cause of this exaggerated HR response but indicates that mechanistic studies may investigate cardiovascular parameters further regarding intra-infusion exercise. It is possible that chemotherapy agents contribute to autonomic dysfunction, resulting in altered cardiac responses (Coumbe & Groarke,2018). Further, the psychological stress of the chemotherapy treatment could also have contributed to increased beta-adrenergic stimulation to elevate HR. Clinically, this finding highlights the importance of monitoring HR and RPE during intra-infusion exercise to ensure that patients exercise within safe physiological limits.

CONCLUSION

The results from this study suggest that chemotherapy may change heart rate and blood pressure responses of aerobic exercise. Of significance, there was a moderate to large effect in BP response during intra-infusion exercise compared to aerobic exercise alone. Our findings highlight that patients performing intra-infusion exercise should have HR and BP regularly monitored to ensure that patients exercise safely within exercise guidelines.

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Chapter 5

The effect of intra-infusion exercise on chemotherapy side effects: the EX-FUSION study

ABSTRACT

Introduction: People undergoing chemotherapy treatment do not meet exercise guidelines, despite known benefits in reducing side effects. Barriers including treatment-related side effects, time, and limited access and education are most cited. Intra-infusion exercise - exercise whilst receiving chemotherapy infusion - may help overcome these barriers to increase exercise during this treatment phase. The EX-FUSION study evaluated the effect of intra-infusion exercise on chemotherapy side effects and physical activity behaviour.

Methods: Forty-five participants with stage I-III breast, colorectal and ovarian cancer were randomised to intra-infusion exercise or usual care for a baseline and three intervention chemotherapy cycles. The exercise group cycled on a foot bike at moderate intensity for 20-minutes whilst receiving chemotherapy infusion. Both groups received exercise education by a clinical exercise professional. Fatigue was the primary outcome, and secondary outcomes included other chemotherapy side effects such as peripheral neuropathy, physical activity, quality of life and resting heart rate.

Results: Adherence to the exercise program was 100% with no adverse events reported. There was an expected increase in fatigue symptoms, resting heart rate and peripheral neuropathy in both groups with each chemotherapy cycle, however no overall effects of intervention nor difference between groups in change over chemotherapy cycles were observed. There was also no difference between intervention groups in terms of physical activity or quality of life.

Conclusion: Intra-infusion exercise has excellent adherence and does not increase chemotherapy side effects compared to usual care. Although 20-minutes of intra-infusion exercise alone did not alter exercise behaviour, the intra-infusion delivery provided an additional opportunity to exercise. Future work should investigate the effects of intra-infusion exercise on tumour blood flow, chemotherapy efficacy and relative dose intensity.

KEYWORDS: exercise, intra-infusion, chemotherapy, side effects

INTRODUCTION

People receiving chemotherapy experience treatment-related side effects which impact QOL with fatigue one of the most commonly reported side effects (Hofman et al, 2007). Aerobic exercise during treatment can improve QoL, reducing chemotherapy-related side effects such as fatigue (Campbell et al, 2019; Cormie et al, 2017; Cramp & Byron-Daniel, 2012; Zou et al, 2014). Indeed, exercise provides the most potent treatment to reduce fatigue when compared to pharmaceutical options (Cramp et al, 2012; Fontvieille et al, 2024; Mustian et al, 2017). Although this effect of exercise is well known and therefore, exercise is recommended in position statements and white papers, less than 30% of people with cancer meet the guidelines for aerobic exercise of 150-minutes of moderate intensity or 75-minutes of high intensity exercise per week (Cormie et al, 2018; Galvao et al, 2015). Barriers to exercise include treatment-related side effects (especially fatigue), limited exercise education, insufficient support from healthcare professionals, and lack of time and access (Clifford et al, 2018; Granger et al, 2017). Further, there often develops a vicious cycle where physical inactivity, driven by these barriers, exacerbates symptom severity and creates additional barriers to exercise initiation and adherence.

Intra-infusion exercise, which involves exercising whilst receiving chemotherapy infusion, is an innovative approach to exercise delivery that allows patients to be active when they would otherwise be sedentary for up to many hours. This approach may help overcome barriers related to time and access to exercise facilities and exercise professionals (Clifford et al, 2018, Elshahat, Treanor & Donnelly, 2021; Galvao et al, 2015; LeMasters et al, 2014). Supervision by a clinical exercise professional in a hospital environment may reduce safety concerns and boost exercise confidence and thereby encourage positive exercise behaviours. Intra-infusion exercise that is supervised by a clinical exercise professional could promote progression along the stage of change continuum and improve self-efficacy by providing an additional exercise opportunity along with exercise education. By providing the opportunity to exercise under supervision, external and internal motivational factors that cause barriers to exercise participation may be counteracted (Husebo et al, 2012). Consequently, there is a shift in stage of change as exercise

adherence is increased, for example from preparation to action, and this can lead to improved self-efficacy.

Although not a focus of the current study, preliminary preclinical data shows that moderate intensity aerobic exercise dramatically increases blood flow to tumours, indicating potential for a drug delivery advantage with intra-infusion exercise (McCullough et al, 2014). If blood flow is increased to the tumour, then greater doses of intravenous chemotherapy can be delivered directly to the tumour, thereby improving treatment efficacy.

This study builds upon existing evidence for safety and feasibility of intra-infusion exercise. We reported the first human study of a single bout of intra-infusion aerobic exercise, demonstrating its safety and feasibility (Thomas et al, 2019). In this randomised crossover trial, ten participants undergoing chemotherapy participated in both a usual care and an intra-infusion cycling session (20-minutes of low intensity cycling on a portable cycle ergometer) during infusion. Adverse events were recorded during and after the infusion. Zero adverse events were recorded during or after exercise. Compared to usual care, participants reported the intra-infusion exercise session was no more difficult and less boring, with no difference in symptoms experienced in the week following infusion.

There have been three other investigations of low-moderate intensity intra-infusion exercise, two studies that assessed the safety and acceptability and one study that assessed mood. Kerrigan et al. (2022) reported a non-randomised trial in participants with early-stage breast cancer (N=14), five of whom performed light intensity cycling during infusion, in addition to a supervised exercise program outside of infusion performed by all participants. There were fewer clinically meaningful adverse events, such as anaemia and fever, in the 3-weeks after each infusion with exercise compared to infusions without exercise (12% vs 53%, respectively). A case report of one patient who performed intra-infusion cycling in addition to a supervised exercise program outside of infusion reported 100% adherence and no adverse events (McLaughlin, Christie & Campbell, 2019). Finally, Dantas et al (2025), recently demonstrated that light intensity intra-infusion exercise provided a positive experience for patients with stages I-IV breast cancer.

The primary aim of the current study was to evaluate the effects of repeated cycles of intra-infusion exercise on fatigue over four chemotherapy cycles. Secondary aims include investigating the impact of intra-infusion exercise on symptom experience, QOL and physical activity behaviour, and evaluating the safety, feasibility and acceptability of intra-infusion exercise.

METHODS

Study design

The **EX**ercise during chemotherapy in**FUSION** (EX-FUSION) study was a randomised controlled trial comparing supervised intra-infusion exercise to usual care. The study was approved by the Sydney Local Health District RPAH Zone (X20-0520 & 2020/ETH03151) and was registered on the Australian and New Zealand Clinical Trials Registry (Trial ID ACTRN12621000677808).

Potential participants who had been referred and approved for participation by their oncologist were screened by the study team against the eligibility criteria (Table 5.1). Participants who self-referred after seeing the study advertisement in the hospital were confirmed for oncologist approval prior to screening.

TABLE 5.1: Eligibility criteria

Inclusion criteria	Exclusion criteria
• English speaking • Aged 18-75 years old • Histologically confirmed stage I-III breast, ovarian or colorectal cancer • Current or planned intravenous chemotherapy (minimum 4 cycles with duration >60 minutes) • Have undergone at least 1 cycle of intravenous chemotherapy • ECOG 0-2 • Medical clearance from oncologist • Willing and able to comply with all study requirements	• Presents with an acute systemic infection or fever • Has a known adverse reaction to infusion • Presents with any condition that is contraindicated to exercise • Presents with significant orthopaedic problems, peripheral neuropathy or pain that would limit lower body exercise • Currently taking beta-blocker medications • Currently receiving immunotherapy • Currently receiving anthracycline chemotherapy

Forty-five adults with stage I-III breast, ovarian or colorectal cancer receiving intravenous chemotherapy at the Chris O'Brien Lifehouse (Camperdown, Sydney) were recruited. Randomisation occurred in a 1:1 ratio via a computer-generated randomisation program in blocks of 10 stratified by tumour type. A member of the study team not involved in screening or recruitment performed the randomisation. Group allocation was placed into a sealed opaque envelope and provided to the researcher to open with the participant following the baseline session.

Participant programs

Data was collected over four chemotherapy infusion sessions after completion of informed consent. The first session (baseline), was used to obtain baseline measures, including questionnaires and physiological measures during infusion. The following three chemotherapy infusions were interventional (Figure 5.1).

After randomisation at the end of the baseline session, participants in the exercise group were offered an optional exercise familiarisation session prior to intervention 1. This involved cycling

as described below, in the absence of chemotherapy infusion. The results of the familiarisation session are detailed in Chapter 4.

Intervention sessions were supervised by a clinical exercise professional and commenced at least 10-minutes into chemotherapy infusion to confirm the absence of acute adverse reactions to the chemotherapy. Participants in the exercise group cycled on a stationary foot bike (Monark 881E), whilst seated in their usual chemotherapy chair, for 20-minutes continuously at a moderate intensity (40-50% HRR and a RPE 12-14) (Supplementary Figure 5.1). HRR was calculated using maximum predicted HR (220 - age) and average resting HR which was captured in a 7-day symptom diary after each intervention. Exercise intensity was maintained once participants obtained target %HRR or RPE, whichever occurred first. Physiological measures were captured for 10-minutes prior to cycling, during cycling (20-minutes) and during 20-minutes recovery. Participants wearing ice gloves, booties and/or scalp cooling continued to wear these whilst cycling.

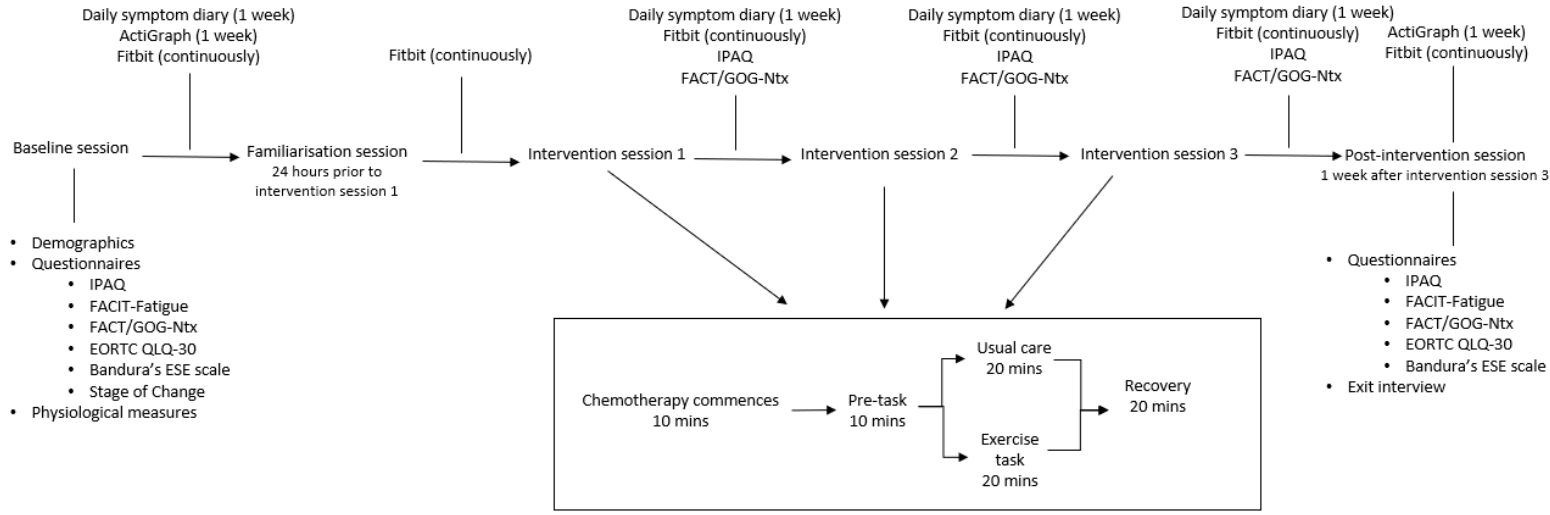
Participants in the usual care group continued with routine clinical care. Physiological measures were recorded during chemotherapy infusion for 50-minutes (to match exercise group monitoring) by a clinical exercise professional.

In recovery following the cycling, participants in both groups were provided with exercise education by the clinical exercise professional. The initial session was patient-driven and may have included discussion of goals, barriers to exercise, current exercise knowledge and exercise history. The clinical exercise professional used an education booklet in subsequent sessions to address strategies to overcome identified exercise barriers. The education booklet included information about the benefits of physical activity during treatment, the amount and intensity of physical activity to aim towards, strategies to maintain consistency and a diary to track physical activity. The education booklet can be seen in the Appendix of this thesis. The “Exercise for People Living with Cancer; A guide for people with cancer, their families and friends” booklet by the Cancer Council was used as a supplementary booklet for participants who would like more information about resistance exercise. General advice on exercise prescription was provided if desired.

Outcomes

All outcome measures and their assessment timepoints are summarised in Figure 5.1. Post intervention questionnaires were completed 1-week after the final intervention.

FIGURE 5.1: Study schema



<u>Primary outcome: Fatigue</u>

Fatigue was determined using two self-reported questionnaires; the FACIT-Fatigue (Webster, Cella & Yost, 2003) at baseline and 1-week after the final intervention and the Brief Fatigue Inventory (BFI) (Mendoza et al, 1999) completed each day for 7-days post each chemotherapy session. FACIT-Fatigue utilises a 5-point Likert scale to assess fatigue experienced over the preceding 7-days and has been designed and validated for use in people with cancer. A higher score indicates reduced fatigue severity. The BFI utilises a 0-10 numeric scale to rapidly assess fatigue severity and impact over the past 24-hours. A higher score indicates increased fatigue severity.

Secondary outcomes

Demographic and clinical information

Participants self-reported demographic and clinical information, including current medications, significant comorbidities, year of cancer diagnosis, location of cancer sites, current and previous cancer treatments, and exercise history. Age, height, weight and ECOG (Oken et al, 1982) were obtained from the chemotherapy day schedule at screening and via the oncologist at approval.

Chemotherapy-induced peripheral neuropathy

CIPN was measured in both groups at baseline and each intervention session using neurotoxicity subscale in the Functional Assessment of Cancer Therapy/Gynaecologic Oncology Group-Neuropathy (FACT/GOG-Ntx) (Calhoun et al, 2003), a 5-point Likert scale assessing symptoms of CIPN experienced over the preceding 7-days. A higher score indicates less neuropathy symptom severity.

Other chemotherapy-related side effects

Symptom burden was captured using the revised version of the ESAS-r (Watanabe, Nekolaichuk & Beumont, 2012). The ESAS-r utilises a 0-10 numeric scale to assess the severity of 9 commonly reported symptoms experienced by people with cancer and was completed as part of the symptom diary for 7-days following each chemotherapy infusion by both groups. Higher total ESAS scores indicate increased symptom severity. Resting HR was also captured as part of the 7-day symptom diary after each chemotherapy infusion.

Quality of life

QOL was measured using the EORTC QLQ-C30 (Aaronson et al, 1993), a widely used tool among cancer patients to measure QOL across the domains of physical, role, emotional, social, and cognitive function. The EORTC has 3 sub-scales which are global health status/QOL, functional, and symptoms. Within each sub-scale, a high score represents a high level of the scale (e.g. a high global health status score indicates high QOL). This EORTC QLQ-C30 was completed by both groups at baseline and post intervention.

Exercise self-efficacy and Motivational readiness for exercise

Exercise self-efficacy was measured using The Bandura Exercise Self-Efficacy Scale (Bandura's ESE Scale) (Bandura, 2006), and was completed by both groups at baseline and post intervention. A higher total score indicates higher self-efficacy. Motivational readiness for exercise in both groups was captured using a single question at baseline and post intervention, categorising participants into one of five Stages of Change (pre-contemplation, contemplation, preparation, action, and maintenance), based upon the Transtheoretical Model (Prochaska & Vilicer,1997).

Physical activity behaviour

Physical activity behaviour was assessed using a questionnaire and wearable devices. The IPAQ (Craig et al, 2003) was completed by both groups at each intervention session and MET-minutes per week for each intensity calculated using the standardised equations. Daily step counts were measured using a commercial wearable activity monitor (Garmin™ Vivofit 4®), worn on the wrist continuously throughout the day by both groups for the duration of the study. The ActiGraph Accelerometer is an accurate and validated tool to quantify physical activity levels. This was worn on the waist by both groups, for 1-week following baseline and for 1-week post intervention.

Physiological measures during infusion

During baseline and at each intervention session, HR and SpO2 were recorded every 2-minutes during the 50-minute data collection period, using a vital signs monitor with a fingertip pulse oximeter. BP was measured every 5-minutes. RPE (Borg, 1982) was collected every 2-minutes using the Borg Scale of Perceived Exertion during the exercise intervention.

Interferences with treatment

Throughout the study, interferences with chemotherapy treatment were recorded by the research team (for example, intravenous lines becoming loose or interfering with nursing duties).

Adverse events

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (US Department of Health and Human Services, 2017) were used. Any adverse event (AE) or serious adverse event (SAE) directly related to the exercise intervention during the study was recorded by the research team and notified immediately to the referring doctor.

SAMPLE SIZE

In the absence of other data, our pilot study (Thomas et al, 2019) was used to determine sample size. Fatigue, assessed by the ESAS-r (Watanabe, Nekolaichuk & Beumont, 2012) was assessed in the week following intra-infusion exercise, with an effect size (Glass' Delta) of 0.39. Using a power of 80% and a significance level of α=0.05, a sample size of n=36 was calculated (G-power) (Faul et al, 2007). With an assumed 80% completion rate, to obtain full results from 36 participants, a sample size of n=45 was required.

STATISTICAL ANALYSIS

R Studio was used for the analysis. Descriptive statistics depict age, height, weight, cancer diagnosis, recruitment, adherence rates, physiological data during exercise, QOL and stage of change. Exercise intensity and RPE were averaged after the 5-minutes of exercise initiation to exclude time to steady state HR. Power output was calculated using 50 revolutions per minute (RPM) which was a fixed setting on the bike. Due to missingness in data because of incomplete 7-day symptom diaries, resting HR, BFI score and ESAS-r scores were averaged on days 2-3 and days 5-6 after each intervention for analysis to represent early and late timepoints in the week post intervention. Mixed linear models were used to compare fatigue and other chemotherapy side effects between groups. Baseline and post intervention physical activity levels, steps and self-efficacy were also analysed using mixed linear models. Sub-group analyses were performed for self-reported walking, moderate and vigorous intensity physical activity. Hypotheses were tested using a significance level of 0.05 and power of 80%. Data are expressed as mean±SD and median [interquartile range].

For the general population, >2000 steps per day are required for analysis of physical activity as this is the lowest feasible step counts for people without major chronic conditions (Tudor-Locke et al, 2011). However, cancer patients are generally less active than the general population and there are no published rules for the minimum valid step count per day. Therefore, for our Garmin results, the number of steps was considered valid for analysis if daily steps were within two SDs from participant's individual mean across the study period. For Actigraph outcomes, data was considered valid for analysis if there was a minimum wear time of >480-minutes per day and a minimum wear of 3-days which was calculated using a reliability estimate of Intraclass Correlation Coefficient (ICC) >0.8 using the Spearman Brown Prophecy formula, and a one-way ANOVA factoring between and within person variability.

RESULTS

Recruitment took place between September 2022 and August 2024. Sixty-four participants were screened, 61 participants were eligible and 45 participants were recruited (74% uptake based on those eligible) (Figure 5.2). Reasons for not enrolling were poor side effects from chemotherapy and time commitment. N=45 were enrolled and n=44 were randomised. N=1 was excluded due to change in chemotherapy regime at baseline prior to randomisation. N=2 became ineligible during the interventions due to change in chemotherapy regime and n=2 did not receive intervention and requested study withdrawal due to increased side effects. Therefore, a total of n=40 participants completed the trial with n=20 participants in each group. Demographic characteristics can be seen in Table 5.2.

FIGURE 5.2: CONSORT diagram

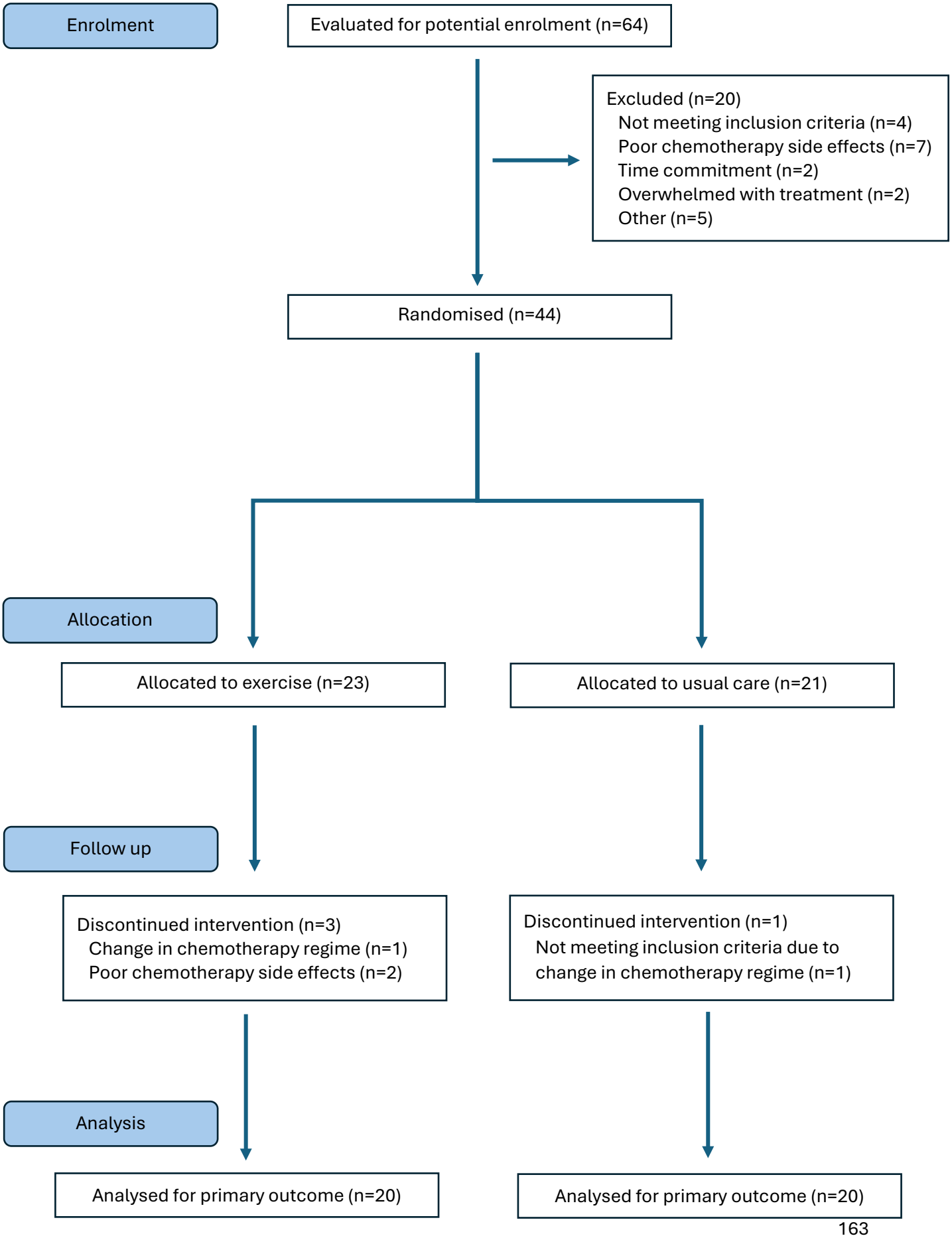


TABLE 5.2: Demographics

	Exercise N=20 (%)	**Usual care N=20 (%)**
Age (years)	48.0±13.6	52.4±11.3
Sex		
Female	16 (80)	18 (90)
Male	4 (20)	2 (10)
BMI (kg/m^2)	26.5±5.1	25.5±6.4
Cancer type		
Breast	8 (40)	10 (50)
Colorectal	7 (35)	5 (25)
Ovarian	5 (25)	5 (25)
Cancer stage		
I	6 (30)	6 (30)
II	6 (30)	4 (20)
III	8 (40)	10 (50)
Chemotherapy agent		
Carboplatin, Docetaxel, Trastuzumab + Pertuzumab (TCHP)	2 (10)	2 (10)
Carboplatin + paclitaxel	4 (20)	4 (20)
Docetaxel + cyclophosphamide	0 (0)	4 (20)
Folinic acid, 5-FU + oxaliplatin (FOLFOX)	7 (35)	5 (25)
Paclitaxel	6 (30)	4 (20)
Capecitabine + oxaliplatin (XELOX)	1 (5)	1 (5)
Timing of chemotherapy		
Adjuvant	15 (75)	17 (85)
Neoadjuvant	5 (25)	3 (15)
Chemotherapy frequency		
Weekly	6 (30)	4 (20)
Fortnightly	7 (35)	5 (25)
3-weekly	7 (35)	11 (55)

Number of chemotherapy cycles prior to baseline		
1	11 (55)	12 (60)
2	7 (35)	3 (15)
3	1 (5)	3 (15)
4	0 (0)	1 (5)
5	0 (0)	0 (0)
6	1 (5)	1 (5)

Data is presented as mean±SD

Adherence and clinical interferences

Adherence to the exercise intervention was 100% with zero adverse events related to exercise recorded. There were no clinical interferences reported.

Physiological responses and intensity during exercise

During the exercise intervention HR increased from 74.2±8.8 at rest to 108.7±11.9bpm during exercise. Intensity was 37.4±0.9%HRR and mean RPE was 12.2±1.2. SpO_2 remained >95% throughout the exercise intervention and BP increased during exercise as expected. Mean power output during exercise was 28.1±18.6 Watts, at 59.8±8.7 RPM. Full physiological data during exercise can be seen in Table 5.3.

TABLE 5.3: Physiological responses during exercise

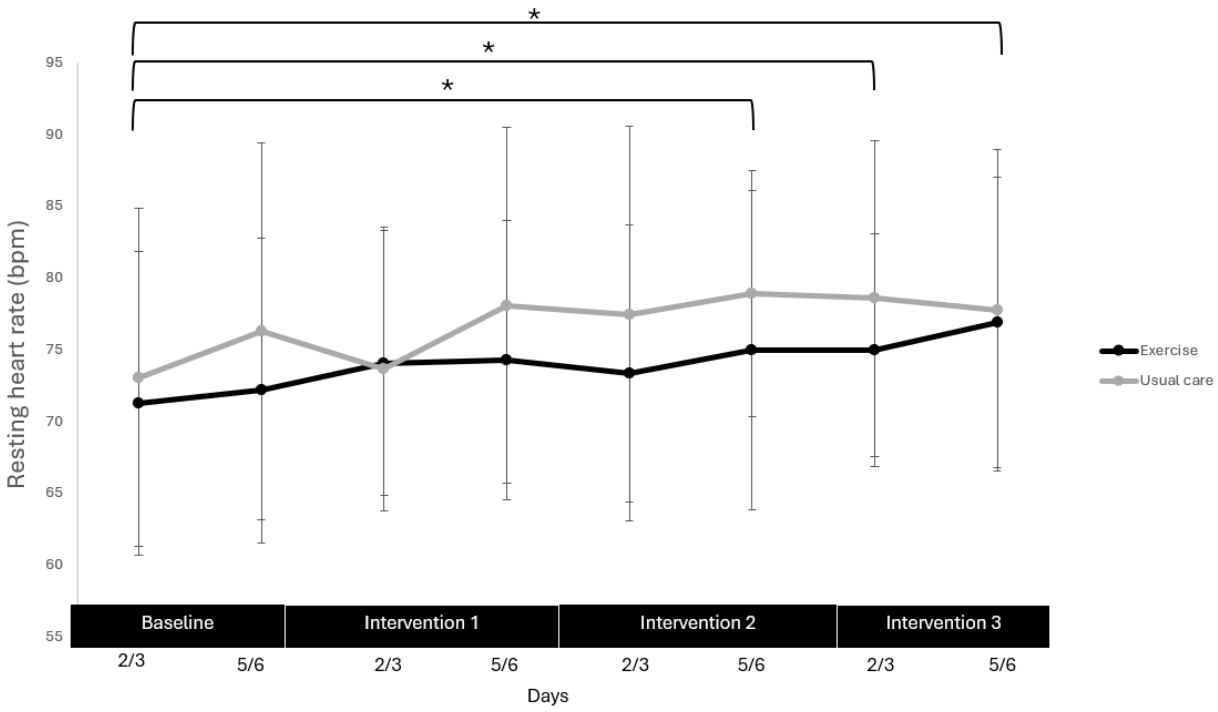
	Exercise (n=20)				
	Resting [+]	**Baseline**	**Intervention 1**	**Intervention 2**	**Intervention 3**
Heart rate	74.2±8.8	77.7±12.5	109.1±11.7	108.2±12.8	108.6±11.8
Oxygen saturation	-	98.1±1.2	97.0±6.2	97.57±3.3	97.2±3.9
Systolic blood pressure	-	125.5±16.1	147.2±16.0	145.8±17.6	144.6±17.6
Diastolic blood pressure	-	82.9±10.2	85.5±11.5	84.2±9.3	86.3±10.3
Rate of perceived effort	-	-	12.5±1.3	12.0±1.4	12.1±1.4
Workload (Watts)	-	-	29.2±19.0	27.2±19.3	28.1±20.2
Revolutions per minute	-	-	59.9±9.3	59.5±7.6	59.4±10.4

Data presented as mean±SD. [+] Average resting HR collected in the 7-day symptom diaries was used to calculate HRR for exercise intensity. RPE was analysed after the first 5-minutes of exercise once participants reached steady state exercise at the prescribed intensity.

Resting heart rate

There was no significant effect of intervention or intervention x time for resting HR, however, there was a significant effect of time such that HR increased in both groups between baseline and post intervention. A post-hoc analysis showed a significant increase in both groups on days 5/6 after intervention 2 ($p=0.029$) and on days 2/3 ($p=0.012$) and 5/6 ($p<0.001$) after intervention 3 compared to baseline (Figure 5.3). Full results can be seen in Supplementary Table 5.1.

FIGURE 5.3: Resting heart rate



Data presented as mean±SD. * $p<0.05$ compared to baseline in both groups

Fatigue

There was no significant intervention or intervention x time effects for fatigue, however there was an expected significant effect of time ($p<0.05$) in global FACIT-Fatigue scores, such that fatigue symptoms were worse in both groups between baseline and post intervention, but not in global BFI scores (Table 5.4 and Figure 5.4).

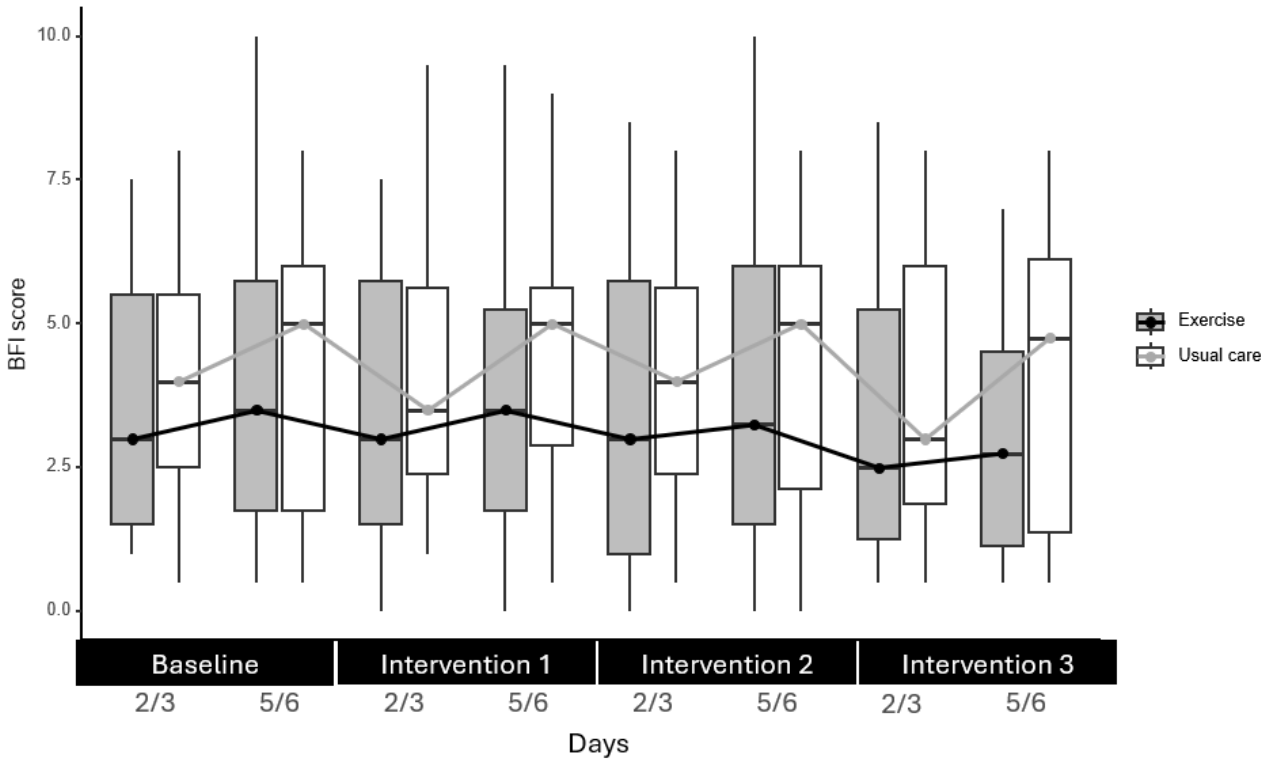
TABLE 5.4: Fatigue, quality of life, exercise self-efficacy and physical activity

	Exercise N=20 (%)		Usual care N=20 (%)	
	Baseline	**Post intervention**	**Baseline**	**Post intervention**
FACIT-Fatigue score *	40.6±8.4	32.0±11.8	41.1±6.35	29.4±12.4
EORTC				
Global health status/QoL	69.2±15.3	51.3±21.2	69.2±16.0	48.3±24.3
Functional scales				
Physical	90.7±14.6	83.0±21.9	92.0±8.8	85.3±16.6
Role	79.2±19.4	60.0±25.0	83.3±16.2	55.0±28.7
Emotional	83.8±14.2	78.3±23.2	80.0±15.9	77.1±21.8
Cognitive	89.2±14.6	77.5±23.1	82.5±18.3	71.7±24.2
Social	65.0±27.5	64.2±32.1	64.2±23.1	54.2±22.2
Symptom scales				
Fatigue	32.8±29.4	55.6±23.9	29.4±16.2	56.7±21.9
Nausea and vomiting	10.0±13.7	20.0±16.8	5.8±9.8	15.0±14.2
Pain	7.5±11.4	20.0±30.9	17.5±19.8	25.0±23.9
Dyspnoea	21.7±22.4	23.3±21.9	10.0±15.7	18.3±22.9
Insomnia	31.7±31.5	40.0±33.5	41.7±32.2	40.0±29.8
Appetite loss	23.3±32.6	23.3±30.8	15.0±17.0	30.0±28.4
Constipation	18.3±22.9	25.0±30.3	18.3±22.9	20.0±25.1
Diarrhoea	18.3±29.6	11.7±19.6	15.0±17.0	15.0±20.2
Financial difficulties	18.3±15.0	18.3±31.5	15.0±27.5	28.3±29.2
Stage of Change				
Precontemplation	0 (0)	2 (10)	1 (5)	1 (5)
Contemplation	7 (35)	6 (30)	1 (5)	1 (5)
Preparation	5 (25)	4 (20)	6 (30)	8 (40)
Action	2 (10)	2 (10)	2 (10)	2 (10)
Maintenance	6 (30)	6 (30)	10 (50)	8 (40)
Bandura's Exercise Self-Efficacy	53.2 [38.5]	39.8 [26.8]	63.2 [32.1]	56.2 [27.2]
Actigraph				
Moderate-to-vigorous	37.1±38.0	39.8±35.3	35.6±28.7	37.2±28.4

physical activity (minutes per day)				
Steps (per day)	5888±4022	6429±4085	5866±2781	6352±2492

Data is presented as mean±SD and median [interquartile range]. * There was a significant effect of time ($p<0.05$) in both groups.

FIGURE 5.4: Fatigue

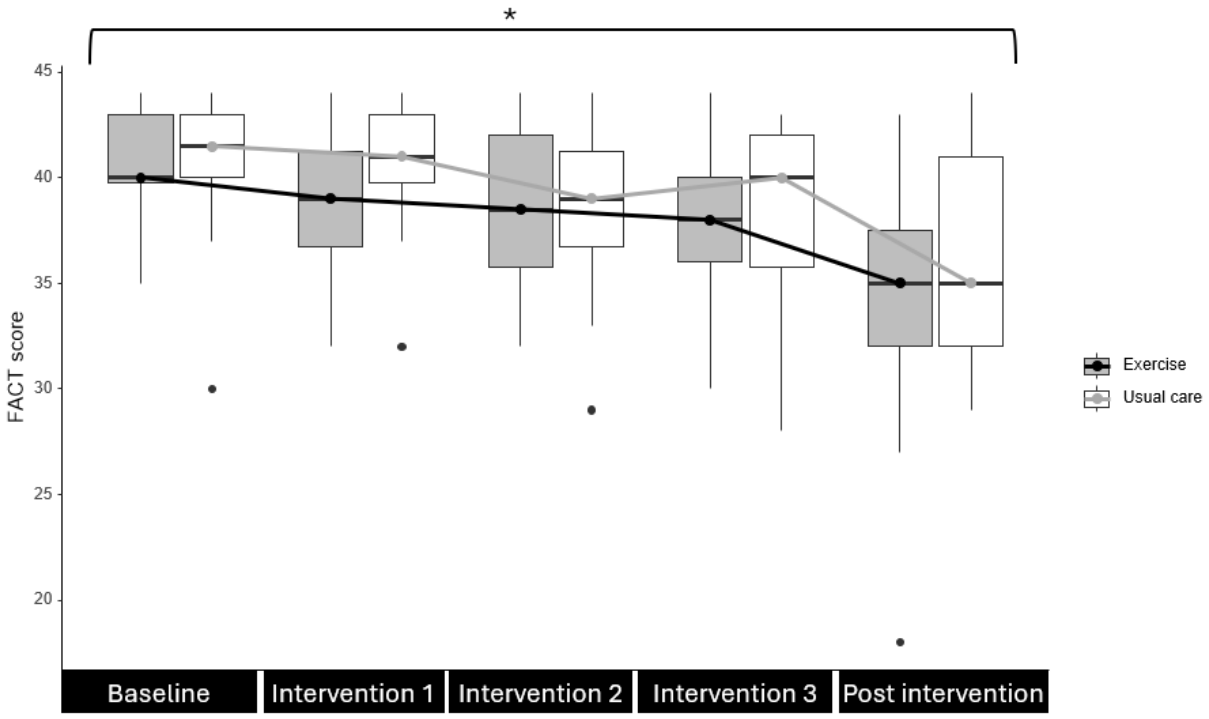


Data presented as median±interquartile range. A higher score represents higher fatigue.

Other chemotherapy-related symptoms and quality of life

There were no significant effects of intervention, nor intervention x time interaction on CIPN symptoms, however there was a significant effect of time such that CIPN symptoms were worse in both groups between baseline and post intervention ($p < 0.05$) (Figure 5.5).

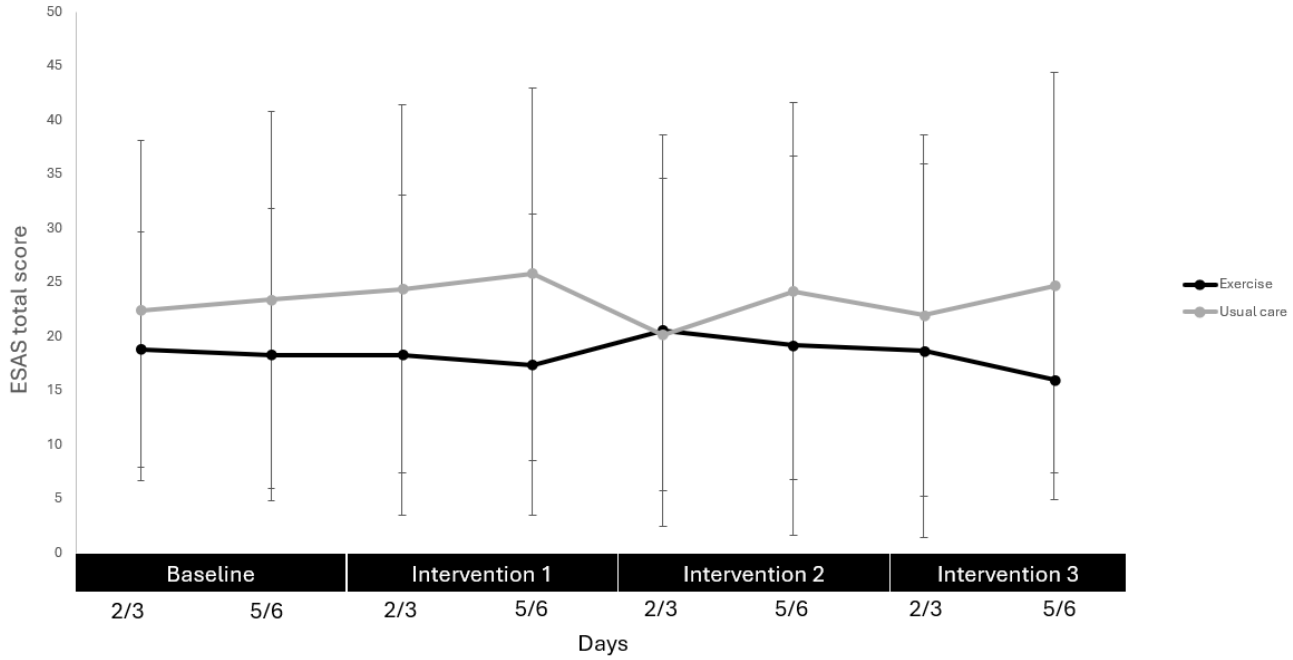
FIGURE 5.5: Chemotherapy-induced peripheral neuropathy



Data presented as median±interquartile range. * $p<0.05$ compared to baseline at all timepoints in both groups. A higher score indicates less neuropathy symptom severity.

There were no significant effects of intervention, time, or intervention x time in total other symptoms (such as pain and nausea) in the ESAS-r (Figure 5.6). Full results can be seen in Supplementary Table 5.1. There were no intervention, time, or intervention x time effects for QOL (Table 5.4).

FIGURE 5.6: Other chemotherapy side effects

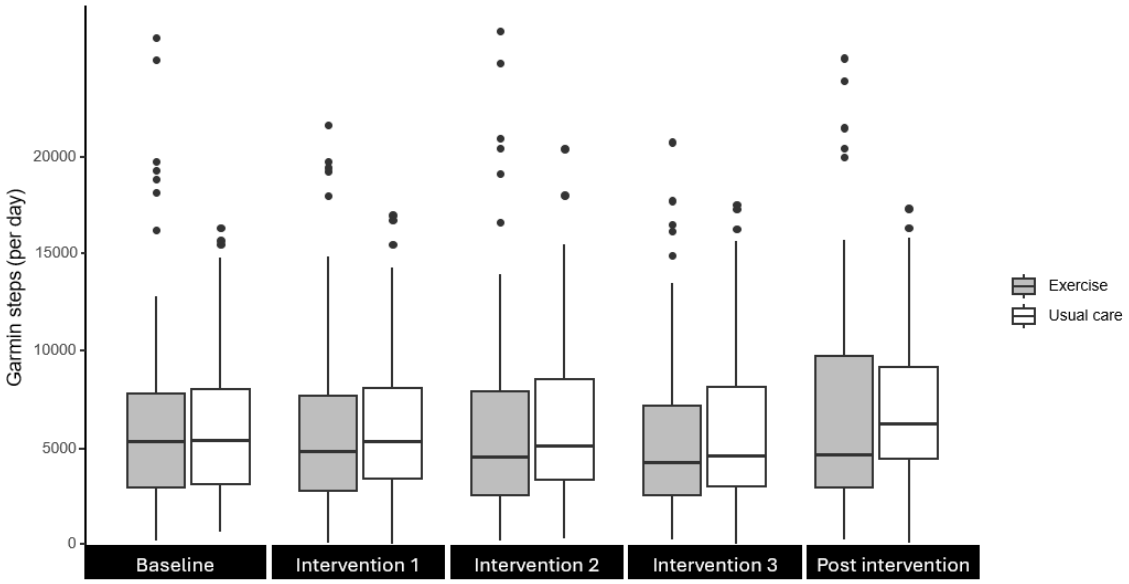


Data presented as mean±SD. A higher score represents increased symptom severity.

Exercise self-efficacy and physical activity behaviours

We found no significant effects of intervention or intervention x time for exercise self-efficacy however there was a significant effect of time such that exercise self-efficacy reduced in both groups between baseline and post intervention ($p<0.05$). There were also no significant effects of intervention, time or intervention x time for self-reported physical activity, steps or Actigraph recorded moderate-to-vigorous physical activity (Figure 5.7). In a sub analysis of separated self-reported walking, moderate and vigorous physical activity, there were no significant effects of intervention, time or intervention x time at each different physical activity intensity. Full results including stage of change can be seen in Table 5.4.

FIGURE 5.7: Steps



Data presented as median±interquartile range.

DISCUSSION

In the current study we report the first randomised controlled trial investigating the effects of intra-infusion exercise on chemotherapy side effects. We found that intra-infusion exercise was well tolerated with no changes in side effects compared to usual care chemotherapy.

We recorded excellent adherence to the exercise intervention reiterating the acceptability of intra-infusion exercise (Thomas et al, 2018). By combining exercise with existing critical medical treatment, this approach overcame several patient barriers to exercise. By providing an opportunity to exercise when patients where already scheduled for multiple hours of chemotherapy, it encouraged participation in an additional exercise session towards meeting minimum exercise guidelines. With the clinical exercise professional supervising the exercise intervention, patients had access to support and education during their treatment time encouraging exercise adherence to the intervention. Furthermore, patients were provided with access to exercise equipment whilst receiving treatment, allowing patients to exercise without requiring their own exercise equipment or access to a gym.

The exercise intensity achieved in the current trial was marginally less that the prescribed 40-50%HRR (achieved 37.4±0.9%HRR) however, the mean RPE was within the 12-14 range (achieved 12.2±1.2). Given the prescribed intensity was based on HRR or RPE we maintained exercise intensity when participants achieved either HR within the %HRR range or described effort within the RPE range, whichever occurred first. Although the RPE scale has been shown to have strong association with HR for exercise intensity in an oncology context, most studies have been performed in cancer survivors who are not on active treatment (Evans et al, 2009). Thus, future work may need to consider the relative difference in RPE and HR indices as measures of intensity during intra-infusion exercise.

There is clear evidence that performing exercise in parallel with chemotherapy treatment (but outside of infusions) improves treatment side effects and QOL (Campbell et al, 2019). However, we saw no significant changes in side effects or QOL with intra-infusion exercise. Campbell et al. (2019) suggests that the exercise prescription required for significant reductions in fatigue is at least 30-minutes of moderate intensity aerobic exercise performed three times per week for 12-weeks. In the current study, the formal exercise prescription only encompassed intra-infusion exercise once for 20-minutes every 1-3 weeks for a total of three exercise sessions, clearly not meeting this recommendation. Given the exploratory nature of our phase 1 study and its limitations in design, it is likely that the exercise dose and intensity was not sufficient to elicit significant improvements in fatigue.

We observed an increase in fatigue with time in the FACIT-Fatigue questionnaire but not the BFI questionnaire. This difference in fatigue outcome may be a due to the timing of questionnaire administration that correlates with the normal patterns of fatigue in patients undergoing chemotherapy. There is an observed a pattern of fatigue during treatment that parallels the biologic activity of chemotherapy (Schwartz et al, 2000; Wu, Dodd & Cho, 2008). After chemotherapy administration, there is an increase in symptom severity followed by a decrease in symptom severity towards the end of the treatment cycle (Schwartz et al, 2000; Wu, Dodd & Cho, 2008). We observed this pattern in the BFI questionnaire as the timing of outcome measures were on days 2/3 and 5/6 after chemotherapy administration where we would anticipate a rise in symptom severity between days 2/3 and 5/6, but then a decrease in symptom severity between days 5/6 and days 2/3 in the next cycle. However, in the FACIT-Fatigue questionnaire, the baseline questionnaire was completed on the day of chemotherapy infusion and then again at 1-week after the final intervention. As there were a number of different chemotherapy agents with varying cycle durations, the severity of fatigue at 1-week after chemotherapy infusion may not have had the same trend in all chemotherapy agents due to the biologic activity of the chemotherapy agent (e.g. weekly chemotherapy infusions may have less fatigue severity at 1-week post chemotherapy infusion as the biological activity of the chemotherapy is significantly decreased compared to a 3-weekly chemotherapy infusion where the biologic activity may still be increasing towards its peak). Therefore, the effect of time was more apparent in the FACIT-Fatigue compared to the BFI.

The direct physiological effects of intra-infusion exercise carry possible risk as well as benefit. The likely increase in blood flow and drug delivery to tumours, may impart a beneficial effect on chemotherapy efficiency, but the parallel increase in blood flow to cardiac tissue and the peripheral tissues may lead to increased risk of cardiotoxicity and peripheral neuropathy symptoms. We measured resting HR in the week following each infusion as an indicator previously reported as a clinical presentation of cardiotoxicity (Kirkham et al, 2019). Although we saw a significant increase over time in both groups, there were no differences between groups or interaction over time which suggests that intra-infusion exercise did not significantly exacerbate a cardiotoxic response. Similarly, the risk for increased peripheral neurotoxicity is implied by the increased blood flow and intravenous chemotherapy delivery to the working muscles, specifically the legs and feet. Although we saw an effect of time with neuropathy symptoms increasing over the four cycles of chemotherapy, we saw no difference in changes between intra-infusion exercise compared to usual care chemotherapy. Our sample included

some patients for whom ice gloves and booties were recommended during infusion, patients continued to wear these through the intervention and our results show that in this sub-group we see a similar pattern of increase over time and no difference between groups. Both these results are positive outcomes that suggest although intra-infusion exercise increases blood flow, 20-minutes of exercise did not result in greater chemotherapy toxicities.

As barriers to exercise include lack of exercise support and education, we hypothesised that supervised intra-infusion exercise may overcome these barriers to increase physical activity behaviour. However, we saw no significant change in exercise self-efficacy with intra-infusion exercise. It is possible that the reason we observed this result is that the integration of exercise with medical intervention is viewed as too clinical by patients and therefore is not transferable to performing physical activity outside of the infusion. It is also possible that the behavioural support was not sufficiently structured, and when combined with infrequent supervised intra-infusion exercise sessions (once every 1-3 weeks), may not have offered enough personalised education or ongoing support to improve exercise self-efficacy. Future use of intra-infusion exercise would benefit from the integration of behaviour change frameworks to improve exercise self-efficacy. Stage of change is a strong predictor of exercise adherence, and adherence has a strong correlation with self-efficacy (Husebo et al, 2012). Therefore, if patients have a lower stage of change (e.g. precontemplation or contemplation), exercise adherence and subsequently self-efficacy is also reduced. We observed that most of the participants in the exercise group were in the contemplation or preparation stage of change (not currently exercising regularly but considering engaging in a regular exercise routine) and therefore would require more frequent theory-based behavioural support to encourage exercise adherence and increase self-efficacy. In a review by Turner et al (2018), exercise programs that involved frequent support with health professionals at least twice weekly resulted in increased adherence to an exercise program that met minimum activity guidelines. Thus, more frequent support outside of the intervention may have been needed to shift participants from contemplation towards action stages of change and increase self-efficacy.

We observed a non-significant increase in step count in both groups between baseline and post intervention. Although the intra-infusion exercise itself may not have provided additional advantage, contact with the clinical exercise professional during each infusion, which included exercise support and referrals onto other supportive care services, may have supported

participants to maintain physical activity when we would otherwise expect to see declines in activity with chemotherapy (Nelson et al, 2019). This small increase in step count is a clinically important finding as every increase in steps by 1000 steps decrease the risk of a clinical event by 21% (Oakley-Girvan et al, 2025). We additionally observed a non-significant increase in self-reported moderate intensity physical activity between baseline and intervention 1 in both groups. At this timepoint in the study after patients had completed the baseline IPAQ questionnaire, patients were already knowingly randomised to either the exercise or usual care group. We theorise that by simply being involved in an exercise study, which included tracking physical activity and exercise counselling, that patients were motivated to engage in some level of exercise irrespective of group allocation.

Although there was not capacity to include outcomes for the possible effect of intra-infusion exercise acutely increasing blood flow to tumours and therefore chemotherapy delivery to the tumour, the possible effect on tumour growth is a critical step for investigation. Whilst there are no studies that investigate the effects of intra-infusion exercise on tumour blood flow, preclinical findings along with our own unpublished data (Chapter 3) suggests that intra-infusion exercise could increase blood flow and subsequently chemotherapy directly to the tumour to significantly improve clinical outcomes without increased side effects. Preclinical evidence, as detailed in Chapter 2, suggests that aerobic exercise may change blood flow to tumours. McCullough et al (2014) demonstrated that an acute bout of moderate intensity aerobic exercise in rats with prostate cancer could increase blood flow to the tumour by 200%. When combined with chemotherapy, Schadler et al (2016) demonstrated that moderate intensity aerobic exercise significantly increased doxorubicin delivery to tumours in mice with melanoma and pancreatic tumours resulting in significantly inhibited tumour growth compared to chemotherapy alone. However, further clinical research is needed to test whether the preclinical evidence is also observed in humans.

The limitations of this study should be acknowledged. The cohort included a mixed cancer type and subsequently mixed chemotherapy agents. This may limit the translation of these findings to specific cancers as the results reflect a broad range of cancer types and treatments. The large missingness in some data due to incomplete questionnaire responses and poor physical activity monitor wear compliance meant that the results may be under-represented.

CONCLUSION

In summary, intra-infusion exercise has excellent adherence, is safe and does not increase chemotherapy side effects. Although 20-minutes of intra-infusion exercise alone did not alter exercise behaviour, it provided an additional opportunity to exercise when patients would be otherwise sedentary and contact with the clinical exercise professional may have supported maintenance of physical activity. Future work will investigate effects of intra-infusion exercise on chemotherapy efficacy and focus on better integrating intra-infusion exercise with additional behavioural support and exercise prescription to promote meaningful changes in exercise behaviour and improve chemotherapy side effects.

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SUPPLEMENTARY MATERIAL

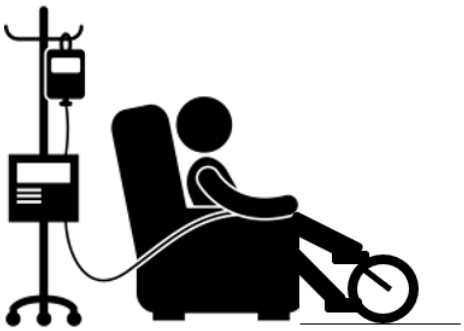
SUPPLEMENTARY TABLE 5.1: Chemotherapy side effects and physical activity

	Exercise N=20					**Usual care N=20**				
	Baseline	**Intervention 1**	**Intervention 2**	**Intervention 3**	**Post intervention**	**Baseline**	**Intervention 1**	**Intervention 2**	**Intervention 3**	**Post intervention**
ESAS total score[+]										
Day 2/3	18.8±10.9	18.3±14.8	20.6±18.1	18.7±17.3		22.4±15.7	24.4±17.0	20.2±14.4	22.0±16.7	
Day 5/6	18.3±13.5	17.4±13.9	19.2±17.5	16.0±8.6		23.4±17.4	25.8±17.2	24.2±17.4	24.7±19.7	
Resting heart rate (bpm)[+]										
Day 2/3	71.3±10.6	74.1±9.24	73.4±10.3	75.0±8.12*		73.1±11.8	73.7±9.87	77.5±13.1	78.6±11.0*	
Day 5/6	72.2±10.6	74.3±9.71	75.0±11.1*	76.9±10.1*		76.3±13.1	78.1±12.4	78.9±8.57*	77.8±11.2*	
BFI[+]										
Day 2/3	3.0 [4.0]	3.0 [4.25]	3.0 [4.75]	2.5 [4.0]		4.0 [3.0]	3.5 [3.25]	4.0 [3.25]	3.0 [4.12]	
Day 5/6	3.5 [4.0]	3.5 [3.5]	3.25 [4.5]	2.75 [3.38]		5 [4.25]	5 [2.75]	5.0 [3.88]	4.75 [4.75]	
IPAQ (MET mins/week)										

Total	363 [1917]	864 [2194]	728 [2715]	1004 [1955]	760 [1227]	729 [1818]	984 [1805]	1408 [1812]	1173 [1298]	1424 [2692]
Walking	363 [742]	429 [1155]	544 [1510]	644 [817]	446 [668]	594 [780]	544 [1130]	544 [528]	644 [1007]	693 [1304]
Moderate	0 [120]	120 [390]	140 [510]	150 [370]	140 [285]	0 [120]	110 [360]	300 [720]	80 [495]	70 [390]
Vigorous	0 [520]	0 [480]	0 [720]	0 [300]	40 [240]	0 [0]	0 [260]	0 [660]	0 [480]	0 [1440]
FACT-GOG NTX	40 [3.25]	39 [4.5] *	38.5 [6.25] *	38 [4.0] *	35 [5.5] *	41.5 [3.0]	41 [3.25] *	39 [4.5] *	40 [6.25] *	35 [9.0] *
Garmin steps (per day)	5408 [4431]	5188 [5479]	4388 [4126]	3801 [4374]	4497 [4879]	5664 [3213]	5117 [3344]	5118 [2435]	4780 [3283]	5994 [1988]

Data is presented as mean±SD and median [interquartile range]. * $p<0.05$ compared to baseline. $^{+}$n=19 in the exercise group

SUPPLEMENTARY FIGURE 5.1: Set up of stationary foot bike with chemotherapy chair



Chapter 6

Discussion

Exercise throughout cancer treatment has been shown to impart improvements in treatment-related side effects and patient-reported outcomes. Intra-chemotherapy infusion exercise is a novel adjuvant treatment for people with cancer, however, there are many unknowns for the mechanistic and clinical effects of intra-infusion exercise, such as potential changes to the tumour microvasculature that may impact chemotherapy delivery and effects on treatment side effects. Prior to commencing the work in this thesis, a single pilot safety and feasibility study and a case study had been published on intra-infusion exercise in humans (McLaughlin, Christie & Campbell, 2019; Thomas et al, 2019). This thesis has provided significant advancement to the literature as this exercise delivery protocol is further examined. My systematic review in Chapter 2 highlighted the preclinical chronic effects of aerobic exercise on tumour blood flow as a background to understanding how acute exercise may affect tumours. The remaining chapters provide evidence for the acute and chronic clinical effects of intra-infusion exercise, providing the foundation for future work to examine the effects on treatment outcomes.

To summarise, the aims of this thesis were to:

1) Examine the existing research that investigates the impact of chronic exercise on tumour vasculature

2) Determine if exercise changes tumour blood flow acutely and if intensity affects tumour blood flow

3) Determine the physiological response to exercise during chemotherapy

4) Determine if intra-infusion exercise affects chemotherapy side effects and physical activity behaviours

Summary of findings

Chronic exercise and tumour vasculature

Chapter 2 reviewed the existing literature on chronic aerobic exercise and tumour vasculature. Pathophysiological descriptions of dysfunctional tumour vasculature suggest that chemotherapy delivery to the tumour centre is prevented thereby reducing treatment efficacy (Wiggins et al, 2018). Therefore, strategies that normalise the vasculature of tumours could improve drug delivery and subsequently improve clinical outcomes. Such tumour vasculature changes include microvessel distribution and increased density, reduced hypoxia and improved microvessel structure (Siemann & Horsman, 2015). The combination of these vascular changes are pro-angiogenic and promote blood flow to the tumour (by increasing the number of normalised open vessels that can supply blood to the tumour), therefore intravenous chemotherapy may be more efficiently delivered directly to the tumour centre (Huang et al, 2021; Wiggins et al, 2018). Indeed, there have been pharmacological approaches to enhance angiogenesis and increase chemotherapy efficacy, however, poly-pharmacy approaches often present with other adverse side effects (Huang et al, 2021; Wong et al, 2015). Unlike these, non-pharmacological approaches such as exercise may promote the desired tumour vascular adaptations to increase blood flow to the tumour, whilst additionally providing well-established benefits in reducing treatment-related side effects.

A notable, but not unexpected, finding from this review was that the majority of the existing literature was preclinical evidence, highlighting a significant gap in clinical evidence. The preclinical studies demonstrated mixed findings for the effects of chronic aerobic exercise on tumour vasculature. Our meta-analysis showed no significant impact of chronic aerobic exercise on tumour hypoxia, vascularisation or blood flow. However, the findings were influenced by a high risk of attrition bias and methodological heterogeneity across studies, which may have contributed to the inconsistent findings. In Chapter 1 of this thesis, I summarised the recent evidence published since the completion of my systemic review and meta-analysis which includes three additional preclinical studies all in favour of improved structural and functional tumour vasculature adaptations. A clear clinical gap remains in clinical research for the effects of exercise training on tumour vasculature, which raises questions regarding the translation of preclinical findings to humans.

Acute exercise and tumour blood flow

Tumour response to treatment relies on adequate concentration of intravenous chemotherapy to be delivered to the tumour. Given the intravenous transport, this can be influenced by tumour blood flow. Chapter 2 found no overall effect for the effects of chronic exercise on tumour vasculature. To explore the potential for acute exercise to influence tumour blood flow, Chapter 3 introduced a novel protocol involving a group of participants with tumours accessible to ultrasounds measurements (liver metastases). My findings supported preclinical evidence showing that acute exercise could increase tumour blood flow. In healthy active tissue, there is an expected positive linear association between blood flow response and increasing exercise intensities. However, in tumours located in inactive tissue like the liver, which typically does not see the same increased blood flow with exercise, the response may be different. Due to the dysfunctional tumour vasculature that includes vessels lacking a vasoconstriction response, aerobic exercise at increasing intensities could increase blood flow to tumours as a function of increased CO.

The findings in Chapter 3 demonstrated that acute moderate intensity exercise dramatically increases tumour blood flow. Unlike in healthy tissue, tumour blood flow changes were not linearly related to intensity. The increase in tumour blood flow at moderate intensity was consistent with the preclinical evidence from McCullough et al (2014) and Garcia et al (2016) who used similar intensity exercise. However, the magnitude of change was slightly lower than reported in the preclinical studies which may be due to the timing of measures taken in the 1-3 minutes after exercise rather than *during* exercise. There was an increase in resistance index after high intensity exercise seen both in the tumour and hepatic artery which is suggestive of a vasoconstrictive response occurring in the liver host tissue and may explain the lack of dose-dependent blood flow increase with high intensity exercise. This chapter demonstrated that irrespective of uncertainty in chronic changes to tumour vasculature (Chapter 2), a single acute bout of aerobic exercise can induce increased tumour blood flow. The findings from this chapter are a critical mechanistic demonstration for the potential development of an intra-infusion exercise intervention aimed at changing delivery of chemotherapy and potentially improving efficacy. However, greater understanding of the physiological impacts of intra-infusion exercise are needed to facilitate safe intervention development.

Physiological response of intra-infusion exercise

When performing exercise whilst receiving chemotherapy infusion, it is important to understand any differences in expected physiological responses. Specifically, due to increased fluid delivery of the infusion and the chemotherapy agent itself, normal physiological responses to exercise may be altered. The findings from Chapter 4 demonstrated that intra-infusion exercise was associated with exaggerated HR and BP responses compared to exercise outside of chemotherapy infusion. Despite exaggerated physiological responses, patients did not feel that the intra-infusion exercise was any more difficult than exercise outside of infusion. These findings highlight that physiological measures during intra-infusion exercise must be monitored to ensure safety.

Intra-infusion exercise, chemotherapy side effects and physical activity behaviours

Prior to this thesis, only pilot safety and feasibility data from a single exercise bout was available to support intra-infusion exercise as an intervention for development. In the final experimental chapter of this thesis, a phase 1 clinical trial was performed using a randomised controlled design investigating the impact of intra-infusion exercise during three chemotherapy cycles on chemotherapy side effects. This trial aimed to provide foundational evidence in preparation for future trials which may examine clinical effects of intra-infusion exercise. The findings from Chapter 5 demonstrated that intra-infusion exercise was very well adhered to, however, did not change chemotherapy side effects. The 100% adherence was likely due to the embedding of exercise within existing medical treatment as well as overcoming patient barriers to exercise such as time constraints and lack of support (Clifford et al, 2018; Elshahat, Treanor & Donnelly, 2021; Galvao et al, 2015; LeMasters et al, 2014). There was no effect of exercise on fatigue in this trial. Although in contrast with the well-established evidence for exercise to reduce fatigue, it is likely that the exercise dose of 20-minutes once per 1-3 weeks was not enough to elicit a change. Evidence suggests that an exercise prescription to reduce fatigue requires a minimum of 30-minutes of moderate intensity aerobic exercise performed three times per week for 12-weeks (Campbell et al, 2019). Notably, the intervention caused no significant increases in cardiotoxicity and neuropathy indicators, important outcomes given the potential safety concern that these side effects could be exacerbated due to increased cardiac and peripheral blood flow leading to greater drug dose exposure. Finally, this trial highlighted the ongoing need to provide more frequent and structured support for regular exercise outside of the chemotherapy infusion. Although intra-infusion exercise was well adhered to, we did not

observe any increase in physical activity outside of the infusion meaning the intervention did not succeed in promoting the meeting of exercise guidelines. However, despite literature that suggests that physical activity decreases linearly with chemotherapy treatment, we saw that physical activity was maintained in both groups between baseline and post intervention. We hypothesise that the clinical exercise professional's support during the intra-infusion exercise protected patients from the usual physical activity decline.

Clinical implications

Governing bodies recommend exercise during cancer treatment based on clear evidence for exercise improving treatment side effects. Despite this, the majority of cancer patients do not perform exercise regularly to meet minimum exercise guidelines and therefore forgo the improvements in treatment side effects and possible benefit of tumour vascular changes (Campbell et al, 2019; Cormie et al, 2018; Van Blarigan et al, 2015). Meeting exercise guidelines is not easy, with patients describing barriers to exercise participation such as time constraints, access to exercise facilities and reduced exercise support, in addition to the side effects they experience (Clifford et al, 2018; Elshahat, Treanor & Donnelly, 2021; Galvao et al, 2015; LeMasters et al, 2014).

Intra-infusion exercise holds many and varied potential benefits as a delivery modality. It may contribute to patients meeting exercise guidelines by overcoming barriers: utilising the time during chemotherapy infusion to provide an additional opportunity to exercise, providing access to exercise equipment, and providing exercise advice and support by a clinical exercise professional. In a positive feedback loop, patients who are helped by intra-infusion exercise to meet guidelines may experience reduced side effects, reducing the barrier of ill-health. Chapter 5 demonstrated that intra-infusion exercise has high adherence, with no adverse events related to exercise recorded, indicating that intra-infusion exercise is safe and acceptable in patients undergoing chemotherapy. Chapter 4 highlighted the significance of monitoring physiological responses such as HR and BP throughout intra-infusion exercise to ensure safety. However, intra-infusion exercise alone over three cycles of chemotherapy did not improve treatment side effects such as fatigue. The findings in Chapter 5 suggest that patients need an exercise dose greater than a single bout of intra-infusion exercise to experience reduced fatigue, emphasising the importance to combine it with additional exercise outside of the infusion (Campbell et al,

2019). Although intra-infusion exercise alone is not enough to change exercise behaviour outside of the infusion, it provides an opportunity for patient education which may allow the additional regular exercise support required to promote chronic exercise outside of the infusion. Future programs should include support to patients to encourage exercise outside of the infusion. These programs should incorporate theory-based behavioural change support to initiate and maintain physical activity changes, such as through structured and individualised exercise prescription.

The findings from Chapters 3 and 5, demonstrating acute moderate intensity exercise increased tumour blood flow and intra-infusion exercise had no change in chemotherapy side effects, provide a strong foundation for future research. Specifically, they support investigating whether intra-infusion exercise could acutely increase chemotherapy delivery. Increased blood flow to the tumour may be achieved without increasing risk for other systemic toxicities, such as cardiotoxicity and neurotoxicity. Chapter 5 demonstrated that indicators of these side effects were not different in exercise and control groups, despite the potential that exercise-induced increases in CO may cause increased chemotherapy delivery to the periphery and cardiac tissue. This suggests that intra-infusion exercise may offer a safe and targeted strategy to optimise chemotherapy delivery.

It is possible if patients performed regular exercise outside of the infusion in addition to performing intra-infusion exercise, patients could experience significantly increased blood flow delivery directly to the tumour (and subsequently increased chemotherapy delivery). Although the preclinical evidence in the meta-analysis included in Chapter 2 suggested an overall no change in tumour vasculature with chronic exercise, there were individual mixed results in the included studies with studies showing increased, decreased and no change in tumour vasculature. With limited clinical evidence, the translation of the preclinical evidence to humans should be cautioned due to the poor methodology and high attrition bias of the included studies. If future clinical evidence supports the results from the current published clinical trials, which demonstrated more normalised tumour microvessel shape and size with chronic exercise, it is plausible to postulate that if patients performed chronic exercise outside of the infusion plus acute intra-infusion exercise, chemotherapy delivery could be increased. This result is achieved by an acute increase in blood flow with intra-infusion exercise which is augmented by tumour vasculature changes that promote blood delivery whilst also reducing

treatment side effects. The overall outcome could demonstrate reduced tumour growth, however further clinical research is needed to demonstrate chronic exercise effects on tumour vasculature in humans as a base to investigate tumour growth changes with intra-infusion exercise.

This intervention, although promising, may not be appropriate within every service that delivers chemotherapy due to patient access because of barriers such as hospital capacity limitations and protocols that limit clinical exercise professional's access the chemotherapy wards to supervise patients. Although clinical exercise professionals are becoming more recognised as integral inclusions in a patient's treating oncology team, not all hospitals have access to a clinical exercise professional in the hospital setting. There may also be challenges with the set-up of intra-infusion exercise. Chemotherapy wards may not have enough space for an exercise bike plus additional personnel. The wearing of ice gloves and booties for neuropathy during intra-infusion exercise is possible, although due to increased body temperature with exercise, the ice gloves and booties melt quicker, requiring more frequent replacement and thus disrupting the exercise. If patients cannot perform intra-infusion cycling due to limitations of space in the chemotherapy ward to accommodate a bike, it is possible that patients who do not wear ice gloves and booties could walk the corridors of the chemotherapy ward whilst pushing the intravenous pole. If patients do not have access to intra-infusion exercise, it could be suggested that patients perform aerobic exercise as close to chemotherapy administration to promote acute tumour blood flow and subsequently chemotherapy delivery when the chemotherapy is the most concentrated. Other practical considerations that need to be acknowledged include medical and nursing staff impressions about priority of exercise during infusion and suitability of patients, concerns about changes in infusion rates, and maintaining IV lines in fragile vessels with movement. Some of these concerns may be overcome with education whilst other concerns will need to be examined in future studies with a larger sample size with different tumor streams and chemotherapy agents.

Limitations

Although limitations have been described in each chapter, there have been a few overarching limitations of this thesis. Firstly, the small sample sizes may overestimate or underestimate the findings. Recruitment, especially for Chapter 3, was difficult given the inclusion criteria of

patients on active treatment and with advanced metastatic disease who were well enough to participate in an exercise trial with no known individual benefit. Another limitation was the heterogeneity of demographics. There were several different cancer types, stages and chemotherapy agents in the participants, thus the findings of this thesis reflect a broad range of cancers and chemotherapy treatments. It is also likely that the participants involved in the studies are more interested in physical activity, for example this was demonstrated in the high self-reported physical activity in Chapter 3. Therefore, the findings of this thesis may represent patients who are more active and physically able than the average cancer patient.

Future directions

The findings from this thesis infer that moderate intensity intra-infusion exercise increases blood flow to tumours without exacerbating critical side effects. However, it did not directly investigate tumour changes with intra-infusion exercise. Future work aims to prove the inferences of this thesis, that intra-infusion exercise increases blood flow and improves chemotherapy delivery to subsequently reduce tumour growth and improve clinical outcomes. These clinical outcomes may be assessed through chemotherapy relative dose intensity, hospitalisations and tumour depth of response. There is some evidence that exercise improves treatment tolerance and subsequently improves treatment relative dose intensity (Wonders, Schmitz & Harness, 2023). This evidence has been based on exercise performed throughout chemotherapy treatment (Courneya et al, 2007; Van Waart et al, 2015). However, most cancer patients do not meet minimum activity guidelines, therefore patients may have reduced treatment tolerance leading to treatment dose reductions (Catala-Vilaplana et al, 2025; Cormie et al, 2018). Consequently, there may be lesser effects on tumour response. Intra-infusion exercise may improve chemotherapy relative dose intensity for improved tumour response and therefore future work should assess these outcomes (Wonders, Schmitz & Harness, 2023). However, intra-infusion exercise needs to be performed alongside exercise support outside of the infusion by clinical exercise professionals to improve treatment related side effects in addition to vasculature changes.

Clinical exercise professionals embedded in cancer care have benefits to the patient and to the economy. The benefits of exercise to the patient have been previously discussed such as improved treatment side effects and QOL, and these benefits can be augmented with

supervision from a clinical exercise professional (Campbell et al, 2019). Exercise has an additional indirect benefit to the economy. Poor management of treatment side effects can lead to hospitalisations which are economically and resource costly (Goldsbury et al, 2018; Numico et al, 2015). Exercise during cancer treatment has been shown to reduce hospitalisation admission and length of stay (Mizrahi et al, 2023). Therefore, to support patients to exercise regularly through treatment, clinical exercise professionals that specialise in oncology, along with exercise equipment and facilities, should be integrated in clinical cancer services. Future work should consider ways to embed clinical exercise professionals into hospital settings such as through intra-infusion exercise.

Clinician researcher reflection

The COVID-19 pandemic in 2020-2023 impacted a number of the studies included in this thesis. Due to lockdowns and strict government restrictions for working with clinically vulnerable populations in a hospital setting, the studies included in Chapter 3, Chapter 4 and Chapter 5 were significantly delayed. During this delay, I conducted a literature review for aerobic exercise and tumour blood flow which led to the systematic review and meta-analysis on chronic exercise effects. As lockdowns ended and government restrictions were reduced, study protocols required amendments to include COVID-19 safety measures such as vaccination status and hygiene practices. The delay in commencing the studies impacted on recruitment rates as these exercise studies were no longer front of mind of referring medical oncologists. Additionally, the previously strict government restrictions for attending and visiting medical facilities affected the confidence for patients to participate in exercise studies with no direct benefit to their clinical outcomes. There was hesitation for referrals to and participation in exercise trials due to potential increased infection risk in comparison to drug trials for example where patients could still wear protective personal equipment during the trial. To combat this, after the lockdowns, I reengaged with the medical oncologists and presented to the oncology department to reassure them of the COVID-19 safety measures established to ensure patients were safe to participate in the exercise trials.

The support from nursing staff was important in implementing a successful exercise trial in a hospital. Nursing staff play an influential role for both medical oncologists and patients. Support from nursing staff encourages medical oncologists to refer to exercise and encourages

patients to consider participating in exercise trials. Patients have great trust in nurses, particularly chemotherapy nurses who establish good rapport with patients that regularly have treatment. As such, the support and encouragement from nursing staff throughout the exercise trial helped to boost patient's confidence to feel that they were in a safe place performing exercise safely. Outside of the exercise trial, chemotherapy nurses have an opportunity to encourage exercise participation outside of chemotherapy infusion. Chemotherapy nurses can see patients more frequently than their medical oncologists and therefore have an opportunity to educate and encourage patients to access exercise support from clinical exercise professionals for individualised exercise prescription and advice.

The patient journey after a diagnosis can be overwhelming with multiple medical appointments, tests and treatments. Unfortunately, as exercise is not yet included in standard cancer care, many patients are not aware of the benefits of exercise during treatment. Despite the evidence for the positive effects of exercise on reducing chemotherapy side effects, improving QOL and improving chemotherapy completion rates, more needs to be done to include exercise in standard cancer care. Further evidence for the effects of exercise on clinical outcomes in addition to patient reported outcomes, such as changes in tumour growth, may support the inclusion of exercise in standard cancer care so that all patients have the education and access to exercise during treatment. The inclusion of exercise in standard cancer care may take many years to achieve and until then, intra-infusion exercise may provide an opportunity for patients to exercise and learn about the benefits of exercise with the support from a clinical exercise professional without needing to attend additional appointments which can contribute to therapy fatigue. A potential challenge with incorporating intra-infusion exercise in a large cancer hospital is resource demands. As cancer hospitals have a limited number of specialised clinical exercise professionals, there needs to be a fair resource allocation so that all patients have access to clinical exercise professionals for tailored prescription and advice.

Concluding remarks

The concept of intra-infusion exercise is still very novel. The first publication of intra-infusion exercise was published as part of my honours thesis, and to my knowledge, this is the first PhD thesis that investigates the broad topic of intra-infusion exercise as a delivery modality and its potential impact on clinical outcomes. The findings from this thesis highlight the mechanism

and effects that modulate tumour vasculature to promote tumour blood flow for improved chemotherapy delivery. Intra-infusion exercise can overcome barriers to exercise participation by embedding exercise with existing medical treatments. It may additionally acutely increase tumour blood flow to increase chemotherapy efficacy. However, intra-infusion exercise alone does not increase exercise outside of the infusion and this thesis highlighted the need for more regular and structured behavioural support to establish exercise behaviour changes for improved treatment side effects. Due to the challenges of the COVID-19 pandemic, such as government restrictions for research trials with vulnerable populations and reduced confidence for participation due to infection control concerns, mechanistic studies have been somewhat limited in the last few years. However, this thesis has provided the foundation for future clinical trials and there is promise for the translation of this work to be included in standard cancer care.

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Appendix

The appendix includes the publications from the studies in this thesis. It also includes the education booklet used in the EX-FUSION study.

Supportive Care in Cancer
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REVIEW ARTICLE

The effect of aerobic exercise on tumour blood delivery: a systematic review and meta-analysis

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Abstract

Purpose Tumour blood vessels are structurally and functionally abnormal, resulting in areas of hypoxia and heterogeneous blood supply. Aerobic exercise may modulate tumour blood flow and normalise the tumour microenvironment to improve chemotherapy delivery. This systematic review and meta-analysis aimed to evaluate the effect of the aerobic exercise mode on tumour hypoxia, vascularisation and blood flow.

Methods Four online databases were searched. Preclinical and clinical randomised controlled trials examining the effects of aerobic exercise training on hypoxia, vascularisation or blood flow in solid tumours were included. The risk of bias was assessed and a meta-analysis performed.

Results Seventeen preclinical studies and one clinical study met criteria. Eleven studies assessed hypoxia, 15 studies assessed vascularisation and seven evaluated blood flow. There was large variability in measurement methods, tumour types and exercise program designs. The overall risk of bias was unclear in clinical and preclinical studies, owing to poor reporting. There was no significant effect of aerobic exercise on hypoxia (SMD = −0.17; 95% CI = −0.62, 0.28; $I^2 = 60\%$), vascularisation (SMD = 0.07; 95% CI = −0.40, 0.55; $I^2 = 71\%$) or blood flow (SMD = 0.01; 95% CI = −0.59, 0.61; $I^2 = 63\%$).

Conclusion There is heterogeneity in methodology, resulting in evidence that is inconsistent and inconclusive for the effects of aerobic exercise on hypoxia, vascularisation and blood flow. Most evidence of aerobic exercise effects on tumour blood flow is in animal models, with very limited evidence in humans.

Keywords Exercise · Tumour · Hypoxia · Vascularisation · Blood flow

Introduction

Cancer incidence worldwide in 2016 was 17.2 million cases, with a 28% increase observed between 2006 and 2016 [1]. There are many modifiable risk factors for cancer development, including obesity and physical inactivity, which combined contribute to 25% of cancer incidence [2, 3]. Exercise during cancer treatment is recognised as an important adjunct to cancer treatment, with significant benefits on quality of life and a reduction in treatment side effects [4–7]. It has been proposed that exercise may also induce adaptations to tumour vasculature and thus stimulate benefit to clinical outcomes of cancer treatment [8].

Unlike in healthy tissues, where blood vessels typically run in parallel, blood vessels in tumours have an unstructured distribution. Tumour centres have high interstitial fluid pressure, leading to the collapse and abnormal compression of vasculature [8]. These structural and functional abnormalities lead to heterogeneous blood flow in in regions of the tumour which is further compounded by necrotic tumour centres [8]. The implication of these abnormal features results in a hypoxic tumour environment that is proposed to suppress immune function, limit the transport of immune cells to tumour regions and increase metastasis, leading to poorer patient prognosis [8–11].

Acute aerobic exercise increases total blood flow to healthy active tissue (e.g. contracting skeletal

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muscle) through the combination of increased cardiac output, increased blood pressure and local vessel vasodilation, with vasoconstriction reducing or maintaining blood flow to inactive tissues [8, 12]. Arterioles in tumours are poorly developed and lack functional smooth muscle [13] and subsequently have a reduced myogenic vasoconstriction response at high pressures such as during aerobic exercise [14]. This inability to vasoconstrict suggests that exercise-induced increased cardiac output, in addition to increased blood pressure, would drive an increase in tumour perfusion through increased total blood flow [14].

Repeated bouts of aerobic exercise cause vascular adaptions in healthy tissue. The combination of angiogenesis and decreased resistance enables increased blood flow to active healthy tissue [12]. These effects have been proposed to be paralleled in tumours, such that aerobic exercise training may cause adaptations that modulate tumour blood flow through increased blood vessel density and improved organisation and vessel function [8, 13]. If these adaptions occur, aerobic exercise training may normalise the tumour microenvironment and facilitate increased blood flow and reduced hypoxia, which may have benefits for reducing cancer progression. Given that many cancer treatments such as chemotherapy are administered intravenously, the aerobic exercise training effects of increased blood flow and improved vessel function have the additional potential for improving the delivery and therefore efficacy of such treatments [14].

The purpose of this systematic review is to: (i) summarise the effects of aerobic exercise training on tumour hypoxia, vascularisation and blood flow; and (ii) evaluate the methodological rigour of this literature.

Methods

This systematic review and meta-analysis is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. This review was registered with the PROSPERO Register of Systematic Reviews (CRD42020159201).

Eligibility criteria

Trials were included if they: (1) were a peer-reviewed journal full-text article in English; (2) used a randomised or quasi-randomised study design with a control group; (3) involved humans or animals with a solid malignant tumour; (4) the intervention group performed repeated bouts of any type of aerobic exercise of ≥2 sessions; (5) the control group performed no structured or unstructured aerobic exercise; (6) measured hypoxia, vascularisation, blood flow or indicators thereof.

Search strategy and study selection

We searched Medline via OvidSP (1946–present), EMBASE via OvidSP (1947–present), Scopus (all years) and CINAHL Complete (all years) in September 2021 using the following search terms: 'exercise' OR 'physical activity' OR 'exercise therapy' OR 'aerobic exercise' AND 'neoplasm' OR 'tumour' OR 'tumor' OR 'carcinoma' OR 'cancer' OR 'tumour microenvironment' OR 'tumor microenvironment' OR 'tumour vasculature' OR 'tumor vasculature' AND 'blood delivery' OR 'blood flow' OR 'vascularisation' OR 'vascular function' OR 'vascular remodeling' OR 'hypoxia' OR 'tumor hypoxia' OR 'tumour hypoxia' OR 'oxygenation'.

Two reviewers (CSL, JY) independently performed the initial screening by title and abstract based on the eligibility criteria. Full-text versions of potential eligible studies were then assessed by two reviewers independently (CSL, JY). A third reviewer (KE) screened full-text studies if there were disagreements between the researchers regarding eligibility.

Outcome measures and data extraction

Two reviewers (CSL, LR) independently extracted data, and a third reviewer (KE) performed data extraction if there were disagreements between the researchers. Data were extracted for the three variables; hypoxia, vascularisation and blood flow. Hypoxia included any measure indicative of hypoxia as identified by the study author. Vascularisation includes microvessel density and changes in vessel physiology (including number of functional and patent vessels). Blood flow included changes in both tumour vessel perfusion and tissue perfusion (by MRI or Hoechst 33342 staining). Data extracted were recorded as the change in mean values from baseline for each group, and the standard error of the mean (SMD), standard deviation (SD) or confidence interval (CI) was also recorded. Study characteristics extracted included study details and design, recruitment source and method, participant results, experimental protocol, adherence and funding.

Quality assessment

The included studies were assessed for internal validity using SYRCLE's Risk of Bias Tool [15] or Cochrane Risk of Bias Tool [16]. The SYRCLE Risk of Bias Tool is used to determine the internal validity of animal studies. The tool contains 10 questions relating to 6 different domains of bias; selection bias, performance bias, detection bias, attrition bias, reporting bias and other biases. Each entry was scored as 'no' (indicating high risk), 'yes' (indicating

low risk) or 'unclear' (indicating unclear risk). Each study was assessed in its entirety, irrespective of whether the study had multiple outcome measures. Baseline characteristics included age, sex, tumour type, site of tumour injection and timing of tumour induction prior to randomisation. Studies had to explicitly report study characteristics to assess the risk of bias. Housing allocation was also assessed in Domain 10, specifically if animals were housed individually and analysed individually.

The Cochrane Risk of Bias Tool is used to determine the internal validity of human studies [16]. The tool contains 5 questions that each covers a domain of bias; selection bias, performance bias, detection bias, attrition bias and reporting bias. Within each domain are signalling questions that draw relevant information from the study to determine risk of bias. The responses to the signalling questions are formulated in an algorithm to determine a judgement of 'low risk', 'some concern' or 'high risk'. The Risk of Bias Tool was used for each outcome measure if the study included multiple outcome measures.

Two researchers (CSL, LR) independently performed these assessments. A third reviewer (JY) performed an assessment if there were disagreements between the researchers.

Statistical analysis

A meta-analysis was performed for hypoxia, vascularisation and blood flow. Data from the outcome measures and indicators thereof were extracted as the mean and standard deviation. Data that were presented as SEM or CI were converted to SD. Data were included if they were quantitative and physiologically plausible. Relative values and fold changes were excluded if raw data were unable to be obtained. Negative values, such as for changes in hypoxia-inducible factor 1-alpha (HIF1α), were excluded as these are not physiologically feasible. For studies that had a range in sample size, the lower end of the sample size was used to avoid overpowering the study. The raw data can be found in the Supplemental materials (Supplemental Table 1).

The meta-analysis was performed in RStudio (R version 4.0.3) using a random-effects model. Heterogeneity was assessed by I^2, and a subgroup analysis was performed on hypoxia, vascularisation and blood flow for animals, species, aerobic exercise mode, tumour location and study duration. The meta-analysis was controlled for the inclusion of multiple datasets that were compared to the same control group within a single study using the following equation; $N_{\text{corrected control}} = N_{\text{control}}$ / number of experimental groups [17].

Results

Study selection

The initial database searches yielded 2692 studies (Fig. 1). After duplicates were removed, a total of 1680 studies were screened by title and abstract. Thirty-nine full-text articles proceeded for further review. A total of 18 studies were deemed suitable for inclusion in this review.

Study and participant characteristics

Details of study and participant characteristics of the included studies are presented in Table 1. Of the 18 studies included, 17 were preclinical and one was clinical. Only one study was published prior to 2010. Multiple studies assessed more than one outcome measure; 11 assessed hypoxia, 15 assessed vascularisation and seven evaluated blood flow. Of the 17 preclinical studies, 14 used mice and four used rats. Study duration ranged from five to 245 days. Aerobic exercise in the preclinical studies included both voluntary ($n = 6$) and forced exercise ($n = 11$). The six studies that used voluntary aerobic exercise adopted wheel running, performed daily or every second day. The remaining 11 studies used treadmill running, of which nine studies were five times per week, two studies were daily and one study was every second day. One study used two different aerobic exercise frequencies in different groups. One clinical study explored cycle training sessions three times per week at 55–100% VO_{2peak} in women with breast cancer.

Risk of bias

Preclinical: SYRCLE Risk of Bias

The risk of bias across the preclinical studies is reported in Fig. 2. Of concern, more than half of the included studies ($n = 11$) had a high risk of attrition bias due to not reporting missing data appropriately. However, all 17 studies were free of selective outcome reporting as all available results were presented as described in the study methods. Nine studies reported clear baseline characteristics and therefore were assessed as having a low risk, while the remaining eight studies did not clearly report all baseline characteristics and were assessed as having an unclear risk. Most other outcomes were assessed as having an unclear risk due to poor reporting of study methodology.

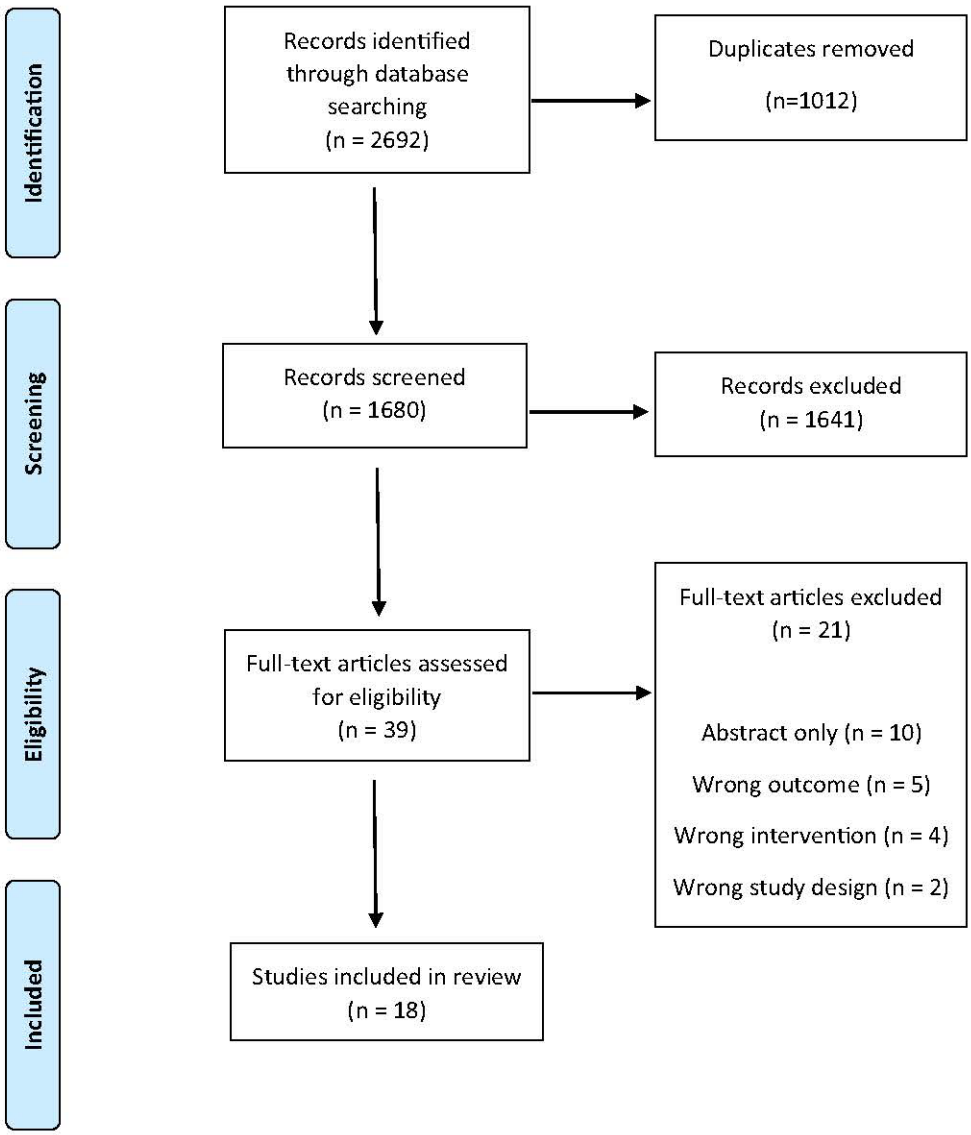


Fig. 1 PRISMA flowchart

Clinical: Cochrane Risk of Bias

Overall, there was a high risk of bias for the clinical study, primarily a result of missing outcome data for hypoxia and vascularisation (Fig. 3). There were additional concerns regarding randomisation, as the study did not detail the concealment procedure. Blinding of participants was not possible due to the pragmatic nature of the exercise intervention, although there was still low risk of bias for deviations from the intended intervention. Although the overall risk of bias was high, there was low risk of bias in the measurement and reporting of all outcomes (Fig. 3).

Hypoxia

Measures of hypoxia included HIF1α (n = 6), EF5 (n = 2), pimonidazole (n = 2), carbonic anhydrase IX (CAIX) (n = 2) and partial pressure of oxygen (PO_2) (n = 1) (Table 2). Some studies assessed multiple measures of hypoxia. Of the 11 studies which assessed hypoxia, six studies reported a decrease in hypoxia [18–23], one study reported an increase [24] and five studies reported no change [23, 25–28]. One study [23] utilised four different datasets, inclusive of two different tumour types and two different measurement methods, finding a decrease and no change in hypoxia within the one paper. Six studies were included in the meta-analysis for hypoxia [18, 20, 23, 25, 26, 28], with three studies having multiple measures analysed [23, 25, 26]. Four studies were excluded from the meta-analysis due to implausible physiological values or lack of continuous mean data [19, 22, 24]. Overall, there was no significant effect of aerobic exercise on hypoxia (SMD = −0.17; 95% CI = −0.62, 0.28; I^2 = 60%) (Fig. 4a).

Table 1 Study characteristics

Variable	Preclinical trials (n = 17)	Clinical trials (n = 1)
Publication year		
2000–2009	1 (6)	0 (0)
2010–2021	16 (94)	1 (100)
Sample size		
≤20	7 (41)	1 (100)
21–49	7 (41)	0 (0)
≥50	3 (18)	0 (0)
Study duration		
<3 weeks	7 (41)	0 (0)
3–8 weeks	8 (47)	0 (0)
>8 weeks	2 (12)	1 (100)
Animal species[a]		
Mice	14 (82)	–
Rat	4 (24)	–
Animal breed/strain		
BALB/c mice	5 (29)	–
Nude mice[a]	3 (18)	–
C57BL/6 mice	3 (18)	–
Sprague-Dawley rat	2 (12)	–
Athymic mice	2 (12)	–
ApoE−/− mice	1 (6)	–
Copenhagen rats[a]	1 (6)	–
American Cancer Institute rats	1 (6)	–
Cancer type		
Breast[b]	9 (53)	1 (100)
Prostate	3 (18)	0 (0)
Pancreatic[b]	2 (12)	0 (0)
Melanoma[b]	2 (12)	0 (0)
Ewing sarcoma[b]	1 (6)	0 (0)
Liver	1 (6)	0 (0)
Lymphatic	1 (6)	0 (0)
Exercise mode		
Type		
Treadmill running	11 (65)	0 (0)
Wheel running	6 (35)	0 (0)
Cycling	0 (0)	1 (100)
Frequency[c]		
1–4×/week	1 (6)	1 (100)
5–7×/week	17 (100)	0 (0)
Outcome measure[d]		
Hypoxia	10 (59)	1 (100)
Vascularisation	14 (82)	1 (100)
Blood flow	6 (35)	1 (100)

Data are presented as n (%)

[a]Some studies included more than one animal species and breed

[b]Some studies included more than one cancer type

[c]Some studies included 2 groups that performed different exercise frequencies

[d]Some studies included more than one outcome measure

Vascularisation

Measures of vascularisation included microvessel density (n = 14), functional vessels (n = 2), vessel length (n = 1), patent vessels (n = 1) and indicators for vessel function and structure (n = 3) (Table 2). Four studies reported an increase in microvessel density [18, 24, 29, 30], three studies reported a decrease in microvessel density [19, 31, 32] and eight studies reported no change in microvessel density [20, 23, 25–28, 33, 34]. Twelve studies were included in the meta-analysis for vascularisation [18–20, 23–30, 33, 34], with four studies having multiple measures [23, 25, 26, 34]. One study was excluded due to no baseline data [32]. One study [31] was excluded from the presented forest plot for vascularisation due to being an outlier, resulting in reduced legibility of the remainder of the meta-analysis results (SMD = 0.07; 95% CI = −0.51, 0.64; I^2 = 79%) (full plot available in Supplemental Figure 1). The exclusion made no difference in the overall effect size. Overall, there was no effect of aerobic exercise on vascularisation (SMD = 0.07; 95% CI = −0.40, 0.55; I^2 = 71%) (Figure 4b).

Blood flow

Of the seven studies that evaluated blood flow, three reported an increase [18, 24, 28], one reported a decrease [27] and three reported no change [25, 26, 35] (Table 2). Four studies were included in the meta-analysis for blood flow [25–28, 35], with two studies having multiple measures, including blood flow and perfusion [25, 26]. One study was excluded from the meta-analysis due to no quantitative data reported, and another study was excluded as there were no mean data [18, 24]. Overall, there was no significant effect of aerobic exercise on blood flow (SMD = 0.01; 95% CI = −0.59, 0.61; I^2 = 63%) (Figure 4c).

Sub-group analysis

Given the heterogeneity of methodology in the studies included in this review, the total meta-analysis is limited. In an attempt to review the data taking into account some of these methodological differences, we performed sub-group analyses for animals, species, aerobic exercise mode, tumour location and study duration. However, it should be noted that these sub-groups included large heterogeneity, such as in exercise mode (for example, treadmill exercise dose). We therefore performed sub-group analyses using broad categories to analyse the data. The results from the sub-analyses showed no significant effect in any of the analyses. Sub-group analyses can be found in the Supplemental materials (Supplemental Figures 2a–e, 3a–e and 4a–e).

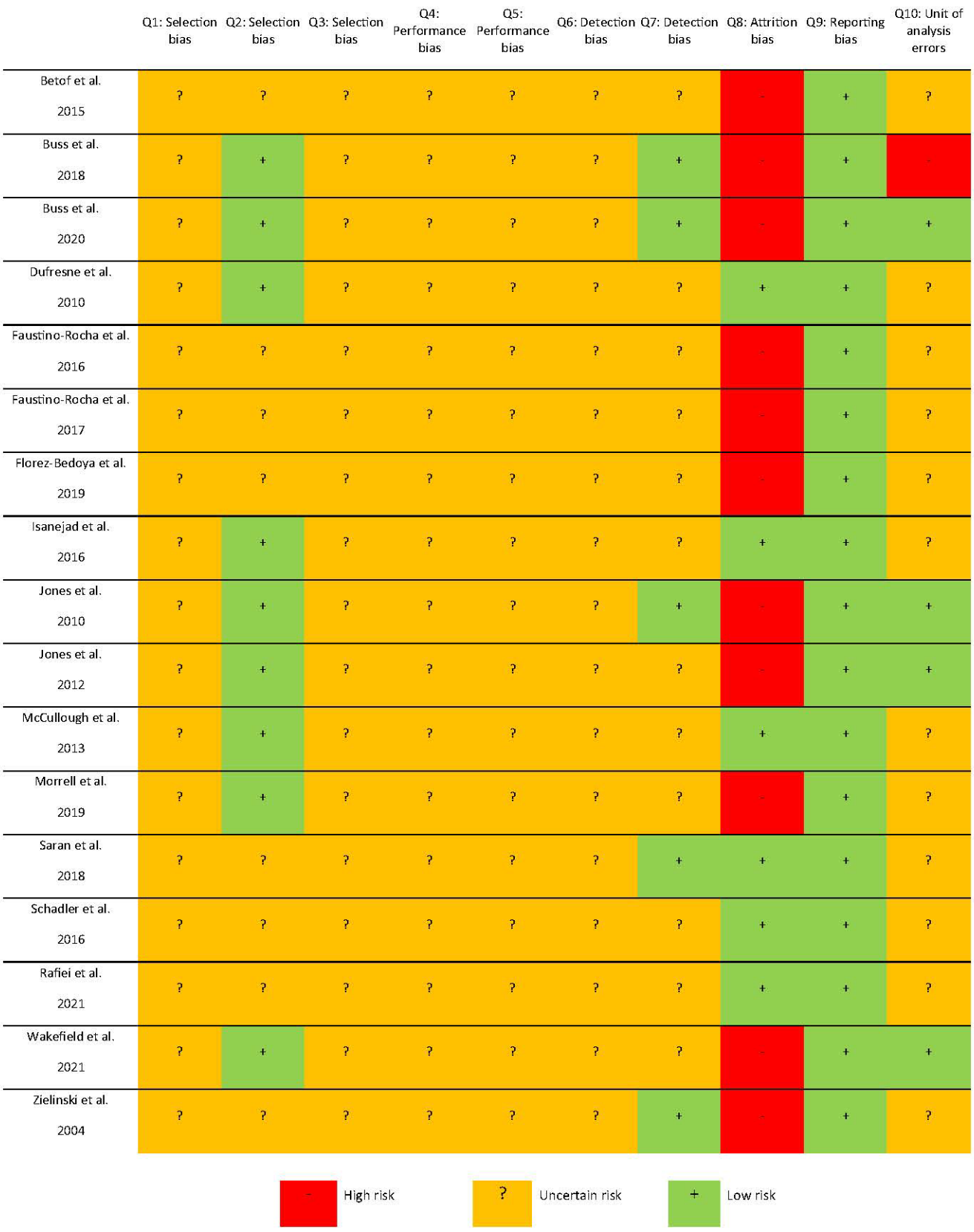


Fig. 2 SYRCLE Risk of Bias for preclinical studies

Fig. 3 Cochrane Risk of Bias for clinical studies

Discussion

The findings from this systematic review and meta-analysis demonstrate that among 17 preclinical studies and one clinical study, aerobic exercise training had no significant effect on tumour hypoxia, vascularisation or blood flow. To our knowledge, this is the only systematic review exploring the effects of hypoxia, vascularisation and blood flow on all three of these related outcomes. Concerningly, there was a high risk for attrition bias in the preclinical studies, and most other domains of bias were of unclear risk. Of the 18 studies included in this review, 17 were published within the last decade, suggesting that there is a growing interest in this area. Although the preclinical body of evidence is growing, the synthesis of data is limited by the variability in animal type and study methodology. The measurement of the outcomes reported herein is possible in a human population, as evidenced in Jones et al. (2013). Therefore, future knowledge gains may be made by exploring these effects in humans for translation to clinical practice.

We anticipated that aerobic exercise would drive an association between our outcome measures; that aerobic exercise training would result in an increase in vascularisation that would cause increased blood flow and consequently decreased hypoxia, representing beneficial changes in the tumour microenvironment with the potential to reduce tumour growth and improve patient prognosis. This assumption has been based on the literature, exemplified by recent narrative reviews [36, 37]. However, the expected pattern of change was not found in this review, with studies reporting aerobic exercise-induced decreases and increases in all outcome measures and combinations of decreases and increases that do not support our assumption. Although our findings contrast with recent narrative reviews, our conclusions are supported by the systematic inclusion of all available data.

In the period between registration and completion of this review, Esteves et al. [38] published a systematic review and meta-analysis that included one of the outcomes we assessed: vascularisation. The authors found an effect of aerobic exercise on vascularisation, which is in conflict with the findings from this review. We have described a number of concerns with the methodology, data extraction and data analysis of their review elsewhere [39]. The current comprehensive review and meta-analysis uses a more appropriate statistical analysis recommended for preclinical studies [40–42] and includes all available studies, providing a more accurate evaluation of the current literature.

Contrary to our expectations, we found that there were inconsistent findings for all explored outcomes. We expected to see decreases in hypoxia; however, of the 11 studies which assessed hypoxia, one [24] found an unexpected increase and five [23, 25–28] found no change. We expected increased vascularisation; however, three of 15 studies [19, 31, 32] showed a reduction and eight [20, 23, 25–28, 33, 34] showed no change. Our expectation of increased blood delivery was also not met; three studies demonstrated no change [25, 26, 35], and one study demonstrated a decrease [27] in blood flow. These unexpected results in hypoxia, vascularisation and blood flow may be a result of many factors, including experimental design differences as well as intra-tumoural factors. For example, physiological mechanisms that may have contributed to these results include angiogenesis development and/or decreased inflammatory immune cells. Aerobic exercise induces the formation of new blood vessels through regulatory factors such as increases in VEGF, which promotes angiogenesis [24]. Although angiogenesis may increase microvessel density and subsequent blood flow to tumours, the dysfunction in tumour blood vessels (specifically leaky blood vessels and low permeability) may mean that this is not translated to changes in hypoxia if oxygen diffusion is poor [9, 10, 31, 40]. Furthermore, tumour blood vessels are heterogeneous in distribution, leading to inconsistent oxygen supply [8], which may mean areas of increased hypoxia within the tumour despite potential increases in overall blood flow through increased microvessel density. Therefore, a single hypoxia measurement may not represent the level of hypoxia elsewhere in the tumour. Immune cell's contribution to angiogenesis has also been suggested to be altered by aerobic exercise training. For

Table 2 Summary of studies

Author (year)	Participants	Housing condition	Cancer type/model	Intervention	Outcome measure: method of measure	Author reported result	Other comments
Preclinical studies							
Betof et al. (2015)	$N = 22–24$ female immunocompetent BALB/c mice	Housing groups: control group were housed individually; experimental group not reported Temperature: not reported Humidity: not reported Light/dark cycle: mice exercised in the dark cycle Wheel diameter: 11.5 cm	Breast: syngeneic 4T1 murine breast cancer cells orthotopically transplanted in the dorsal mammary fat pad	Mode: voluntary wheel running Frequency: continuous access to 11.5 cm diameter wheel Duration: 18 days Speed/distance: not reported	Hypoxia: EF5 Vascularisation: MVD by CD31, vascular maturity Blood flow: Perfusion by magnetic resonance imaging (MRI)	Hypoxia: ↓ Vascularisation: ↑ MVD ↑ vessel maturity Blood flow: ↑	
Buss et al. (2018)	$N = 30–43$ female ApoE+/− mice	Housing groups: control group were housed in pairs or threes; experimental group were housed in pairs Temperature: ~22°C Humidity: not reported Light/dark cycle: housed in 12:12-h light/dark cycle Wheel diameter: not reported	Breast: syngeneic EO771 murine medullary breast adenocarcinoma implanted orthotopically into the 4th mammary fat pad	Mode: voluntary wheel running Frequency: continuous wheel access Duration: until tumours reached 600 m^3 (~17 days) Distance: 10 km/day per pair	Hypoxia: Pimonidazole Vascularisation: MVD by CD31 Blood flow: perfusion by Hoechst 33342 staining	Hypoxia: ‹ › Vascularisation: ‹ › Blood flow: ‹ ›	Mice were euthanised early if impact on welfare occurred due to ulceration of the tumour (n =8) or suspicion of internal tumours (n=5). One mouse was euthanised before the tumour reached measurable size due to malocclusion.
Buss et al. (2018)	$N = 30–43$ female ApoE+/− mice	Housing groups: control group were housed in pairs or threes; experimental group were housed in pairs Temperature: ~22°C Humidity: not reported Light/dark cycle: housed in 12:12-h light/dark cycle Wheel diameter: not reported	Breast: syngeneic EO771 murine medullary breast adenocarcinoma implanted orthotopically into the 4th mammary fat pad	Mode: voluntary wheel running Frequency: wheel access every 2nd day Duration: until tumours reached 600 m^3 (~17 days) Distance: 8 km/day per pair	Hypoxia: pimonidazole Vascularisation: MVD by CD31 Blood flow: Pperfusion by Hoechst 33342 staining	Hypoxia: ‹ › Vascularisation: ‹ › Blood flow: ‹ ›	Mice were euthanised early if impact on welfare occurred due to ulceration of the tumour (n =8) or suspicion of internal tumours (n = 5). One mouse was euthanised before the tumour reached measurable size due to malocclusion.
Buss et al. (2020)	$N = 48$ C57BL/6 female mice	Housing groups: housed in pairs Temperature: not reported Humidity: not reported Light/dark cycle: housed in 12:12-h light/dark cycle Wheel diameter: not reported	Melenoma and breast: B16-F10 melanoma cells or EO771 breast cells injected subcutaneously into the flank or mammary fat pad	Mode: voluntary wheel running Frequency: continuous access to wheel Duration: until melanoma tumours reached 1000 m^3 (median 17 days) or breast tumours reached 600 m^3 (median 21 days) Distance: 8 km/day	Hypoxia: pimonidazole Vascularisation: MVD by CD31 Blood flow: perfusion by Hoechst 33342 staining	Hypoxia: ‹ › Vascularisation: ‹ › Blood flow: ‹ ›	

Table 2 (continued)

Author (year)	Participants	Housing condition	Cancer type/model	Intervention	Outcome measure: method of measure	Author reported result	Other comments
Dufresne et al. (2020)	$N = 17$ athymic male nude mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: housed in 12:12-h light/dark cycle Stimulation for exercise: not reported	Prostate: human prostate cancer PPC-1 cells injected subcutaneously into the dorsal	Mode: treadmill running Frequency: 5×/week Duration: 25–60 min Speed: 18 m/min Slope: 10%	Vascularisation: MVD by CD31	Vascularisation: ‹ ›	
Faustino-Rocha et al. (2016)	$N = 21$ female Sprague-Dawley rats	Housing groups: not reported Temperature: 23 ± 2°C Humidity: 50 ± 10% Light/dark cycle: housed in 12:12-h light/dark cycled; exercised in 12-h dark cycle Stimulation for exercise: not reported	Breast: mammary tumours were induced by a single intraperitoneal administration of the carcinogen agent MNU at a dose of 50 mg/kg	Mode: treadmill running Frequency: 60 min/day for 5×/week Duration: 35 weeks Speed: not reported Slope: not reported	Vascularisation: MVD assessed visually	Vascularisation: ↑	One animal from the MNU exercised group did not adapt to the exercise training and was excluded from the study. During the experiment nine animals died: four animals from the MNU sedentary group (MI = 27%), four animals from the MNU exercised group (MI = 29%) and one animal from the control sedentary (MI = 10%)
Faustino-Rocha et al. (2017)	$N = 21$ female Sprague-Dawley rats	Housing groups: not reported Temperature: 23 ± 2°C Humidity: 50 ± 10% Light/dark cycle: housed in 12:12-h light/dark cycled; exercised in 12-h dark cycle Stimulation for exercise: not reported	Breast: mammary tumours were induced by a single intraperitoneal administration of the carcinogen agent MNU at a dose of 50 mg/kg	Mode: treadmill running Frequency: 60 min/day for 5×/week Duration: 35 weeks Speed: not reported Slope: not reported	Blood flow: Doppler power ultrasound	Blood flow: ‹ ›	One animal from the MNU exercised group did not adapt to the exercise training and was excluded from the study. During the experiment nine animals died: four animals from the MNU sedentary group (MI = 27%), four animals from the MNU exercised group (MI = 29%) and one animal from the control sedentary (MI = 10%) Due to their small size (mammary tumours <1.0 cm were not analysed), only 15 of 28 mammary tumours (54%) from the MNU sedentary group and 11 of 23 (48%) from the MNU exercised group were evaluated by contrast-enhanced US.

Table 2 (continued)

Author (year)	Participants	Housing condition	Cancer type/model	Intervention	Outcome measure: method of measure	Author reported result	Other comments
Florez-Bedoya et al. (2019)	$N = 10–14$ male nude mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Pancreatic: patient-derived xenograft of pancreatic ductal adenocarcinoma tumour tissue implanted subcutaneously into the flank	Mode: treadmill running Frequency: 45 min/day for 5 days/week Duration: 4 weeks Speed: 12 m/min Slope: not reported	Vascularisation: MVD by CD31, functional vessels by lectin perfusion	Vascularisation: ↑ MVD ‹ › functional vessels	
Isanejad et al. (2016)	$N = 16$ female BALB/c mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: housed in 12:12-h light/dark cycle; exercised at the end of dark cycle Stimulation for exercise: gentle tap on the tail or hindquarters	Breast: mouse mammary tumour cells MC4-L2 injected into the flank	Mode: treadmill running Frequency: 10–14 min/day for 5 days/week Duration: 5 weeks Speed: 16–18 m/min that increased each week Slope: 0% Stimulus: gentle tap by investigator on the tail or hindquarters	Hypoxia: HIF1α Vascularisation: MVD by CD31	Hypoxia: ↓ Vascularisation: ↓	
Jones et al. (2010)	$N = 50$ female athymic mice	Housing groups: housed individually Temperature: not reported Humidity: not reported Light/dark cycle: exercised in dark cycle Wheel diameter: 11.5 cm	Breast: human mammary adenocarcinoma cell line MDA-MB-231 injected orthotopically into the right dorsal mammary fat pad	Mode: voluntary wheel running Frequency: continuous access to a 11.5 cm diameter wheel Duration: until tumours reached 1500 m^3 (44 ± 3 days) Distance: ~4 to ~6 km/day	Hypoxia: HIF1α and CAIX Vascularisation: MVD by CD31 Blood flow: perfusion by Hoechst 33342 staining	Hypoxia: HIF1α ‹ › CAIX ‹ › Vascularisation: ‹ › Blood flow: ↑	Histological analysis was only performed on tumours obtained from the 10 animals recording the highest mean exercise running distance and 10 random control animals.
Jones et al. (2012)	$N = 38$ male C57BL/6 mice	Housing groups: housed individually Temperature: 21°C Humidity: 35–45% Light/dark cycle: housed in 12:12-h light/dark cycle Wheel diameter: 11.5 cm	Prostate: transgenic adenocarcinoma of mouse prostate (TRAMP) C-1 cells injected orthotopically into the prostate	Mode: voluntary wheel running Frequency: continuous access to a 11.5 cm diameter wheel Duration: four mice per group were serially killed on days 14, 31 and 36; the remaining 38 mice (exercise, $n = 18$; control, $n = 20$) were killed on day 53. Distance: ~4 to ~6 km/day	Hypoxia: HIF1α Vascularisation: MVD by CD31 Blood flow: perfusion by magnetic resonance imaging (MRI)	Hypoxia: ↑ Vascularisation: ↑ Blood flow: ↑	

Table 2 (continued)

Author (year)	Participants	Housing condition	Cancer type/model	Intervention	Outcome measure: method of measure	Author reported result	Other comments
McCullough et al. (2013)	$N = 27$ male Copenhagen and nude rats	Housing groups: not reported Temperature: 23°C Humidity: not reported Light/dark cycle: housed in 12:12 light/dark cycle Stimulation for exercise: not reported	Prostate: Dunning R-3327 rat prostate adenocarcinoma cell line in both animal species	Mode: treadmill running Frequency: 60 min/day for 5 days/week Duration: 7 weeks (Copenhagen rats) 5 weeks (nude rats) Speed: 15 m/min Slope: 15°	Hypoxia: EF5 and PO_2 Vascularisation: patent blood vessels	Hypoxia: EF5 ↓ PO_2 ↓ Vascularisation: ‹ ›	The duration of intervention in nude rats were shortened by 2 weeks to avoid the potential of tumour size constraints.
Morrell et al. (2019)	$N = 10$–20 male nude mice	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Ewing Sarcoma: A673 and TC71 human Ewing Sarcoma cells injected into the backs of mice	Mode: treadmill running Frequency: 45 min/day for 5 consecutive days/week Duration: 2 weeks Speed: 12 m/min Slope: not reported	Hypoxia: HIF1α and CAIX Vascularisation: MVD by CD31, vessel morphology	Hypoxia: A673 tumours - HIF1α ‹ › - CAIX↓ TC71 tumours - HIF1α ‹ › - CAIX ‹ › Vascularisation: ‹ › MVD ↓ vessel permeability	
Rafiei et al. (2021)	$N = 16$ female BALB/c mice	Housing groups: not reported Temperature: 22 ± 3°C Humidity: 40–60% Light/dark cycle: housed in 12:12-h cycle Wheel diameter: not reported	Breast: MC4-L2 cancer cells injected subcutaneously	Mode: treadmill running Frequency: 30 min in the first 2 weeks and increasing by 5 min every fortnight Duration: 8 weeks Speed: 14 m/min increasing to 20 m/min in the last 2 weeks Slope: not reported	Hypoxia: HIF1α	Hypoxia: ↓	
Saran et al. (2018)	$N = 18$ American Cancer Institute rats (sex not stated)	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Liver: MH-3924A cells were implanted into the liver	Mode: treadmill running Frequency: 770 m for 5 days/week Duration: 6 weeks pre-tumour implantation, 4 weeks posttumour implantation Speed: not reported	Vascularisation: MVD by CD31	Vascularisation: ↓	
Schadler et al. (2016)	$N = 10$–12 male and female wild-type mice from C57B1/6J	Housing groups: not reported Temperature: not reported Humidity: not reported Light/dark cycle: not reported Stimulation for exercise: not reported	Melanoma and pancreatic: B16F10 melanoma and PDAC-4662 pancreatic ductal adenocarcinoma tumours were injected subcutaneously into the flanks of mice	Mode: treadmill running Frequency: 45 min/day for 5 consecutive days/week Duration: 3 weeks Speed: 12 m/min in mice with PDAC4662 10 m/min in mice with B16F10 Slope: not reported	Vascularisation: MVD by CD31, functional vessels by lectin, vessel length	Vascularisation: ‹ › MVD ↑ vessel function ↑ vessel length	

Table 2 (continued)

Author (year)	Participants	Housing condition	Cancer type/model	Intervention	Outcome measure: method of measure	Author reported result	Other comments
Wakefield et al. (2021)	$N = 14$ female BALB/c mice	Housing groups: housed individually Temperature: not reported Humidity: not reported Light/dark cycle: not reported Wheel diameter: not reported	Breast: EMT6 murine mammary cells were implanted into the 4th mammary	Mode: voluntary wheel running Frequency: continuous access to wheel Duration: until tumours reached 200 mm^3 (15 ± 4 days) plus an additional 7 days Distance: 9–14 km/day	Hypoxia: HIF1α and HIF2α	Hypoxia: ↓	
Zielinski et al. (2004)	$N = 137$ female BALB/cByJ mice	Housing groups: housed individually Temperature: 23°C Humidity: not reported Light/dark cycle: housed in 12:12-h reverse light cycle; exercised during dark cycle Stimulation for exercise: not reported	Lymphoma: EL-4 lymphoid cells subcutaneously injected in the back behind the neck	Mode: treadmill running Frequency: 3 h or until volitional fatigue (mean time to fatigue 135 ± 25 min) for 7 days/week Duration: 5–14 days Speed: 20–40 m/min Slope: not reported	Vascularisation: MVD by CD31	Vascularisation: ↓	
Clinical studies							
Jones et al. (2013)	$N = 20$ females	Not applicable	Breast	Mode: cycling Frequency: 45 min/day for 3 days/week Duration: 12 weeks Intensity: 55–100% VO_{2peak}	Hypoxia: HIF1α Vascularisation: MVD by CD31, cell proliferation Blood flow: PET scan	Hypoxia: ‹ › Vascularisation: ‹ › MVD ‹ › vessel structure Blood flow: ↓	Results for hypoxia and vascularisation were only available for 5 participants per group. Blood flow data was limited due to technical difficulties

All results are $p < 0.05$. *HIF1α*, hypoxia-inducible factor 1-alpha; *HIF2a*, hypoxia-inducible factor 2-alpha; *CAIX*, carbonic anhydrase IX; *MVD*, microvessel density; PO_2, partial pressure of oxygen; *MNU*, two *N*-methyl-*N*-nitrosourea

example, inflammatory macrophages in tumours have been shown to have reduced activation after aerobic training [32]. This type of reduced inflammatory activation may down-regulate angiogenesis and reduced microvessel density, resulting in less blood delivery and increased hypoxia [32].

Variability in study methodology and quality can create significant bias in the results. Features of poor study methodology, such as lack of reporting, heterogeneity of baseline characteristics and external influences such as housing (in preclinical research), aerobic exercise outside of the intervention and significant missing data, can largely impact results both within studies and when comparing studies. The risk of bias results from this review should be used to guide future preclinical and clinical methodology to better compare outcome measures across studies. Housing was poorly reported, which is important for social stress effects [43]. Additionally, the impact of stress on animals, such as stimulation for forced aerobic exercise, is particularly significant in cancer outcomes, whereby it has been postulated that the immune response plays a part in the development of tumour vasculature to promote microvessel density and tumour growth in mice [44, 45]. The characteristics of aerobic exercise were poorly reported across studies. Six studies [18, 22, 24–26, 28] used voluntary wheel running, which suffers from a lack of control of intensity and, therefore, dose. Seven papers included a familiarisation period of aerobic exercise for an average of 18 days [18–20, 29, 32, 33, 35], whereby subjects would perform additional aerobic exercise after tumour induction but prior to the aerobic exercise intervention. Exercise duration includes a combination of familiarisation plus intervention, which further complicates the dose of aerobic exercise delivered, particularly in preclinical models. In the current analysis, we saw heterogeneity in animal type and species (14 mice studies with five different species and four rat studies with three different species), making comparison of results between papers problematic and suitability for human models debateable [46].

Tumour type and location likely play a considerable role in determining the effects of aerobic exercise on blood flow. Host tissue is anatomically and functionally different, and thus features of the tumour microenvironment that influence blood flow may also differ based on tumour location. Therefore, it may not be equal to compare tumour type and location. For example, tumours located in host tissue that has a high blood supply at rest, such as the brain, are more likely to use vascular co-option, which encourages greater tumour vascularisation [47]. Furthermore, vasculogenic mimicry occurs in some tumour types, such as melanoma and breast, and can also increase blood supply to and within the tumour [48]. In preclinical models, tumours are induced orthotopically (tumours that are injected into the corresponding host tissue) or ectopically (tumours injected subcutaneously into a different host tissue). Garcia and colleagues [49] directly compared ectopic prostate tumours placed subcutaneously with orthotopic prostate tumours and found increased blood flow in orthotopic tumours after aerobic exercise, suggesting that tumour location plays an important role irrespective of tumour type. We included 13 papers that investigated orthotopically injected tumours and two papers that investigated subcutaneously injected tumours, and we observed no difference in blood flow after aerobic exercise in orthotopic compared to subcutaneous tumours, which is contrary to the results of Garcia et al [49]. However, a majority of the papers in this systematic review investigated breast tumours, whilst Garcia et al. [49] investigated prostate tumours. Therefore, further research needs to be conducted to determine if tumour type, in addition to tumour location, influences blood flow.

Whilst our findings do not favour aerobic exercise training in terms of tumour hypoxia, vascularisation and blood flow, several systematic reviews and meta-analyses have found beneficial effects of aerobic exercise training for tumour growth and development [44, 50]. Given this contrast, we briefly examined tumour growth outcomes, which were reported in 13 studies included in this review [18, 21, 22, 24–26, 28–34]. Of these, there were inconsistent results in tumour growth; one reported increased growth, four showed decreased growth and nine showed no change in tumour size. One study [34] showed both a decrease in one tumour type and an increase in another tumour type. We observed similar inconsistent patterns of change in hypoxia, vascularisation and blood flow even with studies showing the same tumour growth changes. However, it should be noted that tumour growth was not a primary outcome of this review and the reported findings are descriptive only. As the studies included in this review did not mirror the results from the two published systematic reviews on tumour growth, we believe that the mechanisms of action for aerobic exercise on tumour growth are complex and not well described by this literature. It is possible that the mechanisms of aerobic exercise effects could involve interactions between hypoxia, vascularisation and blood flow; however, more consistent data, methodology and reporting are necessary to further explore these associations. It is also likely that other systemic or intra-tumoural factors, such as inflammation, immune responses, myokine signalling and endocrine responses and adaptations, impact tumour growth [44, 51, 52].

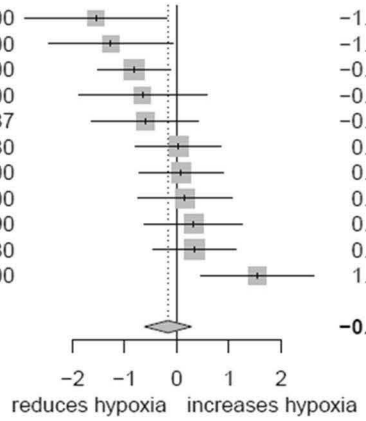
Limitations

Despite using author-reported results, we found that there were two instances where there were differences in the significance of findings between author-reported results

a

Study	Exercise Total	Mean	SD	Control Total	Mean	SD	SMD	95%−CI	Weight
McCullough_2013_EF5	6	4.00	2.4500	6	39.00	29.3900	−1.55	[−2.91; −0.19]	6.5%
Morrell_2019_CAIX_A673	6	0.50	0.3300	8	1.23	0.6500	−1.27	[−2.46; −0.07]	7.5%
Betof_2015	17	25.50	27.0300	17	48.80	29.1400	−0.81	[−1.51; −0.11]	11.4%
Morrell_2019_HIF1a_A673	6	0.42	0.3500	5	0.94	1.0200	−0.65	[−1.88; 0.58]	7.2%
Morrell_2019_HIF1a_T671	7	1.01	0.0064	9	1.01	0.0087	−0.61	[−1.62; 0.41]	8.7%
Buss_2018_Hex	11	9.80	10.2650	12	9.49	7.1880	0.03	[−0.78; 0.85]	10.4%
Buss_2020_B16F10	12	10.74	15.7900	12	9.61	9.8400	0.08	[−0.72; 0.88]	10.5%
Buss_2020_EO771	10	17.43	11.9900	9	15.71	8.5800	0.16	[−0.75; 1.06]	9.6%
Morrell_2019_CAIX_T671	8	1.01	0.0090	10	1.01	0.0090	0.32	[−0.62; 1.25]	9.4%
Buss_2018_Lex	13	12.96	11.9160	12	9.49	7.1880	0.34	[−0.45; 1.13]	10.6%
Jones_2010_total_tum	9	4.20	1.3000	9	2.10	1.3000	1.54	[0.45; 2.62]	8.2%
Random effects model	**105**			**109**			**−0.17**	**[−0.62; 0.28]**	**100.0%**

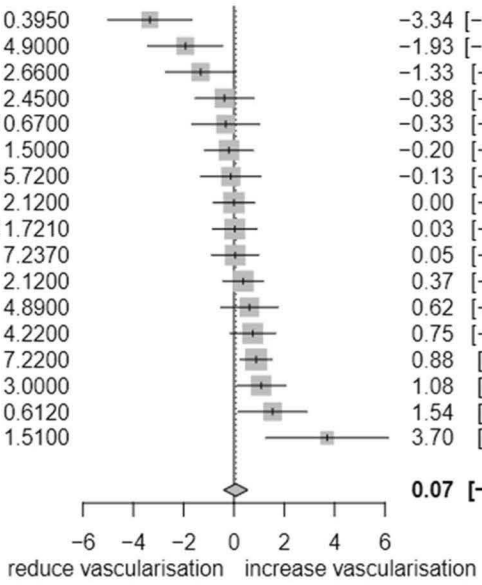
Heterogeneity: $I^2 = 60\%$, $\tau^2 = 0.3391$, $p < 0.01$



b

Study	Exercise Total	Mean	SD	Control Total	Mean	SD	SMD	95%−CI	Weight
Isanejad_2016	8	1.30	0.1950	8	2.40	0.3950	−3.34	[−4.99; −1.68]	4.3%
Zielinski_2004	6	10.62	4.0400	6	20.00	4.9000	−1.93	[−3.40; −0.46]	4.8%
Morrell_2019_TC71	6	11.99	2.0600	5	15.39	2.6600	−1.33	[−2.70; 0.05]	5.1%
McCullough_2013	6	10.00	2.4500	6	11.00	2.4500	−0.38	[−1.52; 0.77]	5.9%
Schadler_2016_B16F10	5	41.70	5.4100	4	44.69	10.6700	−0.33	[−1.66; 1.00]	5.3%
Dufresne_2020	8	1.19	0.3110	9	1.42	1.5000	−0.20	[−1.16; 0.76]	6.5%
Schadler_2016_PDAC	6	60.09	12.2100	5	62.03	15.7200	−0.13	[−1.32; 1.06]	5.7%
Buss_2018_Hex	11	3.82	1.4920	12	3.82	2.1200	0.00	[−0.82; 0.82]	7.0%
Buss_2020_EO771	10	25.37	10.6720	10	24.96	11.7210	0.03	[−0.84; 0.91]	6.8%
Buss_2020_B16F10	10	11.54	5.5990	8	11.21	7.2370	0.05	[−0.88; 0.98]	6.6%
Buss_2018_Lex	13	4.44	1.0100	12	3.82	2.1200	0.37	[−0.43; 1.16]	7.1%
Morrell_2019_A673	7	17.46	3.0400	6	14.82	4.8900	0.62	[−0.51; 1.74]	5.9%
Faustino−Rocha_2016	10	18.35	11.3500	11	11.82	4.2200	0.75	[−0.15; 1.64]	6.8%
Betof_2015	21	32.20	13.6800	23	22.50	7.2200	0.88	[0.26; 1.51]	7.7%
Jones_2010	10	22.90	4.0000	10	18.90	3.0000	1.08	[0.13; 2.04]	6.5%
Jones_2012	6	2.58	1.2000	6	1.00	0.6120	1.54	[0.18; 2.89]	5.2%
Florez_Bedoya_2019	5	24.02	6.5150	5	4.63	1.5100	3.70	[1.28; 6.13]	2.7%
Random effects model	**148**			**146**			**0.07**	**[−0.40; 0.55]**	**100.0%**

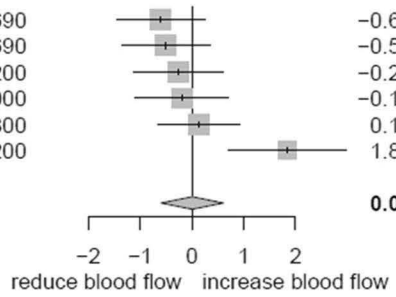
Heterogeneity: $I^2 = 71\%$, $\tau^2 = 0.6720$, $p < 0.01$



c

Study	Exercise Total	Mean	SD	Control Total	Mean	SD	SMD	95%−CI	Weight
Buss_2018_HEx	12	16.96	9.4920	10	23.48	11.6690	−0.60	[−1.46; 0.27]	17.2%
Buss_2018_LEx	12	17.44	11.4320	10	23.48	11.6690	−0.50	[−1.36; 0.35]	17.3%
Faustino−Rocha_2017_PI	10	2.86	2.2800	11	3.50	2.4200	−0.26	[−1.12; 0.60]	17.2%
Buss_2020_EO771	10	8.06	3.0300	9	9.12	7.2000	−0.19	[−1.09; 0.72]	16.6%
Buss_2020_B16F10	12	9.39	4.0700	12	8.60	6.9800	0.13	[−0.67; 0.94]	18.1%
Jones_2010_total_tum	9	29.74	15.0900	9	8.86	2.3200	1.84	[0.69; 2.99]	13.5%
Random effects model	**65**			**61**			**0.01**	**[−0.59; 0.61]**	**100.0%**

Heterogeneity: $I^2 = 63\%$, $\tau^2 = 0.3550$, $p = 0.02$



◂**Fig. 4** **a** Meta-analysis of preclinical studies investigating the effect of exercise on hypoxia. SD, standard deviation; SMD, standardised mean difference; 95% CI, 95% confidence interval (upper; lower limits). **b** Meta-analysis of preclinical studies investigating the effect of exercise on vascularisation. SD, standard deviation; SMD, standardised mean difference; 95% CI, 95% confidence interval (upper; lower limits). **c** Meta-analysis of preclinical studies investigating the effect of exercise on blood flow. SD, standard deviation; SMD, standardised mean difference; 95% CI, 95% confidence interval (upper; lower limits)

and the meta-analysis calculation. Our careful review and meta-analysis showed inconsistencies within a paper, which were confirmed with the authors but may have been the cause of the different outcomes in the analysis. Subsequently, it is possible that the conclusions of this review would differ without the inclusion of a meta-analysis calculation. Another limitation is that although we conducted sub-group analyses, the results were limited due to poor reporting and heterogeneous study design. Furthermore, our meta-analysis examined one mode of exercise, aerobic exercise only, which does not fully represent the effect of all exercise modes, including resistance exercise.

Conclusion

Among the studies included in this systematic review, aerobic exercise training did not have an effect on tumour hypoxia, vascularisation or blood flow. However, there was great methodological heterogeneity, which may have contributed to the inconsistent findings. Future preclinical studies need improved study design and reporting to provide deeper insights into the complex interactions between hypoxia, vascularisation and blood flow. Furthermore, improved reporting and subsequent analysis of aerobic exercise parameters in future studies, specifically intensity and dosage, will give rise to a better understanding of the effects of aerobic exercise on tumour microenvironment for translation to clinical studies. Given the established benefits of aerobic exercise in reducing cancer burden, understanding the mechanisms is an important step towards designing the most efficacious interventions and best practise for translation to clinical studies.

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Thieme

Letter to the Editor on: "The Effects of Physical Exercise on Tumor Vasculature: Systematic Review and Meta-Analysis."

Dear Editor,

We read with interest the recent article "The Effects of Physical Exercise on Tumor Vasculature: Systematic Review and Meta-analysis" [1] and after careful appraisal and consideration we feel that some aspects of the data and analysis warrant further review. The study reported some promising results, namely that both chronic and acute exercise appear to improve intratumoral vascularisation in animal models. This is an important finding given increased vascularisation through tumor modulation may have the potential to improve chemotherapy delivery and efficacy [2]. However, after conducting further investigations, we query several details in the data extraction and analysis decision-making that we believe impact the conclusions of this article.

Critically, the authors performed a random effects meta-analysis using mean difference (MD) as the effect measure. Based on well-established guidelines, MD is used as a summary statistic when outcome measurements from all studies are made on the same scale [3–5].

However, the outcome measures for tumor vascularisation in the authors' meta-analysis used different measurement scales. For example, nine of the eleven included studies assessed microvessel density using CD31, one used VEGF and another Hoeschst-33342. Standard practice is that data, where the scale of measurement differs, are analysed using standardised mean differences (SMD) [3–5]. This allows results to be expressed on a uniform scale, thus appropriate statistical comparisons can be made.

We replicated and re-analysed Dr Esteves and colleagues' data using the more accurate method of SMD and the result revealed no significant effect of exercise on tumor vascularisation (SMD = 0.63; 95 % CI −0.45, 1.72). This contrasts with the original article that states "regular physical exercise improved tumor vascularisation by 2.13; CI 1.07, 3.20."

We then conducted a detailed review of the extracted data and further raise serious concerns about its accuracy. The authors included raw data from several papers, where the measure variation was expressed as either standard error (SE) or 95 % confidence intervals (CI), but Esteves and colleagues did not convert these to standard deviation (SD) as required for meta-analysis of continuous data [3]. We also identified that the authors extracted and analysed total sample size as described in "methods and materials" instead of the analysed sample size from the study results. Furthermore, data from Betof et al. [6] was extracted from a different cancer model than that which was reported. We believe, that in combination, the aforementioned statistical errors would significantly overestimate the overall reported effect size.

Another worrying concern relates to the synthesis of an incomplete set of evidence, which has the potential to introduce bias, including selective outcome reporting. When reviewed, we found two of the included papers, Buss et al. [7] and Morrell et al. [8], reported multiple datasets assessing vascular outcomes (microvessel density) in two different tumors. In their meta-analysis, however, Esteves and colleagues only included one of the data sets from these papers and did not provide an explanation for the exclusion. Given they appear to meet the review selection criteria, it is possible the inclusion of the additional data may further alter the outcome of the study. In addition, we question the depth and comprehensiveness of the literature search strategy, with what appears to be a low yield of papers from database searches. Through our own investigation, we identified an additional three studies eligible according to the reported selection criteria that were not included in the review.

We also note the authors state the review was reported according to the preferred reporting items to systematic reviews and meta-analyses (PRISMA) guidelines; however several items, including details of publicly registering the review protocol, are missing or incomplete.

In conclusion, we raise five concerns for the accuracy of data extraction, data analysis, completeness of the search and protocol registration of Dr Esteves and colleagues' review. We therefore respectfully seek clarification from the authors of the above points.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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The

EX-FUSION

Exercise and Physical Activity Handbook

Introduction

This booklet aims to help you understand the benefits of physical activity for you during and after your cancer treatment. With the help of an Exercise Physiologist, this booklet will assist you to develop a physical activity routine around your cancer treatment to achieve your goals.

The information and advice in this booklet is general advice. If you have specific questions about your health and physical activity, you should discuss this with your doctor or an Exercise Physiologist.

If you have any questions, contact Catherine (Exercise physiologist) on 9351 9380 or csee0522@uni.sydney.edu.au.

What is physical activity?

Physical activity can include any form of activity that increases your heart rate and uses your muscles.

This may include:

- Walking
- Cycling
- Running
- Swimming
- Going to the gym

The term "physical activity" is often used interchangeably with the term "exercise".

Physical activity doesn't need to be very hard – it can be as simple was walking to the local café!

What types of physical activity are you currently doing?

_____

_____

_____

Benefits of physical activity during cancer treatment

In the past, physical activity was not widely performed during cancer treatments. However current research has indicated that people with cancer can experience many benefits from being physically active during cancer treatment and so physical activity is being more widely encouraged. Research tells us that keeping physically active during your cancer treatment is safe and can have many physical and psychological benefits.

These benefits include:

- Improving your physical fitness and strength
- Improving your mood
- Reducing stress and anxiety
- Reducing cancer-related fatigue
- Reduce the risk of your cancer coming back
- Managing weight
- Increasing energy levels
- Improving your sleep

How much physical activity should I do?

The aim for people with cancer is to move more and sit less. People with cancer are encouraged to be as physically active as they feel that they can be. Physical activity does not need to be structured; it can include doing everyday activities that increase your heart rate such as housework or gardening.

The current physical activity guidelines for people with cancer recommends 150 minutes per week of moderate intensity physical activity plus resistance exercise twice per week. This can be broken up into smaller more achievable blocks. For example, if you aim to do 30 minutes of physical activity, you could break this up into 3x 10 minute walks.

However, these guidelines are not always appropriate for everyone, such as people who are currently undergoing chemotherapy treatment and experience strong side effects.

You will still get some benefits from doing a few minutes of light physical activity than doing no physical activity at all.

How much physical activity do you think you are currently doing?

_____

_____

Let's get started!

It can be a little bit daunting to start any sort of physical activity. That's quite normal! The great news is that there are exercise professionals that can help guide you with your exercise. Accredited Exercise Physiologists (AEPs) are allied health professionals that have completed at least a 4-year university degree. They work with people with chronic medical conditions (including cancer) or injuries and use exercise as a form of medicine.

Knowing why you want to become more active and setting goals can help you get started and to stay motivated.

What are some of your goals or reasons for becoming physically active?

_____

_____

_____

_____

_____

Some people decide to make big changes whilst others prefer to take a more step-by-step approach. Everyone is different and you will know what feels right for you.

If you haven't done much physical activity, a great way to get started is to incorporate more walking into your daily routine.

For example:

- Parking a little bit further away from the shops so that you can walk the rest of the way
- Using the stairs instead of the elevator or lift
- Walking during your lunch break

Based on your goals and your current physical activity, what are some ways that you can increase your physical activity?

_____

_____

_____

_____

Safety tips for exercising with cancer

Before starting on your physical activity, it is important to follow some safety tips to keep you safe and reduce risk of injury:

- Start slow and gradually increase your activity. It's not a race!
- It's normal to feel a bit sore in your muscles for a few days after starting a new form of physical activity. If it doesn't go away after a few days, talk to your doctor.
- If you are going out of your house to exercise, carry your mobile phone and let someone know when you

will be back in case you become unwell or fatigued. If you are exercising alone, it is always good to tell someone where you are going and what time you think you might return.

- Wear comfortable and supportive footwear to reduce risk of injury.
- Stay hydrated with water before, during and after exercising
- Eat well before and after exercising.
- Some symptoms are a warning to stop physical activity immediately and get urgent medical assistance. These include pain or pressure in your chest, severe breathlessness, dizziness or fainting, unusually high or irregular heartbeat, nausea or vomiting and extreme fatigue.

How do I know if I'm exercising at moderate intensity?

A simple way to know if you are exercising at moderate intensity is by using the "Talk Test". All that is involved in the "Talk Test" is for you to talk! If you are walking at a moderate intensity, you should still be able to maintain a conversation but not feel like you are able to sing as this would make you feel breathless.

Another way to measure intensity is by using the "Rate of Perceived Exertion" scale (RPE scale) which requires you to rate how hard you feel that your body is working. It is a scale that ranges from 6-20. Moderate intensity exercise equates to working between the 12-14 mark. Below is the RPE scale with some examples of what each intensity should feel like.

Rating	Intensity	How does this feel?	Examples
6	No exertion	No effort; can do this for an unlimited amount of time	Reading a book, watching television
7	Extremely light	Requires very little effort	Tying shoes
8			
9	Very light	Requires little effort	Chores like folding clothes
10			
11	Light	Requires some effort but not enough to increase your breathing	Walking slowly in the grocery shops
12			
13	Somewhat hard	Can carry a conversation but need to pause for breath from time to time	Brisk walk
14			
15	Hard	Can say 1 sentence; your heart rate is increased and you are breathing very fast	Cycling, swimming, running
16			
17	Very hard	Can only maintain this for 1 minute; hard to breath	Cycling, swimming, running
18			
19	Extremely hard	You are working as hard as possible; you can't talk at all	Sprinting at the end of a race
20			

Keeping track of your physical activity

Some people keep a record of their physical activity to help keep themselves motivated as well as to track the amount of physical activity they are doing. There are several ways that people may record their physical activity:

- Exercise diary
- Online (e.g. myfitnesspal.com)
- Mobile apps (e.g. Map My Fitness, My Fitness Pal)
- Wearable devices (e.g. Fitbit, Apple Watch, Garmin)

To help you keep record of your physical activity, an exercise diary has been included at the end of this booklet.

My exercise isn't going to plan!

When starting to exercise, it's very common for things not to go to plan and that's ok! Some of the reasons that things don't go to plan include:

- Time commitment
- Access to equipment
- Cancer-related side effects such as fatigue
- Unmotivated/boring
- Too hard
- Weather

What might be some of your reasons/barriers that exercise might not go to plan?

_____

_____

_____

_____

_____

Now that you've written some potential reasons for things not going to plan, the best chances of successfully starting and continuing with exercise are to try to overcome or prevent these barriers.

What are some things that you can do to overcome the barriers that you stated above?

_____

_____

_____

_____

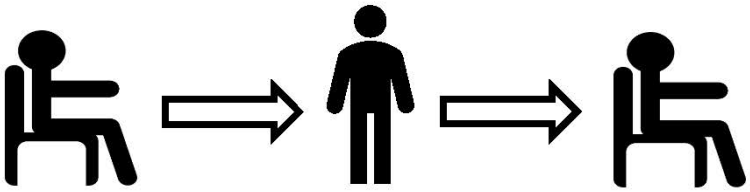
_____

A bit about resistance exercise (strength training)

Resistance exercises uses weights or resistance to increase the strength of your muscles and bones. There are various ways that you can perform resistance exercises:

- Using your own body weight
- Using free weights
- Using machine weights
- Using elastic resistance bands

An easy resistance exercise that you could try at home are "sit to stands" which works the muscles in your legs. You don't need any equipment for this exercise except for a chair! As the name suggests, start seated with your feet hip width apart. Keep your back straight and stand up. You may use your hands on your knees for assistance. Sit back down on the chair. You've just done a strength exercise! If that feels ok, repeat the exercise 10 times.



If you would like more simple resistance exercises, the "Exercise for People Living with Cancer; A guide for people with cancer, their families and friends" by the Cancer Council is a great place to find some easy to follow exercises.

Exercise and activity diary

Week 1: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 2: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 3: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 4: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 5: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 6: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 7: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Exercise and activity diary

Week 8: Dates: ____/____ to _____/_____

Goal: I will be active _____ times this week, for _____ minutes at _____ intensity.

Day	Activity	Duration	RPE (6-20)	Comments
1				
2				
3				
4				

5				
6				
7				

Notes

Notes

Notes

Online resources

Below are also some links to videos of example strength exercises that you can try at home from Cancer Council NSW:

- www.cancercouncil.com.au/cancer-information/living-well/exercise-cancer/strength-training/strength-training-balance-core/
- www.cancercouncil.com.au/cancer-information/living-well/exercise-cancer/strength-training/strength-training-upper-body/
- www.cancercouncil.com.au/cancer-information/living-well/exercise-cancer/strength-training/strength-training-legs/

References

Australia, C. C. (2016). Exercise for People Living with Cancer; A guide for people with cancer, their families and friends. In C. C. Australia (Ed.): Cancer Council Australia.

Gokal, K., Wallis, D., Ahmed, S., Boiangiu, I., Kancherla, K., & Munir, F. (2016). Effects of a self-managed home-based walking intervention on psychosocial health outcomes for breast cancer patients receiving chemotherapy: a randomised controlled trial. Supportive Care in Cancer, 24(3), 1139-1166. doi:10.1007/s00520-015-2884-5