The Influence of Exercise Intensity on Interleukin-8 in Colorectal Cancer Survivors
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BExSS (Clinical Ex Phys) (Hons 1)

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School of Human Movements and Nutrition Sciences
ABSTRACT

Colorectal cancer (CRC) is one of the most common cancers in Western countries and is the second most common cancer for both men and women in Australia. Though 5-year CRC survival has significantly improved in recent years, incidence of the disease has remained relatively stable, therefore increasing the number of people living in CRC survivorship. It is well understood that those living in CRC survivorship are at a heightened risk of again developing the disease.

Though the mechanisms are still not clearly understood, physical activity (PA) levels and intensity are inversely related to the risk of CRC recurrence. A potential factor involved in the relationship between PA and CRC development in CRC survivors is interleukin-8 (IL-8), a recognised molecular driver of cancer growth that can be influenced by exercise. Further justifying the importance of PA to CRC survivors is its ability to improve aerobic fitness and body composition, two health parameters that are often poor in CRC survivors. The primary purpose of the present study was to examine the influence of exercise intensity on IL-8 in a sample of CRC survivors. The secondary purpose was to examine potential changes in peak oxygen consumption (VO2peak) and body composition in response to exercise training completed at two different intensities.

Eighteen CRC survivors were randomly assigned to either a high intensity interval training (HIIT) or moderate intensity training (MIT) group. Participants completed three exercise sessions a week for eight weeks on a cycle ergometer. Each HIIT training session required participants to complete four intervals of 4 minutes each at 85-95% peak heart rate (HRpeak) while in each MIT session, participants completed 50 minutes of exercise at 70% HRpeak. Physiological testing was conducted at baseline (0 weeks), midpoint (4 weeks), and endpoint (8 weeks) of the intervention. Physical activity and diet were controlled in the seven and three days preceding testing respectively. Physiological testing sessions included assessment of VO2peak and body composition using dual-energy X-ray absorptiometry (DXA), with blood collected and analysed for plasma IL-8 concentrations.

IL-8 did not significantly change in either training group at any timepoint. However, secondary analysis of participants from both groups who had elevated baseline IL-8 concentrations (n=4) approached significance for the eight weeks of exercise training to reduce IL-8 values (P=0.068). VO2peak significantly improved in both HIIT and MIT groups after 4 weeks (26.6%, P=0.036 and 3.0%, P=0.043 respectively) and 8 weeks (28.1%, P=0.015 and 7.2%, P=0.018 respectively). After 8 weeks, there was also a group-effect favouring HIIT for improvement in VO2peak (P=0.041). Fat mass and body fat percentage were significantly lower in the HIIT group after 4 weeks (-3.7%,
P<0.01 and (-3.7%, P<0.01) and 8 weeks (-4.9%, P<0.01 and (-3.7%, P<0.01) compared to the MIT group where no changes were observed for either variable. Similarly, lean mass increased in the HIIT group after 4 weeks (+2.2%, P<0.01) and after 8 weeks (+1.9%, P<0.01) but no change in lean mass was found for the MIT group at either timepoint. Changes in lean mass were greater for the HIIT group than the MIT group at 8 weeks (P<0.01). Reductions in fat mass were greater after 4 weeks and 8 weeks for the HIIT group than the MIT group (P=0.046 and P=0.017 respectively).

In summary, exercise failed to elicit significant changes in IL-8 in CRC survivors, likely due to baseline values in IL-8 being within normal ranges. The significant training-induced changes in \( \dot{V}O_2 \text{peak}, \) fat mass, lean mass, and fat percentage found for the HIIT group are potentially clinically meaningful and important, as similar improvements in these markers have previously been associated with reduced cancer specific- and all cause-mortality. The present study has shown that HIIT appears to be a safe and effective mode of exercise for rapidly improving cardiorespiratory fitness and body composition in CRC survivors.
DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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PUBLICATIONS INCLUDED IN THESIS

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CONTRIBUTIONS BY OTHERS TO THIS THESIS

Dr Tina Skinner and Associate Professor David Jenkins made large contributions to the drafting and editing of this thesis. Mr James Devin and Mr Andrew Sax made important contributions in collecting and analysing of data.

STATEMENT OF PARTS OF THE THESIS SUBMITTED TO QUALIFY FOR THE AWARD OF ANOTHER DEGREE

None.
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We’ve achieved a lot over the years – we’ve developed a new international currency in Ice Breaks; we’ve optimised the ratio of rum and cokes to 7eleven $1 coffees for nights on the town; we’ve been the inaugural graduates of a Bachelor of Changing Lives; we’ve produced pun trains that never ran out of track; and now we’ve achieved this. This wad of paper. Your wad will come – much longer, much better, and much more celebrated I’m sure. But until then you can be proud of this wad of paper too, it’s as much yours as it is mine.

Thank you, always.
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cancer, carcinoma, colorectal, exercise, physical activity, interleukin, interleukin-8

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1.0 General Introduction

The term ‘cancer’ describes a range of health conditions characterized by unmediated and uncontrolled cellular growth exceeding that of programmed cell death. This deregulated tissue can infiltrate and harm healthy tissue throughout the body, likely resulting in major disruption to normal physiological function. In addition to the profound physical health implications to the individual, cancer is associated with significant psychosocial challenges to both the individual and their family and friends.

Over 120 000 people are diagnosed with cancer each year in Australia[4] and approximately 50% of the population will be diagnosed with cancer some time in their life[4]. Colorectal cancer (CRC) is the second most common cancer in men and women in Australia[5]. Factors believed to increase the risk of CRC include low physical activity (PA) levels, poor diet, obesity, alcohol consumption, and tobacco smoking [6-12]. While there have been significant advancements in understanding the mechanistic underpinnings of CRC induction and progression[13], how lifestyle factors specifically influence the cellular and molecular drivers of CRC remains unclear.

The ability to modify risk of CRC development through manipulating lifestyle factors is particularly important for people who have previously been diagnosed and treated for CRC. CRC survivors are at a significantly higher risk of developing CRC again when compared to the risk of healthy individuals developing CRC for the first time[14, 15]. With improved awareness of the disease, improved detection of CRC, and more effective treatment strategies, the number of survivors is increasing; reducing recurrence of the disease in CRC survivors is therefore an emerging health focus.

A potential biological mechanism that explains the relationship between PA and CRC risk involves interleukin-8 (IL-8). IL-8 is believed to be intrinsically linked in the molecular processes of CRC and importantly, exercise has consistently been found to acutely influence IL-8 in healthy populations. However, the chronic effects of exercise training on circulating IL-8 in any cancer population are far less studied[16, 17].

CRC survivors are generally characterised by low levels of aerobic fitness and poor body composition[18]. Indeed, improvements in both aerobic fitness and body composition have been associated with vastly improved health parameters in a number of studies examining the benefits of exercise for cancer survivors[19].
The present study examines the response of IL-8 to moderate and high intensity exercise training in CRC survivors. Furthermore, the influence of exercise training at moderate and high intensities on aerobic fitness and body composition in the same population will be explored.
2.0 Literature Review

2.1 Colorectal Cancer

2.1.1 Epidemiology
Cancer has long been a focal point of health research in the medical sciences and in 1996, the Australian Institute of Health and Welfare (AIHW) included cancer as a National Health Priority Area[20]. Of all the cancers, colorectal cancer (CRC) is one of the most common in Western countries[21] and is the second most common cancer for both men and women in Australia[5]. In 2010 there were 14 860 new cases of CRC, a figure which has steadily risen in Australia over the past 30 years[22]. In the same year, 3 999 deaths from CRC were recorded, a decrease from previous years[22]. Thus, while the number of CRC cases is increasing (due to, amongst other factors, improved screening for the disease), patients are living longer following diagnosis and treatment.

When expressed as ‘disease burden’, CRC is second only to lung cancer in Australia in ‘disability adjusted life years’ (DALYs)[23]. DALYs reflect the cumulative number of years lost to premature death and number of years of healthy living lost to disability; in 2003, the total burden of CRC in Australia was estimated to be 63 605 DALYs[23]. Compounding the concern of such a high burden of the disease is that the number of clinical trials being conducted on CRC is disproportionally low when normalised for DALYs and compared to the number of trials being conducted with other cancers[23]. Despite having the second highest burden of disease of cancers in Australia, CRC ranks sixth in number of clinical trials having been conducted in cancer research[23]. Given the high incidence, prevalence and burden associated with CRC, research to better understand how to reduce the risk of CRC and improve outcomes for survivors of the disease is clearly warranted.

Advances in public awareness, screening accuracy and/or treatment effectiveness have led to increases in five-year survival rate to 89.9% for localised CRC cases[24]. However, CRC survivors remain at an elevated risk of CRC recurrence compared to those who have not developed the disease [14, 15]. Additionally, Phipps and colleagues[14] report that survivors of CRC are more susceptible to secondary cancers at other sites including the lungs, stomach, kidneys, endometrium, and small intestine. The combination of increasing incidence of CRC and survival rates following treatment has led to a growing number of individuals living at a heightened risk of CRC recurrence[15]. Of particular concern in this regard is the inconsistent advice and recommendations available to CRC survivors relating to those lifestyle behaviours known to influence risk of the disease recurrence[25].
2.1.2 Lifestyle Factors

The risk of first time CRC development in the greater population is influenced by a number of lifestyle and biological factors. Cross-sectional studies have identified low physical activity (PA) levels, poor diet, obesity, alcohol consumption, and tobacco smoking as significant factors increasing the risk of CRC [6-12]. Levels of PA appear to be particularly important; at least 15% of all CRC cases have been attributed solely to insufficient PA [26]. Furthermore, it has been estimated that a sedentary lifestyle increases CRC risk more than the risks associated with inadequate PA for any other cancer type[26].

In a meta-analysis of 24 case-controlled and 28 cohort studies in CRC, it was consistently found that there was an inverse relationship between PA levels and CRC risk[27]. Such is the strength of the epidemiological literature, in 2006 the American Cancer Society included PA as a recommendation for reducing the risk of CRC[28]. However, given the lack of interventional data and thus specific details of the mechanistic relationship between PA and CRC risk, individuals seeking to reduce their risk of CRC are simply advised to increase the intensity and duration of their PA[28]. Complexity in developing disease-specific PA guidelines arises when one tries to quantify 'high PA levels' with respect to frequency, duration, intensity and type, the cornerstones of exercise prescription. Data from interventional studies involving PA are needed to inform the optimal combination of these variables and therefore ‘exercise dose’ to most effectively reduce CRC risk.

There is convincing epidemiological evidence to suggest that vigorous activity elicits a greater reduction in CRC risk compared to activity performed at a moderate intensity[12, 29, 30]. White and colleagues[29], whose population-based case-control study found no statistically significant trend in risk reduction across groups of increasing total PA duration (hours/week), observed a significantly larger decrease in risk in those individuals who completed more vigorous PA. These findings suggest that high intensity PA reduces risk of CRC to a greater extent than lower intensity exercise.

Despite the weight of research evidence showing that inactive people are at a higher risk of developing CRC and people who regularly undertake ‘high levels’ of PA have a reduced risk of developing CRC, much of the data have been derived from prospective studies in which activity levels were estimated from questionnaires[12, 30, 31]. Research is yet to accurately assess the specific relationship between exercise and risk of CRC recurrence. Six randomised control trials (RCTs) however have been conducted in a population of multiple cancer types, of which CRC was included. Unfortunately, results for the entire cohort were reported rather than for individual cancer
types precluding the examination of CRC in isolation. These interventions varied greatly in overall intervention duration, session duration, intensity, frequency, and type of exercise however it was regularly noted that exercise could improve cardiovascular fitness[32, 33] muscular strength[32], self-reported physical functioning[32-36], levels of fatigue[32, 36, 37], inflammatory markers[37], and quality of life[32, 34, 36, 37]. So, whilst none specifically focussed on CRC survivors, nor was there consistency among interventions, the collective findings from these studies lend additional weight to the role of PA in improving the health of CRC survivors.

Four studies have specifically examined the influence of exercise on the health outcomes in CRC survivors. As summarised in Table 1, it was shown that exercise improved cardiovascular fitness[18, 38, 39]; reduced waist circumference and waist-to-hip ratio[18, 38]; reduced sum of skinfolds[18]; improved quality of life[39, 40]; improved functional capacity[38, 39]; and reduced levels of fatigue[38, 39]. These findings confirm that exercise has a central role in improving the overall health of CRC survivors.
## Table 1: RCTs Examining the Influence of Exercise Interventions on CRC Survivors’ General Health

<table>
<thead>
<tr>
<th>Author</th>
<th>n=</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courneya et al. [40]</td>
<td>INT = 69, CON = 33</td>
<td>INT: 16 weeks of moderate intensity (65-75% of age-predicted maximum HR) exercise 3-5 times/week for 20-30min. Modality was self-selected - walking, swimming, or cycling. CON: 16 weeks usual care.</td>
<td>Improvements in cardiovascular fitness were positively correlated with improvements in quality of life.</td>
</tr>
<tr>
<td>Bourke et al. [38]</td>
<td>INT = 8, CON = 9</td>
<td>INT: 12 weeks of moderate intensity, 3 sessions per week lasting 30-60mins. Exercise sessions included 30 minutes of aerobic exercise at 55-85% age predicted maximum HR followed by 2-4 sets of 8-12 reps of resistance exercises. CON: Usual care.</td>
<td>Improvements in INT with FACT-F, functional capacity, aerobic exercise tolerance, and waist to hip ratio. No difference between groups in FACT-C scores.</td>
</tr>
<tr>
<td>Pinto et al. [39]</td>
<td>INT = 20, CON = 26</td>
<td>INT: 12 weeks of weekly telephone PA counselling as a weekly PA tip sheet. CON: Weekly telephone contact to administer a Symptom Questionnaire. No PA instructions were made.</td>
<td>PA recall levels were higher in INT group at 3 months but not 6 or 12 months. INT group experienced significant increase in estimated VO_{2peak} after three months which was then maintained at 6 and 12 months; greater than CON at all three time points. Both INT and CON experienced improvements in self-reported physical function, fatigue, and quality of life.</td>
</tr>
<tr>
<td>Sellar et al. [18]</td>
<td>INT = 20, CON = 0</td>
<td>INT: 12 weeks of combined aerobic and resistance training. 3 sessions per week: 2 were combined aerobic and resistance, 1 was only aerobic.</td>
<td>Improvements in VO_{2peak}, strength, waist circumference, sum of skinfolds, and trunk forward flexion.</td>
</tr>
</tbody>
</table>


Improvements in cardiovascular fitness are very important to the clinical situation of cancer survivors. Maximal aerobic capacity degrades significantly in CRC survivors throughout the disease-treatment continuum, attributable to the disease itself, disease-related symptoms, and treatment methodologies including surgery and chemotherapy[41-44]. The significance of a decline in VO_{2peak} becomes apparent when examining the prognostic value of cardiovascular fitness for CRC survivors. Compared to CRC survivors with a low maximal aerobic capacity, those with moderate to high levels of cardiovascular fitness have a significantly reduced risk of CRC recurrence [45]. Indeed, maximal aerobic capacity has long been inversely associated to all-cause...
mortality, for an increase in aerobic fitness of 1 metabolic equivalent (MET), the risk of all-cause mortality reduces by 15% and 8% for men and women respectively[46, 47]. As such, greater improvements in maximal aerobic capacity will initiate better prognostic outlooks.

The aforementioned associations suggest a dose-response relationship whereby greater increases in cardiovascular fitness result in more favourable health outcomes. Given that high intensity interval training (HIIT) has been shown to elicit superior improvements in cardiovascular fitness compared to exercise training completed at lower intensities it is can be hypothesised that such training is of greater benefit to the individual[48]. Importantly, such is the case in clinical populations other than CRC survivors[48]. Whilst it is reasonable to assume such findings would be present in CRC survivors, research is yet to examine the influence of different training programs on cardiovascular fitness in this population.

Like cardiovascular fitness, body composition plays a large role in determining the health of a CRC survivor. Survivors of CRC are often characterised by poor body composition, including increased incidence of sarcopenic obesity (atrophy of skeletal muscle cells and hypertrophy of adipose cells)[49]. Such increases in fat mass and body fat percentage are clinically very important as these parameters have been positively associated with cancer risk as well as other significant comorbidities such as cardiovascular disease (CVD) [50, 51].

Exercise is well established to aid in reducing fat mass[52]. The current literature-base and thus professional recommendations have long promoted moderate intensity aerobic exercise as the optimal form of exercise for reducing fat mass[52]. Paradoxically, moderate intensity exercise (approximately 65% \( \dot{V} \text{O}_2 \text{peak} \)) induces a lower energy expenditure than HIIT[53]. However, the rate of fat oxidisation is far greater when exercising at a moderate intensity with a marked drop off in fat oxidization for exercise performed at intensities above 90% \( \dot{V} \text{O}_2 \text{peak} \)[53]. Thus for two exercise sessions with equivalent energy expenditure, the one completed at a moderate intensity rather than a higher intensity will oxidise more fat. However, HIIT inherently has a higher total overall energy expenditure for a given time period, thus total fat oxidation will be a balance of total energy expenditure and rate of fat oxidisation.

This intricate balance, and thus the effectiveness of different exercise intensities in eliciting changes in body composition, is yet to be specifically examined in CRC survivors. The limited research measuring anthropometric data (as estimates for body composition) has been summarised in Table 1.
Examined in only two instances, the influence of exercise body composition is a very under researched area for CRC survivors[18, 38]. Further clouding this research is that both studies used a combination of resistance and aerobic exercise. Whilst this provides excellent ecological validity and their work should be commended, it precludes understanding of each individual exercise type. This is especially the case where anthropometric measures were used as a surrogate to body composition measures. To achieve a strong scientific understanding, research must be conducted with only one exercise type with variations in intensity within that exercise type to determine optimal prescription for these individuals.

In the first study to date, waist-to-hip ratio was found to significantly reduce in CRC survivors following 12 weeks of combined moderate intensity aerobic exercise and resistance training[38]. Participants completed three sessions per week, which included 30 minutes of aerobic exercise at 55-85% age, predicted maximum heart rate (HR) followed by 2-4 sets of 8-12 reps of resistance exercises. Such results are not surprising given participants were completing moderate intensity exercise, the recommended intensity for weight loss as previously discussed. However, the inclusion of resistance exercise and employment of measurement tools that concern anthropometry rather than body composition justify a need for further research. To document the ability for exercise to reduce fat mass, the study should only perform aerobic exercise and have body composition measured directly and accurately such as by dual-energy X-ray absorptiometry (DXA).

Waist circumference and sum of skinfolds were found to significantly decrease following twelve weeks of combined aerobic and resistance training by Sellar et al.[18]. Interestingly though, when assessed by air displacement plethysmography no significant change in fat mass, body fat percentage, or muscle mass were observed[18]. Participants in this study were required to complete three sessions a week of aerobic cycle ergometry at 60-75% peak power output however HIIT began to replace the MIT after week 5. Two of the exercise sessions per week also included a whole body resistance exercise circuit. This research by Sellar er al[18] again provides positive suggestions for the role of exercise in manipulating body composition in CRC survivors. This study also introduces the idea that HIIT could still elicit changes in body composition in this population. However, with confounding factors such as having participants perform both aerobic and resistance exercise as well as contradictions in findings based on the measuring instrument used, more research is required in this area.
Given the paucity of, and discrepancy within, the current findings further examination of how exercise can manipulate body composition variables in cancer survivors is warranted. The specific shortcomings of previous research is in the mixture of aerobic and resistance exercise and in testing methodology. The requirement of future research is to examine aerobic exercise alone given its inherent association with fat oxidisation and in turn body composition. Within aerobic exercise, moderate intensity exercise is widely regarded to be superior in eliciting reductions in fat mass for equal energy expenditure due to the increased rate of fat oxidisation occurring when exercising at this intensity. However previous research into CRC survivors has purported that HIIT may have a role to play in fat reduction, suggesting the inherently greater total energy expenditure at this intensity may offset its lower rate of fat oxidisation [18]. Thus, a dichotomous research approach, which compares the effectiveness of these intensities, is indicated. This study should then ensure body composition is measured most directly and accurately such as by DXA. A study designed in this way would be able to determine the optimal aerobic exercise prescription to counter sarcopenic obesity via reductions in fat mass.
2.1.3 Biological Markers

Despite the available literature showing that regular exercise improves numerous health outcomes with CRC survivors, details of the relationship between exercise and CRC risk are not well understood. This is understandable since calculations of risk commonly use incidence rates in the succeeding years and require very large sample sizes. Furthermore, the justification of a prospective trial to accurately gauge risk of relapse should be preceded with evidence showing that exercise can induce significant benefits to factors associated with risk of CRC. Establishing optimal target outcomes will also allow for RCTs to accurately quantify the influence of variables. One such variable that requires investigation is exercise intensity and how this may be related to the mechanisms influencing CRC. Thus, potential biological markers linked to CRC that may be influenced by exercise need to be examined in an RCT before disease-specific PA guidelines can be developed.

A number of studies have sought to identify potential biomarkers associated with CRC risk[54-56]. Given the multifocal and complex nature of cancer, it is generally accepted that there is no single biomarker that provides a complete diagnostic or prognostic evaluation of disease risk[57]. Indeed, the advancing fields of genomics, proteomics, and bioinformatics create a vast and intricate scientific landscape of several factors and biomarkers known to be associated with CRC[58].

In light of the documentation of the human genome and the progress made in describing the proteomic equivalent, known as The Human Protein Initiative, much of the recent CRC research has concerned itself with serum sampling for proteomic or genomic analysis[54, 56, 57, 59, 60]. For a published review of one possible family of proteins that contribute to CRC, the insulin-like growth factor axis, please see Appendix 5.1. Another potential target area for CRC interventions is cytokines, a small class of proteins vital for immune system regulation. These biochemical factors have a range of regular immune functions including anti-tumour activity[61]. However, deregulation of cytokine production or activity can lead to detrimental accelerations in inflammation, angiogenesis and cell proliferation, thus rendering cytokines as cancer-promoting factors[61-63]. Furthermore, in situations whereby cancer cells are pre-existing, an abundance of cytokines can stimulate tumour growth and cell proliferation[61]. As such, it is believed that chronically elevated serum cytokines regulate, at least in part, tumour development and progression.

Of the cytokines, interleukin-8 (IL-8) has attracted considerable interest in the field of cancer research, including that of CRC [64-66]. While others, such as IL-6, tumour necrosis factor-alpha (TNFα), C-reactive protein (CRP), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-
CA19-9 have been examined by researchers, IL-8 distinguishes itself from other cytokines through its multiplicity of oncogenic roles of angiogenesis, mitogenesis and inflammation[67, 68]. In addition, circulating IL-8 levels show incremental increases as a person moves from normal health, to colorectal adenomas, and then through the stages of CRC (epithelial IL-8 concentrations in adenoma and adenocarcinoma were found to be 114% and 159% of control respectively); this suggests that IL-8 has a regulatory role in CRC [64-66, 69]. IL-8 has also been shown to contribute to the pathogenesis of other conditions including secondary cancers and cardiovascular disease[1, 14, 70-72]. Not only do low levels of PA and poor diet increase the risk CRC, they also increase the risks of type two diabetes; CRC survivors are thus likely to be at a greater risk of developing these other diseases and conditions[73]. As such, research into factors that influence circulating IL-8, and in turn these health conditions, warrants investigation[14].

While research is yet to investigate the effects of exercise on IL-8 in any CRC population, there is research to suggest that exercise training can improve circulating IL-8 levels in clinical populations such as those with metabolic syndrome [16].

Exercise has been shown to influence the levels of several different serum proteins (many being similar in chemical structure to IL-8) in CRC survivors. As summarised in Table 2, exercise has been shown to mediate changes in interleukin-1 receptor antagonist (IL-1ra)[74], resting insulin, and insulin resistance (HOMA-IR) and increase IGF binding protein 3 (IGFBP-3) with CRC survivors – changes that favour a reduction in CRC risk given the roles of each variable within the body and CRC tumour progression[75]. In addition, tumour necrosis factor alpha (TNF-α) was shown to decrease following a 12 week home-based exercise intervention[75]. However, no changes were observed following a 14 day exercise intervention[74]. The influence of exercise on serum cytokines, and in particular, IL-8 with CRC warrants further research.
Table 2: RCTs Examining Influence of Exercise Interventions on Serum Cytokines in CRC Survivors

<table>
<thead>
<tr>
<th>Author</th>
<th>n=</th>
<th>Intervention</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Allgayer et al. [74]</td>
<td>Mod=13 Low=10</td>
<td>2 weeks of 40 min of either moderate (55-65% VO₂_peak) or low intensity (30-40% VO₂_peak) exercise every day.</td>
<td>IL-1ra response to exercise decreased in moderate intensity group but not low intensity group after intervention. NS changes in IL-1β, IL-6, TNF-α, sTNFII in both groups.</td>
</tr>
<tr>
<td>Lee et al. [75]</td>
<td>Frequent INT= 8 Less Frequent INT = 9</td>
<td>12 week home-based exercise program. Frequent INT: Participants were provided one supervised exercise session for first three weeks plus high levels of education and regular support from practitioners throughout the twelve weeks Less frequent: Participants were provided with limited education and support over the 12 week period.</td>
<td>Both groups significantly increased vigorous PA levels with no difference between groups. Significant reductions in body mass, insulin, HOMA-1R, and TNF-α whilst significant increases in IGF-1, adiponectin, and IGFBP-3 were observed when data from both groups was collated.</td>
</tr>
</tbody>
</table>


Given the evidence implicating IL-8 in CRC tumour progression coupled with the data showing changes in IL-8 with exercise, research that specifically examines changes in IL-8 with exercise in CRC survivors is warranted.

2.2 Interleukin-8

IL-8, also known as CXCL8, is a cytokine from the CXC chemokine family encoded by the IL-8 gene[1]. The ligand IL-8 is characterised by 72 amino acids with four cysteine molecules whereby a single amino acid separates the first two cysteines, hence the nomenclature “CXC”[2]. Clark-Lewis[2] went on to document the full sequence of IL-8 illustrating this structure, as shown in Figure 1.

![Figure 1](sequence_of_interleukin-8.png)

*Figure 1. Sequence of Interleukin-8. The 72 amino acid sequence of IL-8 is shown with cysteine molecules indicated with arrowheads and the ELR motif identified by a star[2].
IL-8 is primarily produced by monocytes and macrophages, however any tissues with toll-like receptors such as muscle and fat also possess the capacity to produce it[76-78]. A wide range of stimuli can drive IL-8 production including stress of the microenvironment, inflammatory signalling, and steroidal hormones[1]. Such drivers induce ligands that can bind to toll-like receptors and initiate a cascade of neutrophil activation and in turn IL-8 production[79].

Following production, IL-8 can be secreted into circulation and attend sites of damage as a part of the immune response. IL-8 exerts its biological influence by binding to extracellular transmembrane G-protein coupled receptors CXCR1 and CXCR2 [80]. IL-8 is the only molecule that can bind to CXCR1 and does so with greater affinity than it does to CXCR2, a receptor which is also open to any chemokine that possesses the ELR motif, as indicated in Figure 1[80, 81]. These IL-8 receptors are most commonly found on monocytes and neutrophils whilst presentation is also found on the cell walls of neurons, cancer cells, and endothelial cells[82-88]. Thus, the range of target for IL-8-induced responses is quite broad. Once IL-8 binds to these target cells it can propagate many intracellular processes including inflammation, cell proliferation, angiogenesis, and chemotaxis[89, 90]. It is thought that self-regulation of IL-8 activity occurs via dimerization when IL-8 concentrations reach certain levels[91]. This dimer form of IL-8 is biologically inactive in that it is unable to bind to the N-domain of the CXCR1, thus blocking signalling via this receptor[91]. However, research suggests that this dimer form of IL-8 can still stimulate CXCR1 to a limited extent[92]. The ability for the dimer form to activate CXCR2 is comparable to that of the monomer[92].

2.2.1 Interleukin-8 and Mechanisms of Colorectal Cancer

While cross sectional studies have shown IL-8 to be intrinsically linked with CRC[64, 66, 93], mechanistic research has found CRC cells present with CXCR1 and CXCR2 receptors thus allowing IL-8 to directly exert its biological potential on these cells[67, 94]. The cellular events involving IL-8 that result in mitogenesis and angiogenesis have recently been reviewed by Waugh and Wilson[1]. These pathways are shown in Figure 2 and Figure 3, respectively[1]. Figure 2 depicts the complex cascade of events from initial IL-8 signalling at the cell wall through to eventual gene expression to drive cell proliferation and survival while Figure 3 illustrates the role of IL-8 in the microenvironment to predominantly facilitate angiogenesis. This shows how IL-8 is involved downstream in mitogenesis and angiogenesis. Aspects of these processes are reviewed in greater detail in the following sections.
Source: Waugh and Wilson[1]. Figure 2. Characterised IL-8 Signalling Pathways. A schematic diagram illustrating the range of signalling pathways that are activated after stimulation of CXCR1 and/or CXCR2 receptors with IL-8. These signalling pathways have been shown to promote protein translation (left) and regulate the activity of a range of transcription factors (bottom). Solid bold lines: transcription factors whose activity has been shown to be positively regulated by IL-8 signalling using various reporter assays. Dashed lines: the putative pathways through which IL-8 signalling regulates transcription factor activity[1].
Tumour-derived IL-8 has the capacity to exert profound effects on the tumour microenvironment. Tumour-derived IL-8 will activate endothelial cells in the tumour vasculature to promote angiogenesis and induce a chemotactic infiltration of neutrophils into the tumour site[1].
2.2.2 Interleukin-8 and its Involvement with Mitogenesis and Angiogenesis in Colorectal Cancer

Cancer is characterised by cell proliferation exceeding cell apoptosis. As such, in normal cell functioning, a delicate balance between proliferative and apoptotic factors exists and an imbalance towards the former can result in tumorigenesis. As previously described, IL-8 is a cytokine with proliferative qualities whose overexpression has been found to correlate with CRC and its progression[3, 64, 66].

Cancer cells, which have both CXCR1 and CXCR2 receptors, receive IL-8 via autocrine, paracrine, and endocrine avenues[3]. The binding of this IL-8 to CRC cells triggers a number of intracellular pathways, ultimately leading to proliferation[95]. Although the exact mechanisms underlying this phenomenon are not comprehensively understood, a review of potential molecular pathways has been included in Appendix 5.2. A consequence of these IL-8 triggered pathways appears to be stimulation of transcription factors with the principal task of transcribing cell proliferation genes[1, 3, 96]. Further suggesting IL-8’s involvement in cell proliferation as an autocrine mediator, is that as IL-8 expression increases inside a CRC cell there is a concomitant increase in cell proliferation [95]. So whilst the exact mechanism linking IL-8 and mitogenesis in CRC is yet to be identified, the available evidence supports the existence of such a link.

Along with mitogenesis, one of the major mechanisms of cellular growth, in normal biological functioning and in cancerous tissue, is angiogenesis[97]. With the formation of new blood vessels comes a greater ability for delivery of nutrients, oxygen, growth factors, and other blood-borne elements [97]. Initiated very early in tumour life, angiogenesis is essential to the progression and growth of any tumour, including that of CRC[97-99]. CRC-induced angiogenesis is stimulated by angiogenic factors, such as IL-8, binding to receptors on endothelial cells in the microenvironment[99, 100]. The ability of IL-8 to exert its angiogenic potential on endothelial cells has been demonstrated both in vitro and in vivo[89, 101, 102]. Interestingly, the effectiveness of IL-8 to induce angiogenesis in endothelial cells is more pronounced in microvasculature than macrovasculature[103].

Endothelial cells have been found to exhibit both CXCR1 and CXCR2, however there is suggestion that only the CXCR2 receptor possesses the capacity to initiate angiogenic process[104, 105]. Any chemokine displaying the ELR motif has the potential to bind to CXCR2 and initiate this process, however the abundance of IL-8 produced by the surrounding CRC cells means that it is a potent angiogenic factor[104, 105].
The intracellular process driving angiogenesis is the same as that of proliferation with the additional property of control and direction[106]. Rather than proliferating in a random fashion as is the case in cancer cells, angiogenesis involves the systematic breakdown of the extracellular matrix to direct the proliferation of endothelial cells in certain directions and structures necessary for new vessel formation[107]. Of interest is that IL-8, *in vitro*, can up-regulate the factors responsible for this breakdown, further implicating IL-8 as an angiogenic promoter[107]. This phenomenon has been demonstrated *in vivo* in melanoma, prostate cancer, lung cancer, and Kaposi’s sarcoma[108-111]. Whilst no data for CRC exist, it is reasonable to assume that endothelial cells in the CRC microenvironment would show similar properties. Moreover, Ning et al[112] demonstrated *in vivo* that CRC cells transfected with IL-8 induced angiogenesis in the microenvironment.

As such, IL-8 is a potent mitogenic and angiogenic stimulator, demanding the investigation for interventions with the ability to manipulate this variable. One such method previously discussed in this review is exercise and physical activity.

### 2.3 Interleukin-8 and Exercise

The available evidence supports relationships between IL-8 and CRC as well as PA and CRC risk[30, 65]. What remains to be potentially established is an exercise-induced mechanism that changes circulating IL-8 levels in CRC survivors. It has been well documented that exercise has a major role in the manipulation of cytokines[113]. Indeed, numerous studies have shown that a single bout of exercise can alter circulating levels of IL-8, however relatively few studies have examined the changes in IL-8 in response to exercise training[16, 114, 115]. It is imperative that such research is conducted with CRC survivors to best assist their health and risk of recurrence.
2.3.1 Interleukin-8: The response to Exercise Training

Given the intrinsic link between resting levels of IL-8 and CRC, it is particularly important to understand whether exercise training can be used as a potential normalising agent. Very few studies have examined the effect of exercise training on circulating IL-8 and the findings have been mixed, especially within clinical populations. There are suggestions, however, that exercise can normalise circulating IL-8 concentrations[16, 17].

Troseid and colleagues[16] examined changes in plasma IL-8 in nine patients with metabolic syndrome, another disease associated with inflammatory mechanisms, in response to 12 weeks of exercise training. Thrice weekly exercise sessions each lasted for 45-60 minutes and included a combination of aerobic (walking/running) and resistance exercises. Although exercise intensity was not carefully controlled, training resulted in a significant (p=0.009) reduction of IL-8 from baseline; no change was observed in the control group (n=6).

It is theorised that such a reduction could be due to a multitude of factors. One potential mechanism is a reduction in IL-8 released from the muscle during exercise. Given that hypoxia is the primary trigger of IL-8 production during exercise, it is reasonable that following exercise training, improved delivery of oxygen to the exercising muscles coupled with greater mitochondrial volume will result in less hypoxic stress. As such, muscles will produce less IL-8. This is supported by numerous studies showing that athletic populations characterised by greater levels of aerobic fitness have dampened immune responses to exercise than sedentary counterparts[114, 116-118]. Similarly in older adults, Della Gatta et al.[17] recently examined the influence of exercise training on the acute intramuscular IL-8 response. Eight older adults (age range 60-75 years) completed three sessions of resistance each week for 12 weeks. Each session consisted of two sets of 8-12 repetitions of six multi-joint exercises with intensity increasing from 50% of 1 repetition maximum (1-RM) in week one to 80% 1-RM in week six which was then maintained through to week 12. The authors found IL-8 expression in the muscle to be significantly reduced (p<0.001) two hours post-exercise following 12 weeks of training. A similar trend, though not statistically significant, was found following six weeks of high intensity interval training by Croft and colleagues in healthy individuals[119]. Participants (n=5) completed four sessions per week involving five x 3 minute bouts of running at 90% \( \dot{V}_{O_2}\)max interspersed with 3 minutes recovery. Plasma IL-8 tended to be lower immediately post-exercise compared to pre-training values however, neither intramuscular nor time course measures were reported[119]. Whether or not this can result in a lowering of circulating levels of IL-8 over time is yet to be determined.
Another mechanism that may contribute to a decrease in circulating IL-8 levels in response to exercise relates to changes in body composition. IL-8 can be classed as an adipokine, as it is produced by adipocytes. Indeed, obesity has been associated with higher levels of circulating IL-8[120, 121]. It would thus be reasonable to assume that reductions in fat mass that may occur in response to exercise training, may dampen IL-8 expression and in-turn reduce circulating levels. In line with this, the reductions in IL-8 found by Troseid and colleagues[16] were related to reductions in body mass index (BMI) and waist circumference. However, it must be acknowledged that there is conjecture in the literature over whether the IL-8 produced in adipocytes reaches circulation and contributes to systemic levels[122]. Despite this, the interplay between the changes in IL-8 and body composition in response to exercise training is one that warrants further investigation.

Surprisingly, there exists little research documenting changes in body composition in response to exercise training with CRC survivors. Of the limited number of studies that have examined body composition, most have simply reported BMI, waist-to-hip ratio, and/or skinfolds[18, 38, 39]. To date, the best documentation of body composition in CRC survivors completing an exercise intervention was provided by Sellar et al.[18] who used air displacement plethysmography testing in their investigation. Their 27 participants all completed three exercise sessions per week, two of combined aerobic and resistance exercise on top of one purely aerobic session. It was found that estimations of fat mass and body fat percentage both showed non-significant downward trends over time (p=0.152 and p=0.171, respectively)[18]. Despite methodological shortcomings of the study by Sellar et al.[18] such as an intervention of both aerobic and resistance training and no control group, the conduction of future research into body composition is required. Such further studies will allow inferences to be drawn as to whether changes in body composition resulting from exercise may, at least in part, explain potential changes in IL-8. By understanding the pathways by which exercise influences IL-8, health professionals will be able to prescribe and recommend exercise in a way optimised to reducing IL-8 and in turn CRC risk.

It is also worth recognising that exercise training has not always been found to elicit a reduction in IL-8[123]. In a study conducted by Gomez et al.[123] in breast cancer survivors (n=8), no significant difference in IL-8 was found following 8 weeks of combined aerobic and resistance training. However, by the author’s admission their sample size and cytokine testing methodology could have precluded the detection of significant results[123].

In summary, the available evidence suggests that IL-8 is involved in the development of CRC, whilst exercise appears to impart a normalising effect. However, much of the current IL-8 research
has been in healthy or athletic populations. The few studies that have examined changes in IL-8 with exercise in clinical populations have shown promise yet have lacked the methodological rigor to conclusively detect statistically significant findings. Furthermore, the mechanism underlying how a period of exercise training might affect IL-8 level requires further investigation. Research that controls for, and examines the response of, IL-8 to different exercise intensities with consideration for changes in body composition and aerobic fitness is needed. These findings will inform the development of PA guidelines for CRC survivors that most effectively reduce the risk of recurrence of the disease.
3.0 The Effects of High Intensity Interval Training on Interleukin-8 in Colorectal Cancer Survivors

3.1 Research Aims and Hypotheses
The primary aim of the present study was to determine how exercise training at two different intensities would influence IL-8 in CRC survivors. The secondary aims were to examine potential changes in \( \text{VO}_2\text{peak} \) and body composition in response to high and moderate intensity exercise with the same population.

It was hypothesised that exercise training would reduce circulating IL-8 and that there would be greater reductions following high intensity interval training compared to continuous moderate intensity training. In addition, it was hypothesised that HIIT would result in greater improvements in \( \text{VO}_2\text{peak} \) compared to moderate continuous intensity training. It was expected that reductions in fat mass and body fat percentage would be greatest in the MIT group whilst the HIIT group would have the greatest improvements in muscle mass.

3.2 Methods

3.2.1 Participants
Eighteen women and men who had been previously treated for CRC were invited to participate in this randomized controlled trial. Each was recruited from an existing Cancer Council Queensland (CCQ) CRC registry and inclusion criteria for the study were:

i. aged over 18 years;

ii. not being medicated with insulin;

iii. free of any musculoskeletal, neurological, respiratory, metabolic or cardiovascular conditions that prevent safe completion of the exercise demands of the study.

3.2.2 Recruitment
Participants were recruited by the following methods:

1. Individuals who had previously contributed to a longitudinal study investigating quality of life in CRC survivors were sent a letter of invitation by the Cancer Council Queensland. Those meeting the criteria of the study were directed to contact the Principal Investigator of the current study to express their interest in participating. A CONSORT flow diagram of participants recruited via this method is shown in Figure 4.
2. Queensland Cancer Registry (QCR): The QCR is a population-based registry for almost all individuals diagnosed with cancer in Queensland. The QCR procedure for recruitment included identification of individuals within the QCR aged 18 to 75 years with a histologically confirmed diagnosis of colorectal cancer (ICD0:C18-C20, C218) between 1st January 2005 and 31st December 2012. Patient names and the names of their treating doctor were obtained from the QCR database and entered into a customised study database. A letter requesting permission for their patient to be approached was sent to the treating doctor, followed by reminder telephone calls where necessary. Initially, all individuals meeting the aforementioned criteria diagnosed in 2012 were sent out a letter. Once recruitment began to subside, letters were then sent out to all those diagnosed in 2011, and so on. After this was returned, patients were forwarded a study information sheet and a letter from their doctor informing them of the study. The information flyer included details and instructions for contacting the Principal study investigator to discuss the study in detail, their eligibility and possible participation. A CONSORT flow diagram of participants recruited via this method is shown in Figure 5.
3. Cancer Council Queensland Cancer Helpline: Individuals previously diagnosed with colorectal cancer above the age of 18 who contacted the Cancer Helpline were provided a brief explanation of the study and asked to consider participation. Callers who express an interest were mailed a study information flyer and a brief letter from the Head of Research at Cancer Council Queensland. The information flyer included details and instructions for contacting the Principal study investigator to discuss the study in detail, their eligibility and possible participation.

4. CCQ support groups and contacts: Information about the study and the contact details of the Principal investigator were posted on the CCQ’s website and included in CCQ newsletters, and CCQ volunteer network emails and newsletters. Cancer Support Groups were also asked to advise their members of the study both during meetings and in newsletters.
A CONSORT flow diagram of participants recruited via methods 3 and 4 is shown in Figure 6.

![CONSORT Flow Diagram](image)

**Figure 6**: CONSORT Flow Diagram of recruitment via the Cancer Council Queensland Helpline and Support Groups.

All individuals who contacted the Principal Investigator were first assessed to determine whether they met the eligibility criteria. Those eligible were sent a copy of the participant information sheet, medical history form, and the doctor’s consent form. Participants were asked to complete these forms and return them to the Principal Investigator at the first face-to-face session (familiarisation session). If these were not completed the participant was not allowed to partake in any exercise and would need to reschedule the session.

Ethical approval was obtained for this project through both The University of Queensland’s Medical Research Ethics Committee (#2013000749) and the Queensland Public Health Act (#RD004846).

### 3.2.3 Protocol
Colorectal cancer survivors participated in either a high intensity interval (HIIT; n=9) or moderate intensity (MIT; n=9) exercise-training program. Participants completed 24 training sessions of their respective intensities; three sessions per week were held over the eight weeks. In order to track changes in IL-8, body composition, and aerobic fitness across the intervention, participants completed a series of tests at baseline, midpoint (following 12 sessions and four weeks of training), and endpoint (following 24 training sessions and eight weeks of training). This testing battery included the sampling of venous blood for the later measurement of resting IL-8 concentration; anthropometric measures of height, body mass, and a dual-energy X-ray absorptiometry (DXA)
scan; and a \( \dot{V}O_2 \)peak test on an electronically braked cycle ergometer. This testing battery was all completed in a single testing session and in a standardized order. Four days or more prior to baseline testing, participants completed a familiarisation session.

**Familiarisation**

After recruitment and acceptance into the study, participants returned their completed medical history form and the doctor’s consent form at the familiarisation session. The familiarisation session was conducted seven days prior to their baseline testing session. This session served to familiarise the participant with the environment, equipment and testing procedures. This included a test to determine \( \dot{V}O_2 \)peak on an electronically-braked cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, Netherlands). Inclusion of this test was deemed necessary given the recent findings by Scott et al. [124] examining the reproducibility of maximal cardiopulmonary exercise testing in men with prostate cancer. Scott et al. [124] found high correlations in test-retest results however there was still a significant improvement from the first to the second maximal cardiopulmonary exercise test, indicating performing only one maximal cardiopulmonary exercise test would not yield accurate results. The protocol for this test is detailed below.

**Baseline Testing**

*Control Measures*

Prior to each testing session – baseline, midpoint and endpoint – participants were asked to follow a number of control measures.

**Physical Activity & Diet Control**: Each was asked to record their food consumption for three days and physical activity for seven days preceding each testing session; diaries for recording these data are shown in Appendices 8.4 and 8.5, respectively). In advance of all subsequent testing sessions, participants were asked to reproduce these behaviours as closely as possible; copies of the diaries were retained by the participants for their future reference. Furthermore, in the 24 hours preceding each testing session (except the familiarisation sessions), participants were asked to: (a) abstain from caffeine consumption; (b) abstain from alcohol consumption; (c) abstain from vigorous exercise; and (d) maintain a hydrated state. In the 12 hours prior to the testing sessions, they were requested avoid consuming food, drink, and other consumables with the exception of water and prescribed medications. Upon arrival at the laboratory for testing, each was asked to confirm via checklist that the pre-exercise criteria had been met. For midpoint and endpoint testing, this checklist also included of the three-day food diary (Appendix 5.5) and seven-day physical activity diary (Appendix 5.6). To assess replication a number of factors were examined. The three key
elements of the diet that would have potentially influenced exercise performance between testing sessions are total energy, total CHO and total protein. Replication was assessed as $\pm 10\%$ for each of these components across the three days on each testing occasion. Similarly, for physical activity the three key elements were total number of activities, activity duration, and activity intensity. Using the formula from the Godin Leisure-Time Exercise Questionnaire [125], these factors could be translated to a single number. A $\pm 10\%$ tolerance was placed on this final score. Additionally, consideration was given to the timing of exercise sessions. For example, a participant who completed a week’s worth of exercise in the last three days of the week would not be considered to have acceptably replicated their physical activity levels despite having a similar score for that seven day period. In the instance where not all of the aforementioned criteria were met, the testing session was rescheduled. Physical activity levels were also assessed at baseline as a descriptive characteristic using the Godin Leisure-Time Exercise Questionnaire[125].

**Outcome Measures**

**Biological Markers:** Using a 21 G needle, a qualified phlebotomist collected a 5 mL resting venous blood sample into a 6 mL spray-coated K$_2$EDTA vacutainer (BD Vacutainer, Becton, Dickinson and Company, New Jersey, USA). Samples were then immediately centrifuged with force at a relative centrifugal for 10 minutes at 900 g, from which plasma was extracted into 0.6 mL aliquots and stored in 0.6 mL Eppendorf (PCR Tubes, Neptune Scientific, San Diego, USA) at -80°C. These samples were analysed in duplicate at the completion of the study. Detection of IL-8 in plasma samples was completed using enzyme-linked immunosorbent assay (ELISA) kits as per manufacturer’s instructions by a trained laboratory technician (R&D Systems, Human CXCL8/IL-8 Quantikine ELISA Kit, Minneapolis, USA) (co-efficient of variation (CV) = 11.2%).

**Body Composition:** Body mass and height were measured using an electronic, calibrated scale (Seca, Birmingham, UK) and stadiometer (Seca, Birmingham, UK), respectively. Body composition (i.e. regional and whole body lean and fat mass) for each participant was measured using DXA (Hologic Discovery A, Waltham, MA) (CV = <1.1%). Participants lay on the DXA scanning table for approximately 7 minutes and a scanning arm moved above their body. A low-dosage x-ray passed from underneath the table to the scanning arm. A qualified DXA technician conducted and analysed all DXA scans.

**Peak Aerobic Power ($\dot{V}O_2$peak):** Upon arrival at the laboratory, resting heart rate (HR) (Suunto Ambit2 S heart rate monitor, Suunto Oy, Vantaa, Finland) and blood pressure (BP) using a blood pressure cuff (Durashock Sphygmomanometer, Welch Allyn, New York, USA) measures were recorded. The $\dot{V}O_2$peak test required participants to progressively cycle on an electronically-braked
cycle ergometer to volitional fatigue; oxygen consumption (\(\dot{V}O_2\)) and carbon dioxide production (\(\dot{V}CO_2\)) were measured continuously during exercise. Expired air was analysed for fraction of expired oxygen (\(F_{E}O_2\)) and fraction of expired carbon dioxide (\(F_{E}CO_2\)) every 15 seconds during exercise (ParvoMedics TrueOne 2400, Sandy, USA) from a mixing chamber, while minute ventilation (\(V_E\)) was recorded every 15 seconds using a turbine ventilometer (Morgan, Model 096, Kent, England). The gas analysers were calibrated immediately prior to testing and verified after each test using a certified beta gas mixture (BOC, Brisbane, Australia). The ventilometer was calibrated before and verified after each test using a 3 L calibration syringe (Han Rudolph Inc., Kansas, USA) in accordance with the manufacturer’s instructions. The test was terminated when the participant reached volitional fatigue or met exercise testing termination criteria as outlined by the American College of Sports Medicine (Appendix 5.3). In circumstances where the test was terminated early, participants were required to recomplete the test within three (3) days. \(\dot{V}O_2\)peak was recorded as the average of the highest two \(\dot{V}O_2\) readings recorded. The testing protocol, modified from that of Balke and Ware[126], began with three minutes of rest for respiratory normalisation followed by four minutes of warm up at a resistance of 50 W. Thereafter the resistance provided by the cycle ergometer increased incrementally by 25 W every minute. Participants were required to maintain a cycling cadence of above 50 revolutions per minute (rpm). HR was recorded every minute whilst BP was monitored every two minutes during the \(\dot{V}O_2\)peak test. Participants were also asked each minute to indicate their rating of perceived exertion (RPE) on a Borg’s Scale[127]. Both HR and BP measures were monitored until they returned to within 10 bpm and 10 mmHg of resting levels, respectively, before participants left the laboratory.

**Exercise Intervention**

Following baseline testing, individuals were randomised into one of two treatment arms using a random number generating process and stratified based on gender and age. An individual independent to the study was responsible for the randomisation of participants. All exercise training sessions were conducted on an air- and magnetically-braked cycle ergometer (Wattbike Ltd., Nottingham, England) at the Exercise Physiology Research Laboratory within the School of Human Movement Studies at The University of Queensland.

Once baseline testing was complete and training groups established, participants started cycle ergometer training. Both groups completed 24 exercise sessions (3 sessions per week for eight weeks).
As described by Tjonna et al.[128], each HIIT training session involved 4 x 4 minute bouts of exercise at an intensity of 85-95% heart rate peak (HR\text{peak}). Each 4 minute interval was separated by 3 minutes of active recovery at 70% HR\text{peak}. The HIIT sessions included a 10 minute warm-up and 5 minute cool down at 70% HR\text{peak}.

Participants randomised to the MIT group exercised at 70% HR\text{peak} for 50 minutes. The duration of 50 minutes for the MIT group was chosen for two reasons. Firstly, 3 x 50 minute session would meet the current physical activity guidelines as set by the American College of Sports Medicine for adults[129]. Additionally, data published by Tjønna et al.[128] estimated that 47 minutes of moderate intensity exercise was of equal workload to the previously described HIIT session in patients with metabolic syndrome, as determined by VO\text{2}. Thus, 50 minutes of MIT was comparable to HIIT to the energy expenditure of the 4 x 4 minute HIIT session and met the current physical activity guidelines.

Exercise training sessions were completed individually or in small groups (3-4 people) and supervised by an accredited exercise physiologist. HR was monitored continuously using a heart rate monitor (Suunto Oy, Vantaa, Finland). Examples of these HR graphs for HIIT and MIT sessions can be found in Appendix 5.7. RPE was monitored at the completion of each interval in the HIIT groups and at regular intervals (15 minutes) in the MIT group. All participants had their BP and HR measured prior to commencement of each training session. These measures needed to have returned to within 10 bpm and 10 mmHg of baseline measures, respectively, before participants left the facility. In the instance where a participant achieved a higher HR\text{peak} at midpoint testing than the HR\text{peak} recorded in their baseline testing, this new HR\text{peak} was used to recalculate 85-95\% HR\text{peak} and 70\% HR\text{peak} for exercise prescription of the HIT and MIT sessions, respectively.

**Attendance and Safety**

Attendance and safety was recorded in order to assess and describe the feasibility and appropriateness of high intensity exercise for CRC survivors. Attendance at the exercise training sessions (out of a possible 24) was recorded whilst adverse events as a result of the exercise training were documented at the time of incidence by the Principal investigator.

**Statistical Analysis**

A sample size calculation indicated that to detect a 1.0 pg/mL difference in plasma IL-8 levels between time points, with a SD of 1.1 pg/mL[16], alpha=0.05 and power=80\%, 16 participants (8 per group) would be required to determine changes (G*Power Software, University of Düsseldorf,
Germany). A systematic review of exercise trials in cancer survivors found, on average, an 11.9% dropout rate[130]. Conservatively allowing for an anticipated dropout rate of 15%, a total sample size of 20 participants (10 per group) was targeted to exceed 80% power to detect a significant effect in IL-8 between time points.

Data were analysed using the SPSS statistical software package (version 20.0, SPSS, Inc., Chicago, IL). Normality was assessed using the Shapiro-Wilk test. Outcome measures was analysed using standard descriptive statistics, t tests, Pearson’s correlation coefficients, and regression or the comparable non-parametric test as necessary to examine differences between time points. Potential effect modification of sex, age, baseline IL-8 levels, body composition, and fitness was also tested. Differences in secondary outcomes were evaluated using multi-level analyses.

Analysis of lean mass was further broken down into segments (lower limbs, trunk, and lower limbs) in an attempt to identify the location of any changes or redistributions. Given the non-specific nature of fat mass reduction it was not deemed appropriate to conduct such analysis in the presence of significant changes in fat mass.

For all tests, an alpha level of 0.05 was applied as the criterion for statistical significance.
3.3 Results
Baseline characteristics for participants in both training groups are shown in Table 3. There was no significant difference between groups for any baseline measure with the exception of sex differences (p=0.004). With the exception of baseline VO$_2$peak in the MIT group, all baseline data were normally distributed.
<table>
<thead>
<tr>
<th></th>
<th>HIIT (n=9)</th>
<th>MIT (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>60.9 ± 15.7</td>
<td>61.4 ± 11.2</td>
<td>0.932</td>
</tr>
<tr>
<td>Sex [Men (n (%))]</td>
<td>8 (88.9)</td>
<td>3 (33.3)</td>
<td>0.004 *</td>
</tr>
<tr>
<td>Body Composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.7 ± 7.4</td>
<td>168.3 ± 14.2</td>
<td>0.294</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>80.0 ± 10.7</td>
<td>78.3 ± 20.7</td>
<td>0.202</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>29.07 ± 6.70</td>
<td>29.22 ± 10.14</td>
<td>0.971</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>51.39 ± 7.15</td>
<td>41.17 ± 14.40</td>
<td>0.075</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>35.0 ± 6.05</td>
<td>40.6 ± 6.78</td>
<td>0.085</td>
</tr>
<tr>
<td>Cancer Site</td>
<td></td>
<td></td>
<td>0.303</td>
</tr>
<tr>
<td>Colon</td>
<td>9 (100.0)</td>
<td>8 (88.9)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Cancer Stage</td>
<td></td>
<td></td>
<td>0.493</td>
</tr>
<tr>
<td>Stage [n (%)]</td>
<td>2 (22.2)</td>
<td>3 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Stage II [n (%)]</td>
<td>3 (33.3)</td>
<td>3 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Stage III [n (%)]</td>
<td>2 (22.2)</td>
<td>3 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Stage IV [n (%)]</td>
<td>2 (22.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td>0.842</td>
</tr>
<tr>
<td>Sx [n (%)]</td>
<td>3 (33.3)</td>
<td>3 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Sx + Rx [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Sx + Cx [n (%)]</td>
<td>4 (44.4)</td>
<td>3 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Sx, Cx, + Rx [n (%)]</td>
<td>2 (22.2)</td>
<td>3 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Time Since Diagnosis (yrs) *</td>
<td>2.00 (2.88)</td>
<td>3.00 (7.38)</td>
<td>0.347</td>
</tr>
<tr>
<td>Time Since Treatment (yrs) *</td>
<td>2.00 (2.75)</td>
<td>2.00 (4.38)</td>
<td>0.620</td>
</tr>
<tr>
<td>Physical Activity Levels (Godin Score) *</td>
<td>20.00 (17.50)</td>
<td>35.00 (31.50)</td>
<td>1.000</td>
</tr>
<tr>
<td>VO₂peak (mL.kg⁻¹.min⁻¹) *</td>
<td>21.1 (3.6)</td>
<td>20.5 (2.3) (n=7)</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Values reported as means ± standard deviation unless otherwise indicated
*values reported as medium (interquartile range).
P<0.05 between groups.
HIIT: High intensity interval training; MIT: Moderate intensity training; VO₂peak: peak volume of oxygen consumed; Sx: surgery; Rx: radiation; Cx: chemotherapy; Godin Score: Godin Leisure-Time Exercise Questionnaire Score; IL-8: Interlukin-8.

It was intended that each participant attended four testing sessions and 24 training sessions, totalling 72 testing and 432 training sessions across the intervention. From the time of randomisation, attendance at testing and training sessions for the HIIT group were 100% (36 of 36) and 99.1% (214 of 216 sessions) respectively whilst those in the MIT group attended 100% of testing (36 of 36) and training (216 of 216) sessions. Throughout the intervention there were no significant adverse events nor were hospital admissions, myocardial infarctions or deaths recorded. One participant’s VO₂peak test was terminated early due to systolic BP exceeding 250mmHg; the participant repeated the VO₂peak test seven days later and data from the participant’s retest was included in the analyses. Some participants within the HIIT group experienced mild nausea and light-headedness following the final exercise interval during their first few sessions however this was infrequent and quickly dispelled during the warm down period.

Adherence to the HIIT protocol was assessed by recording how long participants spent within their target HR range of 85-95% HR peak during each interval. These data are summarized in Table 4. Within the four minute high-intensity intervals, participants took an average of 38.6 s to reach their target HR zone and spent the remaining 03:21.36 min within this HR zone. Participants in the HIIT group each spent 322:10.56 minutes exercising in their target HR zones across the whole intervention. This is compared with the participants in the MIT group who completed a total of 1200:00:00 minutes of moderate intensity exercise at a HR of 70% of HR peak.

Table 4: Average adherence to High Intensity Interval Training protocol.

<table>
<thead>
<tr>
<th></th>
<th>Interval 1</th>
<th>Interval 2</th>
<th>Interval 3</th>
<th>Interval 4</th>
<th>All Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to target HR (min:ss)</td>
<td>01:05.62 ± 00:51.35</td>
<td>0:37.88 ± 00:33.07</td>
<td>00:29.33 ± 00:28.59</td>
<td>00:22.09 ± 00:23.78</td>
<td>00:38.64 ± 00:39.27</td>
</tr>
<tr>
<td>Time spent in target HR zone (min:ss)</td>
<td>02:54.38</td>
<td>03:22.12</td>
<td>03:30.67</td>
<td>03:37.91</td>
<td>03:21.36</td>
</tr>
</tbody>
</table>

Values expressed as average times ± standard deviation.
HR: Heart Rate
As shown in *Table 5*, analysis of plasma samples revealed no significant difference in IL-8 concentrations between groups at baseline (p=0.362). Further, neither the HIIT nor MIT training protocol resulted in significant within-group differences in IL-8 at midpoint (p=0.803 and p=0.275 respectively) or 8 week testing (p=0.980 and p=0.065 respectively). A non-significant downward trend was seen within the MIT group between baseline and 8 weeks (p=0.065). Similarly, when participants who were identified as having elevated baseline IL-8 concentrations were pooled (n=4), a trend towards a reduction in IL-8 was observed (p=0.068; *Table 6*). Elevated baseline IL-8 concentrations were defined as being greater than two standard deviations above the mean of apparently healthy individuals (4.39 ± 1.63 pg.mL^{-1})[131]. No between-group differences in IL-8 were observed at midpoint (p=0.915) or 8 week testing (p=0.337).

**Table 5: Plasma IL-8 across the intervention.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (pg.mL^{-1})</th>
<th>4 Weeks (pg.mL^{-1})</th>
<th>8 Weeks (pg.mL^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIIT</td>
<td>5.2 ± 2.7</td>
<td>5.5 ± 3.5</td>
<td>5.3 ± 2.7</td>
</tr>
<tr>
<td>MIT</td>
<td>6.5 ± 3.0</td>
<td>5.4 ± 1.8</td>
<td>4.2 ± 1.5</td>
</tr>
</tbody>
</table>

Values expressed as means ± SD.

HIIT: High intensity interval training; MIT: Moderate intensity training.

**Table 6: Plasma IL-8 concentrations in participants who had elevated levels at baseline.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (pg.mL^{-1})</th>
<th>4 Weeks (pg.mL^{-1})</th>
<th>8 Weeks (pg.mL^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise Group (n=4) (HIIT = 2; MIT = 2)</td>
<td>9.8 ± 2.0</td>
<td>6.8 ± 3.1</td>
<td>5.6 ± 1.6</td>
</tr>
</tbody>
</table>

Values expressed as means ± SD.

\(\dot{V}O_2\)peak significantly increased in both groups in response to training (*Table 7*). The median \(\dot{V}O_2\)peak increases from baseline to 4 weeks in the HIIT and MIT groups were 6.06 (IQR: 6.97) mL.kg^{-1}.min^{-1} (p=0.036) and 1.95 (IQR: 1.80) mL.kg^{-1}.min^{-1} (p=0.043), respectively, however no difference between the groups was observed (p=0.315). When baseline \(\dot{V}O_2\)peak was compared to 8 week testing, the median change in the HIIT group was 7.99 (IQR: 6.49) mL.kg^{-1}.min^{-1} (p=0.015) and in the MIT group was 3.20 (IQR: 2.79) mL.kg^{-1}.min^{-1} (p=0.018). 8 week \(\dot{V}O_2\)peak measures for HIIT and MIT groups were not significantly different to midpoint values (p=0.263 and p=0.071 respectively). However, by 8 week testing, a significant group effect was observed with the HIIT group having a higher \(\dot{V}O_2\)peak than their MIT counterparts (p=0.041).
From baseline to 8 week testing, changes in body composition were observed in both groups; these are detailed in Table 7 and Figure 7. At baseline, no significant differences were observed between the groups for any measure of body composition, despite lean mass (p=0.075) and body fat percentage (p=0.085) approaching significance.

After four weeks of training, the HIIT group experienced significant reductions in fat mass (mean change = -1066.8±535.7 g; p=0.0003) and body fat percentage (mean change = -1.3±0.5%; p=4.1x10^{-5}) as well as a significant increase in lean mass (mean change = 1123.7±570.8 g; p=0.0004). In contrast, there were no significant changes from baseline to four week testing in body composition for the MIT group, though lean mass appeared to trend positively (mean change = 725.6±1041.8 g; p=0.070). There was no significant difference in fat mass, lean mass, or body fat percentage between groups at four week testing (p=0.315, p=0.070, and p=0.166 respectively). However, when comparing the mean changes experienced by the groups, the reduction in fat mass was significantly greater in the HIIT group (p=0.046).

Compared to baseline, significant changes in body composition were observed at endpoint testing in the HIIT group in fat mass (mean change = -1110.9±823.22 g; p=0.004), body fat percentage (mean change = -1.3±0.8%; p=0.0009) and lean mass (mean change = 983.4±663.4 g; p=0.002. Similar to midpoint testing, there were no significant differences from baseline to endpoint testing in any measure of body composition for the MIT group (p=0.235, p=0.139, and p=0.162 respectively). There was also no significant difference between midpoint and endpoint testing in any body composition measure for either group. A between-group effect was seen in lean mass (p<0.003) at endpoint favouring the HIIT group. A non-significant between-group effect was observed in body fat percentage at midpoint (p=0.074) and endpoint (p=0.055) suggesting the HIIT group experienced more favourable outcomes. A group effect was also seen for fat mass when analysing the mean change where the HIIT group experienced significantly greater fat mass loss (p=0.017).

Further analysis of segmental lean mass revealed significant changes in the HIIT group, as expressed in Table 8. Lower limb segments exhibited significant increases in lean mass at midpoint and endpoint testing (p=0.020 and p=0.013 respectively) whilst a significant difference was found in the torso segment (p=0.042) at midpoint only. No differences were observed in the MIT group.
Figure 7. Change in fat mass and lean mass at four week and eight week testing compared to baseline in both groups.
Values expressed as means ± SD
*p<0.05 compared to MIT group
Table 7: VO$_2$peak and body composition measures for both groups across the intervention.

<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MIT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Four Weeks</td>
</tr>
<tr>
<td>VO$_2$peak (mL.kg$^{-1}$.min$^{-1}$)</td>
<td>21.07 (3.60)</td>
<td>26.68 (9.88)$^a$</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>29.07 ± 6.70</td>
<td>28.00 ± 6.94$^a$</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>51.38 ± 7.15</td>
<td>52.51 ± 7.39$^a$</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>35.0 ± 6.05</td>
<td>33.7 ± 6.13$^a$</td>
</tr>
</tbody>
</table>

Values reported as means ± SD unless otherwise denoted. VO$_2$peak expressed as means (IQR)
$^a$p<0.05 versus baseline; $^*$p<0.05 compared to the MIT group
HIIT: High intensity interval training; MIT: Moderate intensity training.

Table 8: Changes in segmental lean mass in HIIT group across the intervention.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Four Weeks</th>
<th>Eight Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Limbs (kg)</td>
<td>6.27 ± 1.21</td>
<td>6.29 ± 1.10</td>
<td>6.22 ± 1.22</td>
</tr>
<tr>
<td>Lower Limbs (kg)</td>
<td>17.34 ± 2.16</td>
<td>17.88 ± 2.09$^a$</td>
<td>17.81 ± 2.01$^a$</td>
</tr>
<tr>
<td>Trunk (kg)</td>
<td>27.71 ± 4.24</td>
<td>28.29 ± 4.46$^a$</td>
<td>28.18 ± 4.21$^a$</td>
</tr>
</tbody>
</table>

Values reported as means ± SD unless otherwise denoted.
$^a$p<0.05 versus baseline; $^*$p<0.05 compared to the MIT group
A correlation matrix showing associations between changes in IL-8, \( \dot{V}O_2 \text{peak} \), and body composition is shown in Table 9 and Table 10. At four-week the MIT group experienced a change in lean mass that was significantly correlated with changes in body fat percentage (negatively; \( p=0.022 \)). There were no significant correlations between IL-8, \( \dot{V}O_2 \text{peak} \), or body composition at eight-week testing (\( p>0.05 \)).
Table 9: Four-week correlation matrix of physiological and anthropometric changes.

<table>
<thead>
<tr>
<th></th>
<th>Δ VO₂peak</th>
<th>Δ Fat mass</th>
<th>Δ Lean Mass</th>
<th>Δ Body Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ VO₂peak</td>
<td>-0.214</td>
<td>-0.119</td>
<td>-0.386</td>
<td></td>
</tr>
<tr>
<td>Δ Fat mass</td>
<td>-0.169</td>
<td>0.901**</td>
<td>-0.467</td>
<td></td>
</tr>
<tr>
<td>Δ Lean Mass</td>
<td></td>
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<tr>
<td>Δ Body Fat %</td>
<td></td>
<td></td>
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|          |            |            |             |              |
| MIT      |            |            |             |              |
| Δ VO₂peak | -0.314     | -0.086     | 0.086       |              |
| Δ Fat mass| -0.643     | 0.944**    | -0.780*     |              |
| Δ Lean Mass |          |            |             |              |
| Δ Body Fat % |          |            |             |              |

*p<0.05; **p<0.01

# Spearman’s correlation used as data was not normally distributed

HIIT: High Intensity Interval Training; MIT: Moderate Intensity training;

Table 10: Eight-week Correlation matrix of physiological and anthropometric changes.

<table>
<thead>
<tr>
<th></th>
<th>Δ VO₂peak</th>
<th>Δ Fat mass</th>
<th>Δ Lean Mass</th>
<th>Δ Body Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ VO₂peak</td>
<td>0.383</td>
<td>-0.133</td>
<td>-0.261</td>
<td></td>
</tr>
<tr>
<td>Δ Fat mass</td>
<td>-0.305</td>
<td>0.224</td>
<td>-0.109</td>
<td></td>
</tr>
<tr>
<td>Δ Lean Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Δ Body Fat %</td>
<td></td>
<td></td>
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</tbody>
</table>

|          |            |            |             |              |
| MIT      |            |            |             |              |
| Δ VO₂peak | 0.429      | 0.257      | 0.200       |              |
| Δ Fat mass| 0.223      | 0.567      | -0.574      |              |
| Δ Lean Mass |          |            |             |              |
| Δ Body Fat % |          |            |             |              |

# Spearman’s correlation used as data was not normally distributed

HIIT: High Intensity Interval Training; MIT: Moderate Intensity training;
3.4 Discussion

The present study is the first of its kind to examine the influence of exercise training intensity on IL-8 in CRC survivors. This study aimed to initiate the scientific discussion surrounding the mechanistic link between exercise training and reductions in CRC risk. The isolation of such a lynchpin would enable guidelines surrounding exercise training to be tailored to best manipulate this variable. Furthermore, it was of particular interest to identify how different exercise training intensities could influence maximal aerobic fitness and body composition given their intrinsic relationship with general and cancer-related health.

It was hypothesised that exercise training would reduce circulating IL-8 and that the magnitude of reduction would be greater in response to HIIT than to MIT. The secondary hypotheses of this thesis were that the HIIT group would experience the largest increases in $\dot{V}O_2$peak and muscle mass whilst the MIT group would experience greater reductions in fat mass.

The primary finding from this study was that circulating levels of IL-8 did not change for either group in response to the training intervention. The secondary findings of this study were that exercise significantly improved $\dot{V}O_2$peak and resulted in changes to body composition. In agreement with our hypothesis, the HIIT group exhibited the greatest increases in $\dot{V}O_2$peak and muscle mass. However, the HIIT group also experienced larger reductions in fat mass that the MIT group, contrary to our original hypothesis. The MIT group experienced no significant change in body composition, further disagreeing with our secondary hypothesis.

3.4.1 Interleukin-8

No change in circulating IL-8 concentrations in response to either MIT or HIIT was observed in the present study. This finding was contrary to our hypothesis but likely explained by the baseline concentrations of IL-8. At baseline, the resting levels of IL-8 were 5.2 pg.mL$^{-1}$ and 6.5 pg.mL$^{-1}$ for the HIIT and MIT groups respectively, which is comparable to reported plasma values of healthy adults when analysed in similar conditions ($4.39 \pm 1.63$ pg.mL$^{-1}$) [132]. This explains the lack of significant change in plasma IL-8 concentrations as without the presence of abnormal levels, the role for exercise to normalise concentrations of this cytokine becomes redundant. The fact that the observed values were not significantly reduced suggests that exercise has a hormetic effect on circulating cytokines. That is, elevated levels would be reduced while low concentrations would be increased towards the optimal level. It was thus unlikely that the present training intervention, at either intensity, would result in significant changes to the normal baseline IL-8 values.
It was expected that CRC survivors would present with elevated IL-8 levels at baseline. There is no current documentation of resting IL-8 levels in this population in the literature however it was anticipated that individuals who are at greater risk of CRC would be accompanied by a systemic elevation in IL-8. Indeed, the median time since treatment for each group was two years, with no participant being more than five years removed from their last treatment. As such no participants were considered to be in remission and were all still at a significant risk of recurrence. Furthermore previous research in other cancer survivors has found such an association between elevated IL-8 concentrations and survivorship. In individuals who had previously been treated for breast cancer, it was found that baseline IL-8 concentrations (10.26 ± 2.34 pg.mL⁻¹) were markedly higher than those found in healthy populations[133]. Given that CRC has been associated with elevated IL-8 levels similar to breast cancer, it was expected that CRC survivors would have higher than normal IL-8 levels[134].

Pre-screening participants for IL-8 concentrations and employing an inclusion criterion to only accept participants with abnormally high levels would have allowed more meaningful investigation of how exercise can improve markers of CRC in CRC survivors. Such a cut-off was not used for the present study due to the aforementioned expectation of participants having elevated circulating IL-8; however in any case funding restrictions would have not allowed such analysis. It is strongly encouraged that any future studies investigating the hormetic role of exercise on biomarkers of any disease employ such pre-screening unless there is strong and consistent literature detailing baseline levels.

Only one of the 18 participants in the current study presented with concentrations greater than those previously observed in apparently healthy individuals. The lack of elevated IL-8 concentrations observed in the overwhelming majority of CRC survivors brings into question the role of IL-8 as a predisposing factor to CRC development. It is well established that plasma IL-8 levels increases concomitantly with cancer stage but whether or not IL-8 is increased in those at risk of, but yet to develop, the disease is less understood [65, 66]. Potentially, IL-8 concentrations may only increase after the cancer has begun developing whereby CRC cells have the ability to constitutively produce IL-8, explaining the lack of elevation in the participants of the present study[3]. In such case it would appear IL-8 is not the chief predisposing factor to CRC development and research into alternative theories for why CRC survivors are at an increased risk of recurrence should be considered in the future. Knowledge of the exact lynchpin behind CRC recurrence would allow the development of effective preventative therapies.
Regardless, IL-8 inarguably has a role in promoting cancer progression in CRC patients and thus manipulation of this variable is still of interest[66, 135]. The proposal that exercise has a role to play in such cytokine modulation should be examined in a future study where participants are only included if their baseline IL-8 levels are elevated. As an example, participants from the present study with IL-8 concentrations greater than two standard deviations above the reported mean of apparently healthy individuals were pooled and analysed(n=4)[132]. Nonparametric analysis of these data identified a reduction in IL-8 concentrations in response to training approaching significance (p=0.068). Whilst this secondary analysis is by no means conclusive, it gives support for the notion that exercise has a role in normalising abnormal IL-8 levels and suggests future research is warranted. Similar non-significant reductions in IL-8 concentrations were also seen in the original MIT group (p=0.065), likely due to the marginally elevated baseline levels observed in this cohort.

It is therefore important that despite the overall findings of the present study, IL-8 remains at the forefront of researchers’ minds when developing new therapeutic avenues or prescriptions. Indeed, given that there were no adverse events in the present study, an experiment conducted with the same or similar exercise prescription but with CRC patients (rather than survivors having finished treatment for the disease) is suggested for future researchers. This would assist in determining how well exercise can normalise elevated levels of IL-8 and what intensity of aerobic exercise is most effective. If exercise prescription could be optimised to best manipulate IL-8 in CRC patients, this may prove to be a very beneficial adjuvant therapy treatment plan.

3.4.2 VO\textsubscript{2}peak
A secondary outcome measure of this study was VO\textsubscript{2}peak, which significantly improved in both groups at midpoint and endpoint testing. A group effect was also seen by endpoint testing, indicating that HIIT was more beneficial than MIT for improving VO\textsubscript{2}peak.

Increases in VO\textsubscript{2}peak are clinically very important. It is well established that maximal aerobic capacity (e.g. VO\textsubscript{2}peak) is an independent predictor of all-cause mortality[136]. For men, it has been suggested that for every increase in aerobic fitness of 1 MET, the risk of all-cause and cardiovascular disease mortality will reduce by 15% and 19%, respectively[46]. Similar associations between aerobic fitness and mortality have been observed in women, with an estimated risk reduction of 8% and 21% for all-cause and cardiovascular disease mortality, respectively[47,
Reducing mortality risk by improving aerobic fitness is important for cancer survivors, as many have severely reduced levels of fitness following various treatment regimes[138]. Furthermore, every 1 MET increase in fitness has been shown to reduce cancer-specific mortality by 5%, however this trend was non-significant (p=0.10)[139]. The ability to reduce risk of mortality from a variety of disease by improving aerobic fitness outlines the importance of exercise training following cancer treatment.

*Table 11* shows how the observed improvements in cardiovascular fitness over eight weeks of training in the current study may translate to reductions in mortality based on previously published literature[46, 139, 140]. Median increases in aerobic fitness of the HIIT and MIT groups can be expressed as 2.82 and 0.91 METs, respectively (1 MET = 3.5 mL.kg\(^{-1}\).min\(^{-1}\)).

<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MIT</th>
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<tbody>
<tr>
<td>All-cause</td>
<td>42.3%</td>
<td>13.7%</td>
</tr>
<tr>
<td>Cancer-specific</td>
<td>14.1%</td>
<td>4.6%</td>
</tr>
<tr>
<td>CVD</td>
<td>53.6%</td>
<td>17.4%</td>
</tr>
</tbody>
</table>

CVD=cardiovascular disease.

With regard to the time-course of changes, most of the improvements in \(\dot{V}O_2\)peak observed in the present study occurred within the first four weeks (i.e., after only 12 training sessions). The effectiveness of the intervention is further highlighted by considering that the HIIT group only spent 114 minutes exercising each week including warm ups and cool downs. This is considerably less than the currently recommended 150 minutes per week of MIT proposed as the minimum duration necessary to gain health benefits[141]. It should be noted however that the minimum of 150 minutes per week may be required for other health benefits and that the present findings only give suggestion that cardiovascular fitness improvements can be attained with less exercise of a higher intensity.

Differences in absolute changes of \(\dot{V}O_2\)peak in response to training at different exercise intensities has been well documented in clinical populations[48]. Indeed, the present data are in agreement with previous findings and strongly supports HIIT to be more effective than MIT over relatively brief periods[48]. The present study stands out from previous exercise physiology research in CRC survivors when examining the time-course and magnitude of changes of \(\dot{V}O_2\)peak. The absolute
and percentage change increases in VO₂peak for the present HIIT group (8.0 mL.kg⁻¹.min⁻¹ and 44.2%, respectively) are far greater than those reported in previous CRC research – the two studies presently reporting such data include increases in VO₂peak of 3.0 (95% CI = 1.4 – 4.6) mL.kg⁻¹.min⁻¹ following 12 weeks of combined aerobic and resistance training and 4.68 mL.kg⁻¹.min⁻¹ following three months of a home-based physical activity intervention [18, 39]. Of these, Sellar et al.[18] measured VO₂peak whilst Pinto et al.[39] only estimated VO₂peak using submaximal testing. The study conducted Sellar and colleagues[18] comprised 12-weeks of combined aerobic (thrice weekly) and resistance exercise (twice weekly). The aerobic component required participants to cycle on an ergometer at a moderate intensity for the first four weeks. Through weeks five and six participants completed one session of HIIT and two sessions of moderate intensity exercise on the cycle ergometer. Thereafter two sessions per week constituted HIIT with the remaining session being of moderate intensity. All sessions were supervised by the research team with the HIIT sessions being comprised of long and short intervals as described by Guiraud et al.[142]. Despite this highly controlled and well-prescribed intervention, the ‘4x4’ protocol utilised in the present study yielded far greater improvements in VO₂peak than those reported by Sellar et al.[18]. Indeed, following 12 weeks of exercise training, Sellar et al. reported a mean increase in VO₂peak of 3.0 (95% CI = 1.4 – 4.6) mL.kg⁻¹.min⁻¹ compared to 8.0 (IQR=6.5) mL.kg⁻¹.min⁻¹ observed in the present study across just 8 weeks. Moreover, greater increases in VO₂peak were recorded in the first four weeks of the present study (6.1 (IQR=7.0) mL.kg⁻¹.min⁻¹) than were found across the entire 12-week intervention conducted by Sellar et al.[18]. A summary comparison of the two studies can be found in tabulated form in Appendix 5.8. For the present study to have elicited greater benefits despite only being one third of the intervention duration of the study by Sellar et al.[18] highlights the exceptional efficacy of the ‘4x4’ HIIT protocol. Given the marked variation in results between the current study and that of Sellar et al.[18], it is most logical to deduce that the intensity of exercise completed by the participants of the ‘4x4’ was significantly higher. This cannot be conclusively stated however, as exercise prescription for the Sellar et al.[18] study was done so as a percentage of peak power output, thus restricting the ability to conduct straight comparisons. It should also be noted that participants in the previous study were concurrently completing regular resistance training[18]. Whether the adaptive processes of the two systems combatted each other somewhat thus damping aerobic improvements or perhaps participants were simply less capable of completing HIIT at such an intensity due to fatigue, further research is required to conclusively determine the optimal training regime for this population.

The discrepancy between the HIIT and MIT groups in the present study favouring the former is consistent with previous research comparing exercise intensities in untrained populations[48, 143,
It has been established that HIIT can elicit such benefits through a myriad of physiological processes, predominantly improvements in mitochondrial enzyme activity, increases in mitochondrial size, and greater rates of mitochondrial biogenesis[144]. By way of increasing the stress placed upon the muscle cells during exercise, HIIT can provoke a greater magnitude of these adaptive responses[144].

3.4.3 Body Composition

Changes in body composition (reduced fat mass, increased lean mass, and reduced body fat percentage) were observed for the HIIT group but not for the MIT group in response to training. The significant improvement from baseline in body composition in the HIIT group were present at both midpoint and endpoint testing, with no further improvements from week four to week eight.

The two studies that have previously assessed fat mass, muscle mass and/or body fat percentage in response to an exercise intervention in CRC survivors did not report any significant changes [18, 75]. Differences in findings between the present study and those of Lee et al. and Seller et al. may relate to the exercise training protocols used as neither employed HIIT[18, 75]. The study conducted by Lee et al.[75] involved aerobic exercise at a significantly lower intensity than that used in the present study and was largely a home-based intervention. The intervention conducted by Sellar et al.[18], previously detailed in Section 3.4.2, included both aerobic and resistance exercise. In the latter weeks of the Sellar et al. study, the aerobic exercise sessions began to include interval training, however there is little evidence to suggest that participants in this study were exercising at the same relative intensities as those in the present HIIT group.

This suspected discrepancy in intensity is again the likely mechanism explaining the difference in findings of the present study. It has been found that completion of a HIIT program can lead to a reduction in dependence on glucose utilisation and an increase in fat oxidisation in untrained individuals[145, 146]. In fact, such metabolic changes have been observed following only two weeks of HIIT. This would mean the participants in the HIIT group of the present study completed the final six weeks of training with a vastly different balance of energy utilisation, thus promoting reductions in fat mass and body fat percentage.

Whilst not as strongly linked with health outcomes as aerobic fitness, body composition is nonetheless related[51]. Historically, a 5% reduction in body mass was considered to be clinically significant, however this does not account for the various types of body tissue that make up body mass[147]. In more recent times, with the enhanced capacity to differentiate between lean mass and
fat mass, fat mass and body fat percentage have been positively associated with CVD and cancer risk[50, 51]. Lean mass has also been associated with a reduction in all-mortality risk, whilst the relationship between fat parameters and mortality is less conclusive given the protective effect fat mass can have in later life[148]. It is reasonable to conclude that the changes in body composition observed in the present study were favourable for health.

It is important to acknowledge how efficient the HIIT training program was in eliciting changes in fat mass. Whilst the HIIT group only experienced a moderate reduction in fat mass (1.1 ± 0.8 kg on average), the amount of exercise required to achieve this was well below the current guidelines for fat mass loss. It is currently recommended by the American College of Sports Medicine that to achieve sustained weight loss, one needs to complete a minimum of 250 minutes of moderate aerobic exercise per week[52]. In this study however, participants in the HIIT group were only performing 114 minutes of aerobic exercise per week including warm up, recovery intervals, and warm down. It cannot be concluded, however, from this study whether this acute weight loss would result in sustained weight loss, nor whether it is clinically significant. This raises an important future research question to determine whether HIIT alone can elicit weight loss of such character or if such training protocols should be used to complement the current recommendations of MIT.

Another important change relating to body composition was the increase in lean mass for the HIIT group. These participants were cycling against greater resistance than those in the MIT group and this appears to have resulted in skeletal muscle hypertrophy as indicated by the increases in segmental lean mass in the lower limbs and trunk. The increases in trunk lean mass may not seem commonsensical in response to cycling, an exercise modality almost solely of the lower limbs, until it is recognised that the DEXA analysis software includes the pelvis, and thus gluteal muscles, as a part of the trunk. It is very conceivable that cycling would induce hypertrophy in such muscles in addition to those of the lower limbs.

Further group-effect analysis was conducted on the absolute change values revealing the HIIT group experienced a reduction in fat mass of a significantly greater magnitude than the MIT group at both midpoint and endpoint. Such a discrepancy is contrary to the traditional school of thought that MIT is more beneficial for weight loss than HIIT, as is outlined in the current American College of Sports Medicine Position Stand on exercise prescription for weight loss[52]. This consensus was brought about due to fat oxidisation being at its maximal rate when exercising at approximately 65% \( \dot{V}O_2 \)peak with a marked drop off at intensities above 90% \( \dot{V}O_2 \)peak[53].
However, a more recent review has outlined the potential for HIIT to elicit greater reduction in fat mass than MIT[149]. Given the absolute energy expenditure of HIIT is vastly greater than MIT, the deficit in fat oxidation rates can be annulled. Furthermore, as previously discussed, implementing a HIIT program in previously untrained individuals will lead to increases in fat oxidation at higher intensities of exercise[146]. The present study supports this notion and extends this field of the literature by being the first to document the phenomenon in cancer survivors. It should be noted, however, that MIT may be more appropriate for long-term, sustained weight loss which this study was not designed to evaluate[150]. To accurately evaluate whether HIIT or MIT is more effective in reducing fat mass, future studies need to be conducted over a greater time course and should be performed in conjunction with tight dietary control.

The present data show that HIIT can produce significant changes in body composition very rapidly, i.e. within the first four weeks then plateauing. The reasoning behind this plateau is not entirely clear but alternative approaches (e.g., dietary modification and resistance training) to continue improvements in body composition beyond 4 weeks may be required [151, 152]. The plateau observed in a number of variables after 4 weeks of training in the HIIT group may reflect over-training. Anecdotally, some participants were expressing minor signs of fatigue nearing the end of the intervention. This is also supported by the lack of significant improvement in VO2 peak between midpoint and endpoint. To accurately identify whether either of these theories are indeed the case a longer-term intervention would need to be conducted with greater reporting of psychosocial parameters. A further, avenue would be to then develop strategies to avoid this burnout effect. Potentially, an exercise program with shorter blocks of HIIT (for example of four weeks in duration) that is interspersed with less intense exercise, or even rest, may effectively combat this problem.

3.4.4 Safety and Adherence

There were no adverse events in in either training group in response to the present training intervention, therefore this study provides evidence to suggest that supervised HIIT and MIT are safe for CRC survivors. Specifically regarding the HIIT group, the current study, of 297 training hours, mirrors previous research in clinical populations utilising the ‘4x4’ HIIT protocol, with zero adverse events reported[153].

Strangely, the comparison of safety between HIIT and MIT is not something that has been well documented in the exercise physiology literature. There is a theoretical acceptance that the HIIT
could elicit more adverse events due to the higher intensity inherent to the training regime as well as transient immune suppression following high intensity exercise. The available literature, however, does not appear to support this with numerous HIIT protocols eliciting no adverse events in various clinical populations[18, 153-157]

The described safety of this study is most attributable to the stringent screening and safety procedures employed. As this was a pioneering study in utilising the ‘4x4’ HIIT protocol in CRC survivors, those with comorbidities or complications deemed possible to limit or prevent participation in the program were excluded. Whilst this is a clear limitation of the study – the inability to generalise safety statistics to persons with comorbidities as regularly found in the general population – such a study design is a requirement of early stage research to strongly describe the basic physiological mechanisms at play and how they impact on health and fitness parameters within this sample population. As such, this greatly reduced the risk of adverse events during training and testing. It is therefore suggested that future research begin to include CRC survivors who are experiencing comorbidities such as diabetes, cardiac abnormalities, or musculoskeletal problems with an adapted training protocol as is necessary. For the meantime, it is recommended that for patients whom could be classified in numerous clinical categories, HIIT be undertaken with consideration for the evidence base of both groups.

Furthermore, for the sample populations, regular safety measures were put in place to prevent any adverse events. Upon presentation to all testing and training sessions resting HR and resting BP were assessed and participation in the session was not initiated unless these values were permissible according to the American College of Sports Medicine’s Guidelines for Exercise Testing and Prescription[158]. A subjective assessment of general wellness was also conducted each session. Throughout the testing sessions HR, BP, and RPE were regularly measured whilst HR and RPE were measured throughout training sessions. In the instance where BP was abnormal at rest (though not contraindicative of exercise), numerous exercising BP measurements were taken throughout the training session to monitor the response to exercise. Lastly, participants were not permitted to leave the facility until resting parameters were representative of their pre-exercising values ensuring participants were adequately recovered. It is thus recommended that similarly strict supervision be carried out whenever CRC survivors are completing HIIT in both the research and clinical setting in order to prevent adverse events.

Previous clinical research has found great discrepancies in attendance rates to exercise interventions of both high and moderate intensities (range of 82-100% and 80-100% respectively). The present
study experienced very high adherence (HIIT = 99.1%; MIT = 100%)[155, 156, 159], further suggesting HIIT to be a suitable form of exercise for this population. As to why the recorded attendance of the current intervention was at the higher end of the previously seen ranges could be due to a number of factors. The participants were recruited on a volitional basis meaning those who partook in the study had an inherent will to complete the program. Furthermore, training sessions were commonly conducted in small groups with numerous supervising researchers. As such participants were given almost one-on-one attention from supervisors whilst also being able to develop relationships and support networks with fellow participants. This combination of interaction appeared to be very effective in building rapport and enjoyment at the anecdotal level. Further research could be conducted in a similar study to assess how different participant-supervisor dynamics affects adherence and ultimately biophysical outcomes.

The present study goes beyond those existing in the literature by documenting how well participants adhered to their target HR zones during the high intensity intervals. This is very surprising omission from previous research as differences in this parameter could largely alter a study’s or participant’s results. Given that this has not been explored in previous research, the findings of this study cannot be compared to the data from other groups. However, it is interesting to note that performing only four bouts of high intensity exercise lasting on average 3:21 minutes each is enough to elicit such significant changes in body composition and fitness of CRC survivors. This highlights the exceptional efficiency of HIIT.

3.4.5 Limitations and Future Directions
The greatest limitation of the present trial was the lack of pre-screening for elevated IL-8 levels in participants recruited for the research. As explained earlier, it was expected that the recruited participants would present with elevated levels, however time and financial constraints meant that pre-screening would not have been viable in any case. In the absence of existing data on the concentrations of IL-8 in CRC survivors, this expectation stemmed from previous research in breast cancer survivors who clearly demonstrated elevated IL-8 concentrations[133]. It was the belief of the authors that CRC survivors would demonstrate exhibit, if not higher, concentrations of IL-8 given the fact IL-8 plays a more important role in CRC than breast cancer[134]. Going forward, the present study has shown that CRC survivors are not exposed to higher levels of circulating IL-8. Therefore, as previously discussed, the role of IL-8 in survivors also must be questioned and it may be worthwhile for future studies to investigate other potential markers.
Additional control measures regarding parameters such as time since diagnosis, time since treatment, treatment type, cancer site, and cancer stage would also allow more specific understanding. Alternatively, a greater sample size would allow for subcategories to be formed within the data set based on these parameters and allow comparative analysis. Similarly, the unequal distribution of sex between the two groups, despite using this as a randomising variable, is an unfortunate occurrence and with a greater sample size such a discrepancy would have likely been eliminated. It also may be advisable for future research to stratify participants based on previous PA levels as well as age and gender. Although this study detected no significant difference between PA levels at baseline, the Godin Leisure-Time Activity Questionnaire does not specifically determine whether participants previously engaged in HIIT, potentially influencing the findings. While it is possible that participants may have been already engaging in high intensity exercise, all available evidence relating to activity levels of cancer survivors, including the large improvements in physiological measures following the present intervention, suggests that this was highly unlikely. Indeed, recently published data [160] suggests that only 12.3% of prostate cancer survivors in Australia are meeting current physical activity guidelines and it is reasonable to assume that CRC survivors from the same geographical location would be of a similar standing.

As it has now been established that HIIT is a very effective means of exercise training for this populations, an effort should be made to assess how well CRC survivors tolerate HIIT. Conducting a similar study with the inclusion of psychosocial measures such as Functional Assessment of Chronic Illness Therapy and Functional Assessment of Cancer Therapy questionnaires would allow such evaluation. Further to this point, training programs that integrate both MIT and HIIT to prevent burnout are suggested.

Likewise, assessing the viability of HIIT in CRC survivors with comorbidities that were used as exclusion criteria for the present study is recommended, as indicated in the previous section.
4.0 Conclusion

The present investigation has shown that IL-8 concentrations in CRC survivors did not change in response to 8 weeks of HIIT and MIT. This may have been related to the normal baseline concentrations of IL-8 in all but four of the study’s participants. When those four, who had higher than normal resting IL-8 concentrations, were examined there was a trend towards lower levels as a result of the training intervention.

The HIIT intervention did, however, succeed in positively altering body composition in this population. Significant improvements in maximal aerobic capacity were observed in both groups however much more pronounced for those completing HIIT. Indeed, changes in maximal aerobic capacity and body composition were present within only 4 weeks of HIIT.

The study has furthered the current research bed demonstrating the effectiveness and, most importantly, the safety of the ‘4x4’ HIIT protocol in clinical populations. HIIT in this form and setting proved to be a feasible and suitable method of exercise for CRC survivors.
4.0 References


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5.0 Appendix

5.1 The Insulin-like Growth Factor Axis: a biological mechanism linking physical activity to colorectal cancer survival

Citation

Abstract
Physical activity (PA) is related to colorectal cancer (CRC) mortality, with approximately 15% of CRC deaths worldwide attributable to physical inactivity. Moreover, higher levels of PA in CRC survivors have been associated with a reduced risk of the disease recurring. Despite the recognised nexus between PA and the risk of CRC, the physiological mechanisms underlying the inverse relationship between PA and mortality following CRC diagnosis are less apparent, with evidence primarily drawn from epidemiological studies. The insulin-like growth factor (IGF) axis plays a central role in cellular growth, proliferation regulation, differentiation and apoptosis. Specifically, high levels of insulin-like growth factor 1 (IGF-1) have been consistently linked to the severity of CRC tumours. Further, insulin-like growth factor binding protein 3 (IGFBP-3) regulates the bioavailability of IGF-I and therefore plays a central role in CRC prognosis. Decreasing levels of IGF-1 and increasing levels of IGFBP-3 may thus be a plausible mechanism underlying the inverse association between PA and CRC survival.

Introduction
Of all cancers, colorectal cancer (CRC) has the fourth highest incidence rate worldwide and it is estimated that CRC is responsible for the deaths of approximately 608,000 people each year [1]. Given these statistics, reducing CRC incidence, recurrence and improving survival have emerged as major public health priorities.

Physical activity (PA) has been specifically linked to CRC mortality, with approximately 15% of CRC deaths worldwide being attributable to physical inactivity [2] Further, epidemiological data show a significant decrease in disease-specific mortality for individuals who are physically active after diagnosis compared to those who are not [3,4]. However beyond recognising a relationship between PA and CRC survival, the biological mechanisms that underpin this association are not
entirely clear. Given that the insulin-like growth factor (IGF) axis has been implicated as a key host pathway responsible for the association between PA and CRC specific mortality [5-7], using PA to influence the IGF axis may represent an effective means of reducing CRC mortality and improving survival. This paper will review the available evidence relating to PA following CRC diagnosis, the IGF axis and survival from the disease.

1.0 Physical Activity and Colorectal Cancer
An inverse relationship between PA and the incidence of CRC has consistently been reported in the literature [8-10]. Furthermore, engaging in PA after CRC diagnosis is associated with 50-60% reductions in disease-specific mortality [11-14]. Despite the important role PA has for the health of survivors following CRC diagnosis, specific PA guidelines for reducing the risk of CRC-related mortality following diagnosis do not yet exist.

1.1 Physical Activity and Colorectal Cancer Incidence
Over the past ten years, three meta-analyses [8,9,15] have reported an inverse relationship between PA and the incidence of colon cancer. Samad et al. [8] analysed nineteen cohort and twenty-eight case-control studies and identified a relative risk (RR) for developing colon cancer of 0.79 when comparing the leisure-time PA of the most to the least active men. For women, a RR of 0.71 was identified for recreational PA when comparing the most active to least active [8]. More recently, Wolin et al. [9] found a RR of 0.76 for both men and women when comparing the most to the least physically-active individuals. Further, Boyle et al. [10] highlighted an inverse dose-response relationship between PA and colon cancer risk in eleven of the twenty-one studies included in their analysis.

Whilst these results offer support for the promotion of PA to reduce the risk of colon cancer, there is little evidence that PA can decrease the risk of developing rectal cancer [8,9,15]. The reason for this disparity is unknown. The closest understanding to the relationship between PA and rectal cancer is derived from studies that demarcate the colon into proximal and distal sub-sites during statistical analysis. However, findings from these studies offer no significant differences between proximal and distal colon regions with a RR of 0.73 and 0.74, respectively, when comparing the most to the least physically-active individuals [10]. A greater understanding of the physiological link between PA and colon cancer from a survival perspective may explain why rectal cancer incidence does not appear to be mediated by PA.

1.2 Physical Activity and Colorectal Cancer Survivorship
Following conventional treatment, CRC survivors who remain or become physically active have a >50% reduction in cancer-specific mortality over those who are inactive [11-13]. Indeed, researchers recommend exercise as an adjuvant to conventional treatment for those diagnosed with the disease [8,9,11-15].

Meyerhardt et al. [11] found an inverse relationship between PA and hazard ratio for CRC-specific mortality in male survivors. In a cohort of 661 men, those who engaged in more than 27 metabolic equivalent of task (MET) hours of PA per week had a CRC specific mortality hazard ratio of 0.47 compared to those who engaged in less than 3 MET-hours per week [11]. In a cohort of 573 female CRC survivors, a RR of 0.39 was found for those who engaged in at least 18 MET-hours of PA per week compared to those who engaged in less than 3 MET-hours per week [12]. Both studies found no change in statistical significance following adjustment for cancer stage (I-III), body mass index (BMI) and pre-diagnosis levels of PA. Such evidence highlights the importance of PA following diagnosis irrespective of pre-diagnosis activity levels. Although the specific frequency, intensity, type and mode of PA required for reductions in CRC specific mortality is uncertain, Meyerhardt et al. [11] have indicated that a protective effect for this measure occurs at approximately 9 MET-hours per week. This volume of PA aligns well with the current adult PA guidelines for health benefits [16].

The majority of studies that have investigated the relationship between PA and CRC survival have not reported the frequency, intensity, duration and/or mode of activity of the participants [8,9,11-15]. To a large part, this can be attributed to the limitations of self-report PA measures used in these studies, which typically estimate activity levels using MET values. It has been shown that participants tend to over-report than under-report PA when recalling previous activity levels [17]. This limits the conclusions that can be drawn from studies with respect to the ‘dose’ of PA required to elicit a protective effect. Research that involves structured PA interventions is required to better understand the relationships between CRC survival and PA that have been identified in prospective, case-control studies. Results from these intervention trials will help to determine the optimal ‘dose’ of exercise required to reduce CRC incidence and disease-specific mortality post-diagnosis. The Colon Health and Life-Long Exercise Change trial (CHALLENGE) [18] aims to address this limitation; this ongoing randomised controlled trial incorporates a multicentre PA intervention utilising instrumented measures of PA and aerobic fitness for people with stage II and III colon cancer. The primary outcome of this trial is disease-free survival, with cardiovascular fitness a secondary endpoint. This study will also track key biological markers believed to underpin the relationship between PA and colon cancer risk.
2.0 Insulin-like Growth Factors and Colorectal Cancer

Changes in gastrointestinal transit time, inflammation, immune function, genetic mutations, insulin and the IGF axis have all been suggested as mediators to explain the relationship between PA and CRC incidence and disease-specific mortality [19,20]. Specifically, it is believed that the IGF axis plays a central role in cellular growth, proliferation regulation, differentiation and apoptosis [21,22]. Given these mechanisms, IGFs and their binding proteins (IGFBPs) have been identified as a key research focus in CRC pathology [23].

The IGF axis has been linked to the primary and secondary development of CRC in meta-analyses of prospective studies [7,24]. Cross-sectional research has also found associations between the IGF axis and the graded severity of CRC carcinomas [23,25,26]. Manipulation of the IGF axis through PA may therefore be a promising therapy for preventing CRC, as well as reducing the likelihood of CRC-specific mortality post-diagnosis.

2.1 Insulin-like Growth Factor Axis

The IGF axis consists of two polypeptide ligands (IGF-I and IGF-II), two cellular membrane receptors (IGF-IR and IGF-IIR), and six binding proteins (IGFBP-1 through IGFBP-6). IGF-I and IGF-II are produced via the endocrine, paracrine and autocrine systems [27]. Growth hormone (GH) plays a dominant role in the upregulation of IGF-I with serum levels peaking around puberty and then decreasing throughout life [28,29]. IGF-I levels are also influenced by sex and nutritional status with higher levels found in females [30], periods of excess energy intake [31] and obesity [28]. Unlike IGF-I, the release of IGF-II is GH independent and levels remain stable after puberty [27]. At a cellular level, IGF-I and IGF-II accelerate cell cycle progression through the growth phase where DNA replication occurs [32]. Analogous to this growth-facilitating effect, IGF-I and IGF-II have the capacity to block cellular apoptosis. These processes have been reported in healthy [33] and malignant tissue [34], highlighting the potential role of IGF-I and IGF-II in the progression of CRC following diagnosis.

The biological actions of IGF-I and IGF-II are mediated via two cell-surface receptors; IGF-IR and IGF-IIR [33]. Because of the structural similarities between IGF-I and IGF-II, the IGF-IR is able to bind both molecules albeit at different affinities. IGF-IR favours IGF-I, binding the molecule at a 2-15 fold higher affinity than IGF-II [35]. Unlike the IGF-IR, the IGF-IIR does not bind IGF-I; this receptor specifically binds IGF-II, and at a 500-fold affinity greater than the IGF-IR [22]. Because binding of IGF-II to the IGF-IIR results in degradation of the molecule, the intra-cellular actions of
IGF-II are thought to be primarily mediated through the IGF-IR [36]. This complex association underpins the uncertainty that exists for the role of the IGF axis within the relationship between PA and CRC.

The majority (~75%) of IGF-I and IGF-II produced via the endocrine system are bound in a ternary complex with IGFBPs and an acid labile subunit (ALS) [37]. The remaining IGF-I and IGF-II circulates in free form or in a binary unit with IGFBPs only [37]. Because ALS only has an affinity for IGF-I/IGF-II that is bound in a IGFBP complex, IGFBPs are thought to control the bioavailability of IGF-I and IGF-II [38]. This is actioned via three distinct pathways; 1) transportation, 2) prolonging the half-life of IGFs and protecting them from degradation, and 3) modulating the interaction between IGFs and their receptors [39]. When combined in the ternary unit, IGF-I and IGF-II are unable to bind to the cell surface receptors, IGF-IR and IGF-IIR. This is due to the up to 50 fold higher affinity of IGFBPs for IGF-I and IGF-II over their respective receptors [39]. The outcome of this affinity is thought to be the inhibition of IGF receptor activation, which in turn prevents IGF-I and IGF-II mediated cellular proliferation and reduces anti-apoptosis. In contrast, IGFBPs prolong the half-life of IGF-I and IGF-II via the prevention of proteolytic degradation that would normally occur if IGF-I and IGF-II were circulating in isolation [37]. This results in a lengthening of IGF-I and IGF-II bioavailability [40]. Given these differing processes, IGFBPs can facilitate or inhibit the mitogenic actions of IGF-I and IGF-II. These mechanisms indicate that IGFBPs may be of equal importance to IGF-I and IGF-II in mediating cellular growth and understanding how PA influences CRC incidence and mortality.

In serum, IGFBP-3 is the most abundant IGF binding protein, carrying approximately 90% of all bound and free circulating IGF-I and IGF-II [6]. Independent to its association with IGF-I and IGF-II, IGFBP-3 has been found to have a pro-apoptotic and anti-proliferative capacity [41]. This has led to a focus on this specific binding protein as a mediator for the development and progression of colorectal neoplasms.

A limitation to the measurement of these biomarkers in existing cancer research is that they may not reflect downstream cellular growth. Assays used to measure IGF-I and IGF-II do not discriminate between free form IGF-I/IGF-II, and that which is bound in binary and ternary units [38]. Because of this, current techniques may not reflect the bioactive IGF-I and IGF-II that are able to interact with cellular receptors. Furthermore, given that IGFBPs have both growth facilitating and inhibiting effects, direct measures of these biomarkers cannot accurately predict pro- or anti-proliferative processes. An assay that overcomes these limitations will enhance the understanding of how
exercise influences the IGF axis interaction with carcinomas. Identifying the action of intracellular growth processes rather than merely measuring circulating levels of these biomarkers is needed. Nonetheless, reductions in plasma IGF-I and IGF-II, and increases in IGFBP-3 suggest a more favourable outcome for the prevention of CRC and reductions in disease-specific mortality.

2.2 Insulin-like Growth Factors and Colorectal Cancer Survival

There is strong epidemiological evidence linking IGF-I, IGF-II and IGFBP-3 to the primary development of CRC [7,24,42-45]. What is less apparent is the nexus between the IGF axis and the development of secondary tumours. Although 5-year relative survival for localised CRC is promising (89.9%), those who experience secondary and tertiary tumours following diagnosis have a poorer prognosis, with expected survival rates of 69.6% and 11.9%, respectively [46].

Research has identified significant associations between tumour severity and IGF-I, IGF-II and IGFBP-3 [23,25,26,47,48]. These studies have focussed on tumour grade and metastasis, where a greater tumour grade and extent of metastasis infers a heightened CRC-specific mortality risk [23,25,26,47,48]. In an animal model, IGF-I has been found to influence colon cancer tumour growth and metastasis in liver-IGF-I deficient (LID) mice, which have approximately 75% less endogenous IGF-I than controls [47]. Following transplantation of colon adenocarcinomas, LID mice had smaller and fewer tumours with less liver metastases than controls. Further, exogenous IGF-I administration in both controls and LID mice increased the rate of tumour progression and metastases compared to mice treated with saline [47].

Findings from human in vivo research examining IGF and CRC staging have produced varying results [23,25,26,48,49]. Prospective samples from men and women undergoing a colonoscopy identified high-risk adenomas were positively associated with serum IGF-I and inversely associated with IGFBP-3 [26]. Supporting this, in a cohort of 125 CRC patients, higher serum concentrations of IGF-I were found in those with metastases compared to those with localised CRC [25]. Similar findings for IGF-I and adenoma severity were reported by Jacobs et al. [49]. IGF-II has also been linked to CRC severity with higher serum levels found in concurrence with secondary cancers [23]. In contrast to these results, no significant differences in serum IGF-I were reported with patients who had moderate (adenoma) and more advanced (carcinoma) colon cancers [48]. Nonetheless, the weight of the limited available evidence tends to support a positive relationship between IGF-I/IGF-II and CRC specific mortality and an inverse relationship between IGFBP-3 and CRC specific mortality.
Given the pathways by which IGF-I and IGF-II stimulate cellular proliferation, the expression of IGF-IR and IGF-IIR in colonic carcinomas is likely to influence tumour progression. A high presence of these receptors in tissues would allow for enhanced activation of intracellular growth processes via IGF signalling. Positive tissue staining for IGF-IR has been more frequently identified in primary and high risk CRCs in comparison to non-cancerous adenomas and normal tissue [50,51]. Further research has identified IGF-IR and IGF-IIR gene expression to be 2.5 and 5 times higher, respectively, in malignant tissue compared to adjacent non-cancerous tissue [52]. Despite this finding, the IGF-IIR is not thought to influence tumorigenic potential as it lacks the capacity to initiate mitogenic behaviours [36].

In vitro research has examined markers of the IGF axis after CRC diagnosis to determine their relationship with disease-specific mortality. Wolpin et al. [53] addressed this among 373 participants over 13 years, and found no associations between pre-diagnosis IGF-I and IGFBP-3, and mortality in those who developed CRC. Given that lifestyle factors such as diet, obesity and PA are known to influence IGFs [28,38], failure to include these confounders into the analysis may have contributed to the lack of significant findings with regard to IGF-I and IGFBP-3. Following CRC diagnosis, higher circulating IGFBP-3 has been correlated with a greater response to chemotherapy, arrested rate of tumour progression, and an increase in overall survival [54].

The varied physiological findings reviewed above highlight the need for robust interventional designs to clarify the relationships between IGF-I, IGFBP-3 and CRC mortality. Larger sample sizes coupled with improved assays that more precisely measure how IGF-I, IGF-II and IGFBP-3 influence cellular growth will advance understanding of these relationships. In addition, confounding factors such as energy intake and PA following CRC diagnosis need to be either controlled for or accurately monitored. Notwithstanding these limitations, there is strong evidence to support a mechanistic link between IGFs and heightened CRC mortality following diagnosis, whereby increased levels of IGFBP-3 and decreased circulating levels of IGF-I and IGF-II are associated with reduced disease-specific mortality.

3.0 Physical Activity and the Insulin-like Growth Factor Axis

The physiological response of IGFs to PA is inconsistent; increases, decreases and no change in the IGF axis have been reported in cancer and non-cancer populations [55-61]. While the reason for this inconsistency is not clear, some researchers believe that negative energy balance may underlie the mechanism/s [57], while others purport that it may be more closely related to energy flux [55] and physical conditioning [56]. Although limited, there is evidence from trials involving CRC
populations to indicate that changes in these biomarkers driven by PA can have favourable outcomes for disease-specific mortality [14].

Only one intervention study has investigated the influence of PA on the IGF axis in a CRC population. Following 12 weeks of unsupervised PA of 18 to 27 MET-hours per week, significant increases in IGF-I and IGFBP-3 were found [62]. Given that the intervention was unsupervised and adherence was measured via a self-reported questionnaire, the precise quantity and intensity of PA completed is unknown. Furthermore, prospective evidence demonstrated a one standard deviation increase in IGFBP-3 was associated with a 51% reduction in cancer-specific death for those who were physically active [63]. The same association was not seen for inactive individuals or for IGF-1 [63]. This indicates that PA is capable of eliciting a beneficial shift in disease-specific mortality that is manifested by a measureable biochemical change. Whilst it cannot be concluded without further research why the IGF-I response does not reflect that of IGFBP-3, there likely exists a delicate interplay between the IGF axis and other exercise-induced biochemical responses.

Although not within the subset of CRC, research in other cancer pathologies has examined the relationship between the IGF axis and PA. In a cohort of 26 males diagnosed with prostate cancer and currently receiving androgen deprivation therapy, it was found that IGFBP-3 significantly increased and IGF-I significantly decreased in men undertaking a six month resistance training program [58]. No significant changes in these biomarkers were reported for men in the aerobic training arm. Although no analysis of this dissonance was mentioned in the paper, it is interesting to note the discrepancy with regard to the exercise prescription in both treatment arms. Those in the resistance training group were given a set of exercises to complete in the program whereas those in aerobic group were encouraged to exercise at 60-80% of their age-predicted maximum heart rate via feedback from a wristwatch monitor. No baseline test was completed to confirm this heart rate range, therefore the intensity range completed by participants in the aerobic group may have been inadequate to elicit changes in the IGF axis that those in the resistance training group experienced.

Several studies have measured the response of IGFs to a PA intervention in breast cancer survivors. Fairey et al. [61] tracked the changes in IGF-I, IGF-2 and IGFBP-3 following 15 weeks of moderate intensity aerobic exercise; significant increases in IGFBP-3 and decreases in IGF-I were found, including decreases in their molar ratio, which is thought to reflect bioactive IGF-I. More recent research by Irwin et al. [59] tracked breast cancer survivors over a 6 month randomised controlled trial, and found similar results for IGF-I but significant decreases in IGFBP-3 compared to pre-intervention levels.
Schmitz et al. [64] investigated the role of resistance training on the IGF axis in breast cancer survivors. Six months of resistance training resulted in significant reductions in IGF-II however no significant changes in IGF-I were reported. Compared to Fairey et al. [61], a higher percentage of participants in this study were undergoing chemotherapy, which is known to alter IGF-I levels (Bonani, 2001) and therefore may have muted the biochemical response to PA.

In addition to PA, negative energy balance through dietary restriction may lead to a reduction in IGF-I [31]. Several studies involving healthy populations have attempted to address this by employing dietary controls in addition to a PA intervention. Nemet and colleagues [57] found IGF-I to only decrease following seven days of aerobic exercise when participants were in a negative energy balance (assisted via dietary restriction). In agreement, Smith et al. [65] found no differences in groups who experienced the same negative energy balance through PA or diet alone. The volume and intensity of PA required to lower IGF-I is not known and it may be that without a negative energy balance, longer interventions at a higher intensity or volume of PA are required for significant IGF-I changes to occur. Rarick et al. [55] addressed this idea of energy flux which accounts for the absolute level of energy expenditure and intake under conditions of energy balance. They found that energy balance and baseline aerobic fitness had no impact on IGF-I or IGFBP-3 response. These findings are in contrast to those of Smith et al. [65] and Nemet et al. [57], further confounding the relationship between PA and the IGF axis.

Given that dietary intake influences IGF-I levels, controlling for this variable in the days preceding blood sampling is crucial to understanding the precise impact of PA on the IGF axis [31]. Many of the studies investigating the PA/IGF association have not reported dietary intake control prior to testing. Further, assays that only measure total IGF-I in circulation rather than free IGF-I or IGF-IR activation have generally been used. This measure does not differentiate between IGF-I that is free/bioactive or bound in binary and ternary units (and therefore unable to interact with cell surface receptors).

4.0 Conclusions and Future Directions

While the benefits of PA for the prevention of CRC and reduction in disease-specific mortality post-diagnosis are well established, the physiological mechanisms by which PA can mediate these outcomes remains to be determined. Of the several biological pathways that have been considered, the IGF axis is the most plausible mechanism and has subsequently received the most interest. Following diagnosis from CRC, there is evidence to suggest that a decrease in IGF-I and an
increase in IGFBP-3 can reduce disease-specific mortality. While there is evidence to suggest that PA may influence IGF-I and IGFBP-3, intervention studies involving a structured PA regime for CRC populations examining changes in the IGF axis are required. Understanding the frequency, intensity, duration and mode of exercise that each will potentially influence the IGF axis, reduce the incidence of CRC and improve survival is needed to inform the development of specific PA guidelines for CRC survivors - guidelines that currently do not exist.
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5.2 The influence of high-intensity compared with moderate-intensity exercise training on cardiorespiratory fitness and body composition in colorectal cancer survivors: a randomised controlled trial

Abstract

**Purpose:** Following colorectal cancer diagnosis and anti-cancer therapy, declines in cardiorespiratory fitness and body composition lead to significant increases in morbidity and mortality. There is increasing interest within the field of exercise oncology surrounding potential strategies to remediate these adverse outcomes. This study compared four weeks of moderate (MIE) and high intensity exercise (HIE) training on peak oxygen consumption ($\dot{V}O_2$peak) and body composition in colorectal cancer survivors. **Methods:** Forty-seven post-treatment colorectal cancer survivors (HIE=27 months post-treatment; MIE=38 months post-treatment) were randomised to either HIE [85-95% peak heart rate (HRpeak)] or MIE (70% HRpeak) in equivalence with current physical activity guidelines and completed 12 training sessions over four weeks. **Results:** HIE was superior to MIE in improving absolute (p=0.016) and relative (p=0.021) $\dot{V}O_2$peak. Absolute (+0.28 L.min⁻¹, p<0.001) and relative (+3.5 ml.kg⁻¹.min⁻¹, p<0.001) $\dot{V}O_2$peak were increased in the HIE group but not the MIE group following training. HIE led to significant increases in lean mass (+0.72 kg, p=0.002) and decreases in fat mass (-0.74 kg, p<0.001) and fat percentage (-1.0%, p<0.001) whereas no changes were observed for the MIE group. There were no severe adverse events. **Conclusions:** In response to short-term training, HIE is a safe, feasible and efficacious intervention that offers clinically meaningful improvements in cardiorespiratory fitness and body composition for colorectal cancer survivors. **Implications for Cancer Survivors:** HIE appears to offer superior improvements in cardiorespiratory fitness and body composition in comparison to current physical activity recommendations for colorectal cancer survivors and therefore may be an effective clinical utility following treatment.

Introduction

Colorectal cancer is one of the most prevalent cancers worldwide; with the second and third highest mortality rates for men and women, respectively, it represents a significant proportion of the health burden attributable to cancer [161]. Following diagnosis and anti-cancer therapy (locoregional and systemic), many cancer survivors experience acute and chronic toxicities that increase morbidity and mortality [162, 163]. There is increasing interest in the clinical utility of exercise-oncology as an adjunctive therapy for improving prognosis following colorectal cancer diagnosis via remediation of adverse clinical outcomes [163].
Cardiorespiratory fitness appears to be a critical prognostic factor across the oncology continuum [164]. Compared with low cardiorespiratory fitness (measured by peak oxygen consumption [$\dot{V}O_{2}^{\text{peak}}$]), moderate and high cardiorespiratory fitness has been associated with a 33% and 44% reduction in colorectal cancer incidence, respectively [164]. Following a cancer diagnosis, cardiorespiratory fitness has been shown to predict cancer mortality in men [139] and women [165]. Indeed meta-analytical conclusions indicate that compared with lower cardiorespiratory fitness levels, higher cardiorespiratory fitness is associated with a 45% reduction in cancer-specific mortality [166]. In addition to the strong relationship between cardiorespiratory fitness and mortality, cardiorespiratory fitness has also been shown to be an important predictor of morbidity following major colonic [43] and rectal [44] cancer surgery. Recent data have shown that in pre-operative rectal cancer patients, neoadjuvant chemotherapy leads to a decrease in $\dot{V}O_{2}^{\text{peak}}$ of between 1.4 and 4.0 ml.kg$^{-1}$.min$^{-1}$ [41, 42]. These decrements following anti-cancer therapy in conjunction with the poor prognostic relationship between low cardiorespiratory fitness and cancer morbidity and mortality demonstrate the clinical importance of improving cardiorespiratory fitness within the oncology setting.

Mechanistic underpinnings of the reduction in cardiorespiratory fitness following anti-cancer therapy appear to be determined by a multitude of central factors such as cardiac and pulmonary function, as well as peripheral factors including haematological, vascular and skeletal muscle function [163]. Recent pilot data strengthens the mechanistic relationship between reductions in cardiorespiratory fitness and skeletal muscle function, with significant reductions in mitochondrial function following neoadjuvant chemotherapy observed in rectal cancer patients [42]. This reduction in mitochondrial function and subsequent impairment of oxidative phosphorylative capacity may be further compounded by a reduction in skeletal muscle mass. Skeletal muscle atrophy or cachexia is a common comorbidity following diagnosis and anti-cancer treatment characterised by ongoing loss of muscle mass, with or without loss of fat mass, leading to progressive impairment in function [167-169]. The presence of cachexia has been shown to increase premature mortality rates in patients with advanced colorectal carcinoma [170]. Whilst the clinical presentation of advanced cancer cachexia is physically distinguishable, less obvious or ‘hidden’ conditions such as pre-cachexia or sarcopenic obesity (muscle mass loss masked by adipose tissue hypertrophy) are of perhaps greater clinical importance as these stages may be more responsive and amenable to therapeutic interventions as compared with advanced states of cachexia [167, 169]. Alluding to the clinical importance of body composition following cancer diagnosis, the presence of sarcopenic obesity in colorectal cancer survivors is associated with impaired physical function as well as an increase in premature mortality [49].
Collectively, reductions in cardiorespiratory fitness, skeletal muscle mass and an increase in adipose tissue present a series of adverse comorbidities for colorectal cancer survivors that can severely impair prognosis following treatment. Aerobic exercise training has been suggested to be one of the most effective therapeutic strategies to combat cancer cachexia through remediation of mitochondrial and oxidative phosphorylation dysfunction [171]. Additionally, aerobic exercise training is arguably the most effective method of facilitating improvements in cardiorespiratory fitness and thereby presents itself as a potentially effective therapy to combat the aforementioned adverse clinical outcomes associated with colorectal cancer survivorship. Within the scope of aerobic exercise, a recent meta analysis demonstrated high intensity exercise (HIE) to be more efficacious in improving cardiorespiratory fitness compared with moderate intensity exercise (MIE) in patients with cardio-metabolic disease [172]. Whilst one previous trial has utilised an intervention that included HIE in colorectal cancer survivors [173], no study to-date has investigated the comparative effectiveness of HIE and MIE to determine the most effective aerobic intensity prescription for improvements in cardiorespiratory fitness and body composition. The aim of this randomised controlled trial was to compare the effect of a short-term HIE or MIE aerobic exercise intervention as a potential clinical utility to promote improvements in cardiorespiratory fitness and body composition in colorectal cancer survivors. It was hypothesised that HIE would result in greater improvements in cardiorespiratory fitness and body composition compared to MIE.

**Methods**

**Participants**

Men and women previously diagnosed with histologically-confirmed colorectal cancer were recruited from Brisbane (Queensland, Australia) for this randomised controlled trial. Participants recruited for this intervention are included as part of the first phase of a larger ongoing trial. Inclusion criteria were as follows: (i) aged ≥ 18 years old; (ii) ≥ one-month post-treatment for colorectal cancer and not anticipating undergoing treatment during the study period; and (iii) free of any musculoskeletal, neurological, respiratory, metabolic or cardiovascular conditions that may have prevented safe completion of the exercise demands of the study. Potential participants were contacted either through access to the population based Queensland Cancer Registry according to previously described procedures [174] or from an existing cohort of colorectal cancer survivors [175]. Participants were required to obtain physician consent for participation in the program, and were individually screened via a medical history form and interview with the investigators to determine eligibility.
Study Protocol

This study was granted ethical approval by the Human Ethics Committee of The University of Queensland and written informed consent was obtained from all subjects prior to commencing the study. Date of diagnosis and cancer stage information was extracted from pathology reports. Following recruitment, participants completed a familiarisation session consisting of a test of peak oxygen consumption (\(\dot{V}\text{O}_2\text{peak}\)) to assess cardiorespiratory fitness (detailed below). Following familiarisation (\(\geq\) seven days), participants completed a baseline testing session consisting of an assessment of body composition (detailed below) and a \(\dot{V}\text{O}_2\text{peak}\) test. Following baseline testing, a researcher independent to the study stratified the participants according to age (< 55 or \(\geq\) 55 years) and sex and then randomised them via a random number generating process to either HIE or MIE (detailed below) at a ratio of 2:1, respectively. This randomisation ratio was implemented for two reasons: firstly to account for a potentially inflated dropout rate as the feasibility of a HIE intervention in this population has not been reported; and secondly to allow for appropriate sample sizes for future phases of this ongoing trial. Both HIE and MIE groups trained three times per week for four weeks. Between three and seven days following the final exercise session, participants completed endpoint testing involving identical procedures to those used at baseline testing.

Physiological Performance

\(\dot{V}\text{O}_2\text{peak}\) testing was completed using a cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, Netherlands) and a portable metabolic cart system (ParvoMedics TrueOne 2400, Sandy, USA). Expired air was analysed for oxygen consumption (\(\dot{V}\text{O}_2\)), carbon dioxide production (\(\dot{V}\text{CO}_2\)); the fraction of oxygen and carbon dioxide in expired air (\(\text{FEO}_2\) and \(\text{FECO}_2\)) were sampled every 15 seconds during exercise from a mixing chamber, while total ventilation (\(\dot{V}\text{E}\)) was recorded every 15 seconds using a turbine ventilometer (Morgan, Model 096, Kent, England). The gas analysers were calibrated immediately prior to testing and validated after each test using a certified beta gas mixture (BOC, Brisbane, Australia). The ventilometer was calibrated before each test using a 3 L syringe (Hans Rudolph Inc., Shawnee, USA) in accordance with the manufacturer’s instructions. Blood pressure was measured against contraindications to exercise testing [176]. The \(\dot{V}\text{O}_2\text{peak}\) testing protocol, modified from Wasserman et al. [177], began with three minutes of rest for respiratory normalisation, followed by four minutes of warm up at a resistance of 50 W. Thereafter, the electronic resistance provided by the cycle ergometer increased incrementally by 20-30 W.minute^{-1}. Participants cycled at a cadence between 60 and 70 revolutions per minute throughout the test. Heart rate was continuously recorded throughout exercise using a heart rate monitor (Polar FT1, Polar, Kempele, Finland) and blood pressure (DuraShock Sphygmomanometer, Welch Allyn, New York, USA) was recorded every two minutes throughout the test. At the
conclusion of each minute, participants indicated their rating of perceived exertion (RPE) on the Borg 6-20 scale [178]. The test was terminated when participants reached volitional fatigue or at the discretion of the researcher in accordance with the indications for exercise test termination as outlined by the American College of Sports Medicine [176]. \( \dot{V}O_2 \)peak was recorded as the mean of the two highest 15-second \( \dot{V}O_2 \) epochs. Peak power output (PPO) was determined by the addition of the highest completed power level and the fraction of time spent in the incomplete stage multiplied by the stage wattage increments:

Equation 1:

\[
PPO = \text{final completed workload} + (\text{workload increment} \times \text{seconds in final stage} \times 60^{-1})
\]

**Body Composition**

Fat mass, percentage body fat and lean mass were derived by dual energy x-ray absorptiometry (DEXA; Hologic Discovery A, Waltham, MA). The coefficients of variation values in our laboratory for whole body fat and lean masses are <1.1%. All scans were conducted and analysed by two accredited DEXA technicians. All fat and lean mass results are subtotal values (whole body minus the head) rather than whole body totals. Height and body mass were measured using a stadiometer (Seca, Birmingham, United Kingdom) and electronic scales (A & D Mercury, Pty Ltd, Thebarton, Australia), respectively.

**Control Measures**

For each testing session participants were asked to: (i) maintain a hydrated state in the 24 hours prior to testing; (ii) abstain from caffeine and alcohol intake for 12 hours prior to testing; and (iii) avoid any vigorous, high or unaccustomed moderate intensity exercise or physical activity for the 48 hours prior to testing which were confirmed via checklist prior to commencing the session. All participants were asked to maintain their current diet and level of physical activity outside of the training sessions for the duration of the study (i.e. not commencing any unaccustomed dietary or physical activity behaviours throughout the duration of the intervention). To quantify and track the weekly physical activity behaviours of participants the Godin leisure-time exercise questionnaire [125], was completed at both testing time points. The Godin questionnaire has been shown to have a modest correlation compared with accelerometry derived measures of physical activity (\( r = 0.45 \)) [179] but demonstrates high test-retest reliability (\( r = 0.75 \)) [180].
Exercise Intervention

An Accredited Exercise Physiologist (Exercise and Sports Science Australia) supervised all testing and exercise training sessions. Both the HIE and MIE sessions were conducted on air- and magnetically-braked cycle ergometers (Wattbike Pro, Wattbike Ltd., Nottingham, England) with heart rate continuously measured throughout each session (Suunto Ambit2 S, Suunto Oy, Vantaa, Finland). The HIE training sessions commenced with a 10 minute warm up at 50-70% HRpeak before commencing 4x4 minute bouts of cycling at 85-95% HRpeak. Each four minute interval was interspersed with a three minute period of active recovery at 50-70% HRpeak, totalling 38 minutes for the session. The MIE training protocol consisted of 50 minutes of cycling at 50-70% HRpeak. The frequency and volume of MIE was established according to current physical activity guidelines recommended for cancer survivors [181, 182] and for adults by the American College of Sports Medicine for adults (≥ 150 minutes of moderate intensity physical activity per week) [178]. RPE was measured at the conclusion of each 4-minute interval in the HIE session and at similarly regular intervals during the MIE session (15, 30, 40 and 50 minutes). Power output and cadence were continuously measured throughout the sessions and analysed using specialised software (Wattbike Expert, Wattbike Ltd., Nottingham, England).

Rates of Completion, Adverse Events, Attendance and Adherence

Completion rates were calculated as the number of participants that completed baseline testing divided by the number that were randomised at the beginning of the intervention. Adverse events were defined as ‘any untoward medical occurrence in a participant subject to the intervention’. A severe adverse event was defined as any event requiring hospitalisation or causing an inability to carry out usual activities. Adverse events were assessed by monitoring and recording during all exercise sessions by the supervising Exercise Physiologist. Attendance to the intervention was measured as the number of sessions attended (nattended) divided by the number of sessions prescribed (nprescribed):

Equation 2: attendance = nattended × nprescribed⁻¹ × 100

Adherence to the intervention was assessed within the prescriptive domains of duration and intensity. Duration adherence was measured as the duration of the completed session divided by the prescribed duration in minutes:

Equation 3: duration adherence = ncompleted × nprescribed⁻¹ × 100
Adherence to the prescribed intensity for both the HIE and MIE groups was measured as the mean heart rate (HR\text{mean}) achieved within the 12 sessions relative to the recorded HR\text{peak}:

\text{Equation 4: } \text{intensity adherence} = \left( \sum HR\text{mean} \times 12^{-1} \right) \times HR\text{peak}^{-1} \times 100

The MIE HR\text{mean} was calculated as the mean HR recorded at four time points (15, 30, 40 and 50 minutes) throughout the 50-minute session whereas the HIE HR mean was calculated as the mean of the peak HRs recorded during each of the four intervals. Intensity was also concurrently assessed as the mean session RPE recorded throughout the intervention, with session RPE calculated as the mean of RPE recorded at four time points during both sessions.

\textit{Statistical Analysis}

All data were analysed using SPSS (version 22.0; Chicago, IL). Data were assessed for normality of distribution using the Shapiro-Wilk test. Analyses included standard descriptive statistics, independent t-tests, Mann-Whitney U tests or Chi-squared tests, as appropriate to test for differences between the groups at baseline. Non-parametric data were log-transformed and re-checked for normality prior to univariate within- and between-groups analyses. Differences within groups were assessed using paired samples t-tests or the Wilcoxon Signed Rank non-parametric test, as appropriate. Differences between groups were assessed using independent t-tests and the non-parametric equivalent Mann-Whitney U test where necessary. Statistical significance was accepted at an alpha of p < 0.05. Normally distributed data are presented as mean and standard deviation (SD) whereas data-requiring log-transformation were re-transformed and are reported as the geometric mean and 95% confidence intervals (CI). Data unable to be normally distributed using log-transformation were analysed non-parametrically and presented as median, interquartile range and 95% CI.

\textit{Results}

\textit{Participant recruitment and baseline characteristics}

A consort diagram of participant flow throughout the study is detailed in Figure 1. Following recruitment and baseline testing, 47 colorectal cancer survivors were randomised. The characteristics of participants included within the analyses are shown in Table 1. There were no significant differences (p > 0.05) between groups for any of the measured baseline characteristics. A previous diagnosis of colon cancer was more prevalent than rectal cancer within both the HIE (colon = 70.0%; rectal = 30.0%) and MIE (colon = 82.4%; rectal = 17.6%). Only 30.0% of
participants in the HIE and 41.2% of MIE participants underwent surgical treatment without any adjunctive therapy whereas the prevalence of treatment with a chemotherapeutic agent was high with 66.6% of HIE and 58.6% of MIE participants undergoing adjunctive chemotherapy. Participants in the HIE intervention were at a median 27.0 months post-treatment whereas participants in the MIE training were 38.0 months post-treatment. Cancer staging was unable to be determined for 13 participants, 9 due to insufficient pathology report information and 4 sets of data were unable to be accessed due to consent reasons.

Rates of Completion, Adverse Events, Attendance and Adherence

Study completion rates for the MIE and HIE were 94.1% and 96.7%, respectively, with only one participant dropping out in either group (MIE: discontinued the intervention due to personal reasons prior to commencing training, HIE: discontinued after six sessions due to ongoing interruptions resulting from additional medical diagnostic testing for an unrelated condition). No severe adverse events occurred during any exercise testing or training throughout this study. In terms of non-severe adverse events during the study, on three occasions for separate participants (two following a HIE session and one following exercise testing), participants experienced a symptomatic episode of post-exercise hypotension, which was resolved following active recovery, consumption of fluids and recovery in the supine position if necessary. On one occasion, a participant experienced an aggravation of pre-existing sciatica following a HIE session. Two participants also experienced an acute exacerbation of knee pain during a HIE interval related to pre-existing osteoarthritis. In both instances the respective sessions were terminated and the issue did not impact subsequent sessions. The number of non-severe adverse events between HIE and MIE groups were not statistically significantly different (p=0.082). Additionally following the completion of HIE intervals, two participants occasionally reported brief feelings of nausea, which were resolved following active and passive recovery as well as fluid intake. Attendance rates at sessions across the intervention were 100% in both MIE and HIE groups for the 16 and 29 participants that completed the intervention in either group (including all participants: MIE = 97.1%; HIE = 97.0%), respectively. The mean ± SD duration of the MIE sessions was 50 ± 0 minutes, indicating a 100% adherence rate to the prescribed duration. MIE training was completed at a mean ± SD intensity of 73.4% ± 8.4% HR\text{peak} (prescribed: 70% HR\text{peak}) and a RPE of 12.0 ± 1.7. The mean ± SD duration of the HIE intervals completed during each session was 15.95 ± 0.1 minutes, representing 99.7% of the prescribed duration. HIE intervals were completed at an intensity of 91.9% ± 4.2% HR\text{peak} (prescribed: 85-95% HR\text{peak}) at a RPE of 14.6 ± 1.2.

Physical activity levels of participants are displayed in Table 2. There were no significant
differences between groups at baseline. No significant changes were observed within-groups across the intervention, nor between-groups following the intervention (p>0.05).

Physiological Performance
Changes in cardiorespiratory fitness and power output are shown in Table 2. There were no significant differences between the HIE and MIE groups for any variables at baseline. HIE training was associated with a mean increase of 0.28 L.min\(^{-1}\) ± 0.28 L.min\(^{-1}\) and 3.5 ml.kg\(^{-1}\).min\(^{-1}\) ± 3.5 ml.kg\(^{-1}\).min\(^{-1}\) in absolute and relative \(\dot{V}O_2\)peak, respectively. Improvements in both absolute \(\dot{V}O_2\)peak (p<0.001) and relative \(\dot{V}O_2\)peak (p<0.001) in the HIE group were significant from baseline to endpoint whereas no significant within-group changes were observed in the MIE group. Mean changes in absolute (p=0.016) and relative (p=0.021) \(\dot{V}O_2\)peak were significantly higher in the HIE group than the MIE group. PPO significantly increased following the intervention in both the HIE (p<0.001) and MIE (p=0.030) groups. The mean increase of 29.3 W ± 20.7 W in response to HIE training was significantly greater than the 11.7 W ± 19.3 W MIE group increase (p=0.018). Similar trends in relative PPO were observed with both groups improving from baseline to endpoint (p<0.05), with the mean increase in the HIE group being significantly greater than the HIE group (p=0.043).

Body composition
Body composition data are shown in Table 2. A subset of DEXA data from 10 consecutive participants (8 HIE and 2 MIE) was unable to be analysed due to equipment malfunction, which consequently reduced the sample size for all body composition analyses (excluding body mass) within each group. No significant differences were found between HIE and MIE groups at baseline for any of the body composition variables. For the HIE group, body mass significantly decreased from baseline to endpoint (p=0.005), with mean changes in the HIE group being significantly different compared to MIE (p=0.005). Within the HIE group, lean mass significantly increased by a mean (SD) change of 0.72 kg ± 0.80 kg from baseline to endpoint (p=0.002). In the HIE group, both fat mass (p<0.001) and body fat percentage (<0.001) significantly decreased from baseline to endpoint by a mean (SD) change of 0.74 kg ± 0.65 kg and 1.0% ± 1.0%, respectively. No significant within-group changes in body composition were observed for the MIE group. No significant differences were observed across the intervention between groups for measures of lean mass, fat mass or fat percentage, however the whilst the mean decrease in fat mass in the HIE group was greater than the MIE group, the difference was not statistically significant (p = 0.060).
Discussion
This study compared the influence of HIE training with MIE training, on cardiorespiratory fitness and body composition in colorectal cancer survivors. The findings show that 12 sessions of HIE completed over 4 weeks is significantly more effective than MIE in improving absolute and relative VO₂peak and PPO. Furthermore HIE was found to elicit increases in lean mass and decreased body mass, fat mass and fat percentage, with no changes associated with MIE in colorectal cancer survivors.

To assess the feasibility of the intervention and the subsequent influence on program efficacy we assessed the rates of completion, adverse events, adherence and attendance. We found excellent completion rates (MIE = 94.1%; HIE = 96.7%) for participants enrolled in this trial, comparable to rates reported in other interventions in colorectal cancer survivors [40, 173, 183]. Data regarding program safety and adverse events are severely underreported in the current colorectal cancer-exercise literature [184]. Only one study to date has reported these data in colorectal cancer survivors [173], with which our data compares well. Post-exercise hypotension was the most common adverse event, and is a well-known post-exercise phenomenon thought to be resultant from a peripheral vasodilatory response in the active musculature coupled with a relative decrease in sympathetic vasoconstrictive signals to restore homeostasis following exercise cessation [185]. As compared with moderate intensity, high intensity resistance training has been shown to induce greater reductions in post-exercise blood pressure, which may be due to an increased vasodilatory response of the working musculature due to the increased muscle volume recruited and metabolic demand of higher intensity exercise [186]. To assist in the maintenance of blood pressure, activation of a skeletal muscle-pump mechanism, which augments venous return from the periphery to central circulation is a critical process to prevent post-exercise hypotension [185]. As such, it is strongly recommended that all HIE sessions and VO₂peak tests are concluded with a period of active recovery to promote activation of this muscle-pump mechanism, as abrupt cessation of exercise and withdrawal of this muscle-pump can lead to significant decreases in venous return and blood pressure [185]. As an additional prospective countermeasure, we recommend that participants undertaking HIE maintain adequate fluid intake prior to, during the session and during recovery. Water ingestion is known to induce an acute pressor response, leading to increases in sympathetic drive and increases in blood pressure [187], a response that has been shown to effectively maintain post-exercise blood pressure and prevent hypotension [188]. Exercise-induced gastrointestinal discomfort and nausea is also known to occur with greater frequency following high intensity exercise when compared with light exercise [189]. The precise causes of this remain to be determined however it has been suggested that dehydration, consumption of foods that delay gastric
emptying, and as well as splanchnic hypoperfusion resulting from sympathetic splanchnic vasoconstriction to shunt blood to the working musculature and organs (inverse of the post-exercise hypotension mechanism) may all contribute to symptoms [189]. This further illustrates the importance of appropriate nutritional and hydration-monitoring prior to HIE. Given the small number and nature of the adverse events from this study and coupled with previous reports [173], HIE training appears safe in the clinical setting with appropriate supervision for colorectal cancer survivors.

Our attendance rate of 100% for participants in both the HIE and MIE groups who completed the intervention (when including all randomised participants: MIE = 97.1%; HIE = 97.0%) was higher than previous trials in colorectal cancer survivor populations, which have reported attendance rates ranging from 75.8% to 91.0% [40, 173, 190]. Throughout this intervention we also report excellent adherence to the duration of both the MIE (100%) and HIE (99.7%) sessions which again is higher than previous measures of duration adherence (87.3%) [173]. When considered in conjunction with strong rates of adherence to the prescribed exercise intensity, these outcomes suggest that aerobic MIE and HIE are highly feasible within a supervised clinical environment for colorectal cancer survivors. The median time since treatment for participants undertaking HIE was 27.0 months. Whilst HIE was highly feasible at this time point along the post-treatment continuum more research is needed to establish the feasibility of HIE at more proximal post-treatment time points. It is the view of the authors that establishing the suitability of a patient for HIE at a point along the post-treatment continuum should not follow a ‘one size fits all’ approach. Rather, decisions regarding a patient’s suitability to undertake HIE should be made with consideration of their overall clinical presentation and comorbidities, which therefore necessitates consideration on an individual-by-individual basis with input from the patient’s primary healthcare physician. Given the relatively brief duration of our intervention, these feasibility outcomes may be inflated compared to longer duration interventions (≥12 weeks), however given our results pertaining to the efficacy of the intervention on cardiorespiratory fitness and body composition, these data provide novel considerations for the design of shorter, highly feasible and efficacious clinical exercise programs for colorectal cancer survivors.

The present study demonstrates HIE to be significantly more efficacious than MIE in improving absolute (0.28 L.min⁻¹, p=0.016) and relative (3.5 ml.kg⁻¹.min⁻¹, p=0.021) VO₂peak as well as PPO (29.3 W, p=0.018). Only one previous study has measured changes in VO₂peak in response to exercise in colorectal cancer utilising a maximal graded exercise test with direct ventilatory measurement similar to the present study. Sellar et al. [173] implemented a longer intervention than
the current study (12 weeks) and utilised a combination aerobic and resistance training program (aerobic: three sessions/week; interval training; moderate to high intensity progressively increasing from 60-75% PPO to 110% PPO; resistance training: two sessions/week; 2-3 sets, 6-15 repetitions at 65-85% 1 repetition maximum) and reported significant increases in absolute (0.24 L.min⁻¹; p<0.001) and relative (3.0 ml.kg⁻¹.min⁻¹; p<0.001) $\dot{V}O_2$peak as well as PPO (24.0 W), similar to the present results. A meta-analysis including three other studies in colorectal cancer survivors demonstrated evidence for increases in physical fitness in response to exercise and physical activity interventions, however submaximal treadmill tests were used to predict physical fitness, which rely on extrapolation of values (e.g. heart rate) to estimate maximal capacity and are therefore less accurate than direct measurement at maximal exercise [40, 183, 184, 190]. Meta-analytical data of interventions of all cancer patients suggest mean (95% CI) improvements in relative $\dot{V}O_2$peak of 2.9 ml.kg⁻¹.min⁻¹ (1.16 – 4.64) following supervised exercise training [191]. Whilst our results are of a similar magnitude and provide further support for the efficacy of exercise interventions to improve cardiorespiratory fitness in oncology populations, previous trials have been of a substantially longer duration than the present study, ranging from 8 to 24 weeks of predominantly MIE. As no studies have previously used an intervention of such a short duration, it is not possible to conclude that similar cardiorespiratory fitness changes similar to the present study were not also observed at 4 weeks in other studies. However our data provide evidence that short-term exercise training of a higher intensity offers greater physiological adaptations when compared to moderate intensity training of the same duration. This discrepancy between our intervention duration (4 weeks) and others (≥ 8 weeks) may also explain the lack of $\dot{V}O_2$peak improvement in response to MIE in the present study. Cardiorespiratory fitness improvements in response to MIE may require longer interventions when compared to HIE, which is largely supported by previous studies in cancer survivors of longer durations that are predominantly of a moderate intensity [173, 191]. The present data strongly suggest that exercise intensity is a key prescriptive variable in short-term exercise interventions, with HIE offering significant increases in cardiorespiratory fitness compared with MIE.

In the present study, HIE training led to significant increases in lean mass (0.72 kg ± 0.80kg; p=0.005) and reductions in fat mass (-0.74 kg ± 0.65 kg; p<0.001) and body fat percentage (-1.0% ± 1.0%; p<0.001), whereas no significant changes were observed in response to training for the MIE group. Comparably, of the seven exercise-intervention studies conducted exclusively in colorectal cancer survivors [40, 75, 173, 183, 190, 192, 193], only two incorporated discrete measurement of separate tissues in body composition analysis similar to the present study. Lee et al. [75] used bioelectrical impedance analysis in response to a 12 week home-based physical activity program
whereas Sellar et al. [173] utilised air displacement plethysmography. No changes in fat percentage, lean or fat mass was observed in response to either intervention. Recent meta-analytical data demonstrate that in cancer survivors, resistance training interventions conducted over 12 to 52 weeks are associated with mean (95% CI) increases in lean mass of 1.1 kg (0.8 to 1.4) and decreases in body fat percentage of -2.1% (-3.5% to -0.7%) with non-significant changes in fat mass [194]. Whilst the magnitude of these changes is greater than in the current study, it is promising that improvements in lean mass were seen at all, given that the intervention in the current study did not involve resistance training and was shorter in duration than those in the meta-analyses. These findings however are limited by our reduced sample size due to the unavailability of 10 data sets and should be interpreted with caution.

To our knowledge, this is the first trial to demonstrate improvements in both physiological performance and body composition following aerobic HIE in cancer survivors. These increases with HIE but not MIE provide novel insight into the importance of exercise intensity prescription. The HIE prescription within the current study utilised four sets of a four-minute interval duration followed by three minutes of active recovery. Within the breadth of high-intensity interval training literature, a four-minute interval is considered a ‘long-interval’ (> 60 seconds) and is associated with a high physiological demand, incorporating both anaerobic (phosphocreatine system and anaerobic glycolysis) and aerobic (oxidative phosphorylation) energetics [195]. The physiological demands in terms of energy systems leads to both central and peripheral fatigue in response to long-interval training [196]. Within this study, the use of an air-braked, high-intensity interval cycle ergometer training regime specifically targets the hip and knee extensor musculature, which has been previously shown to increase the activity of various oxidative and glycolytic enzymes (citrate synthase, succinate dehydrogenase and phosphofructokinase) concurrently with PPO and \(\dot{V}O_2\)max [197-199]. Whilst specific enzymatic measures were not included in the current study, significant improvements in PPO in response to HIE (29.3 W; \(p <0.001\)) suggest an increase in peripheral oxidative and glycolytic capacity to offset peripheral muscular fatigue of the lower limb musculature. Increases in mitochondrial biogenesis has been suggested as a primary mechanism underlying the HIE-induced peripheral adaptions leading to increases in oxidative capacity [143]. Specifically, increases in peroxisome proliferator-activated receptor \(\gamma\) co-activator 1\(\alpha\) (PGC-1\(\alpha\)), which is considered the ‘master regulator’ of mitochondrial biogenesis has been shown to increase in response to HIE [143, 200-202]. In patients with heart failure, HIE of the same prescription used in the present study increased PGC-1\(\alpha\) whereas no changes were observed in response to MIE [153]. Increases in muscular PGC-1\(\alpha\) content have been reported to occur through cellular activation of both the 5’-adenosine monophosphate-activated protein kinase and the p38 mitogen-
activated protein kinase (MAPK) as a result of HIE [143, 201]. The latter is a downstream target of the MAPK pathway, which in addition to other key pathways (akt/mammalian target of rapamycin, calcium (Ca\(^{2+}\))-dependent pathways), is concurrently an important cellular pathway leading to muscular hypertrophy and anabolic development, however this is generally only considered in response to resistance training [203, 204]. Evidence to date suggests that HIE is capable of promoting MAPK activation and subsequent cellular signalling which can induce both increases in mitochondrial content as well as muscular hypertrophy which may explain the increases in lean mass observed in HIE but not MIE. Furthermore HIE has been suggested to be more effective than MIE in reducing fat mass, hypothesised to occur as a result of exercise-induced increases in fat oxidation, catecholamine mediated fat metabolism or appetite suppression [149]. This presents a mechanistic hypothesis for the interpretation of the current results that is capable of explaining increases in aerobic and anaerobic performance (\(\dot{V}O_2\)peak and PPO) as well as simultaneous increases in lean tissue and decreases in fat mass in response to aerobic HIE but not MIE. Future cellular analysis of these pathways is required to provide greater mechanistic scope for the interpretation of these findings.

These results have important clinical implications for the therapeutic use of targeted HIE programs within the oncology setting. Cardiorespiratory fitness is strongly and inversely associated with both mortality following cancer diagnosis [139, 164-166] and morbidity following major colonic and rectal surgery [43, 44]. Recent data in male cancer survivors suggest that an increase of 1-metabolic equivalent (MET; 3.5 ml.kg\(^{-1}\).min\(^{-1}\)) leads to a concurrent 10% decrease in cancer-specific mortality risk [164]. The present four-week HIE intervention observed a mean increase in \(\dot{V}O_2\)peak of 3.5 ml.kg\(^{-1}\).min\(^{-1}\) (p<0.001), demonstrating the potentially clinically meaningful improvements of HIE on cancer prognosis following diagnosis and adjuvant therapy. The prevalence of adjuvant chemotherapy was high in the current cohort (HIE = 66.6%; MIE = 58.8%) and given the known decrements in \(\dot{V}O_2\)peak (1.4-4.0 ml.kg\(^{-1}\).min\(^{-1}\)) following treatment, the clinical importance of the rapid improvements in \(\dot{V}O_2\)peak observed in the present study are further illustrated [41, 42]. The decrease in cardiorespiratory fitness following chemotherapy is thought to be determined primarily by a decrease in mitochondrial content and function [42], the amelioration of which is an important component of this high intensity prescription as improvements in mitochondrial density and function have been reported in response to HIE [205]. Sarcopenic obesity has been associated with impaired functional status and increased mortality risk following colorectal cancer diagnosis [49]. The present increases in lean mass (0.72 kg; p=0.002) and decreases in fat mass (-0.74 kg; p<0.001) in response to HIE but not MIE, may present HIE as an effective intervention to assist in remediating reductions in skeletal muscle and increases in adipose tissue following colorectal
cancer treatment. With the observed improvements in cardiorespiratory fitness and body composition, combined with the short-term accrual of these changes, aerobic HIE appears to offer clinically meaningful improvements in colorectal cancer prognosis following diagnosis and treatment, superior to that of MIE.

This study has several limitations that are worthy of comment. Firstly we did not implement any restrictions on inclusion criteria relating to age, cancer stage, treatment received or time since diagnosis or treatment. Given the known effects of adjuvant chemotherapy on cardiorespiratory fitness, participants who underwent this treatment may be more receptive to cardiorespiratory fitness improvements as a result of lower baseline values when compared to participants who did not receive chemotherapy. Furthermore this relationship may extend to differences in time since treatment, whereby participants more proximal to final treatment may be more receptive to improvements in cardiorespiratory fitness and body composition. Additional sub-analysis according to these variables was not possible in the present study due to sample size restrictions, however future trials should endeavour to make these considerations wherever possible. Within this study, we used the MIE group as a ‘usual care’ condition as this aligns with current physical activity guidelines for cancer survivors and as such there was no true ‘control group’. Following from this, our comparison with current physical activity guidelines for cancer survivors meant that controlling for total metabolic expenditure in each intervention was not possible. This may limit the conclusions that can be drawn regarding the isolated effect of exercise intensity between non-isoenergetic protocols. Whilst we did not directly compare the exercise volumes of the HIE and MIE protocols, a previous study estimated that the same HIE protocol implemented in the present study was approximately isoenergetic with 47 minutes of MIE (at 70% HRpeak) in a group of patients with the metabolic syndrome [206]. This data provides basic support that the volume of exercise completed between the HIE and MIE protocols was somewhat similar, however the non-isoenergetic nature of these protocols should be acknowledged as a limitation when drawing conclusions from these results. In the present study we found no increases in leisure-time exercise across either HIE or MIE groups, providing support for the observed changes being resultant from the exercise intervention rather than external participation in exercise or physical activity. Whilst self-reported levels of physical activity using the Godin questionnaire have been shown to be sufficiently reliable [180], recent data has questioned the validity of this method when compared with objectively measured levels of physical activity such as tri-axial accelerometry [207]. Whilst discrepancies between methods may originate from the focus of self-report data on recreational exercise, whereas objective measures capture total physical activity, future research should endeavour to use more accurate measures to quantify external exercise and physical activity so as to
strengthen conclusions made as being resultant of the intervention rather than external factors. This extends to changes in dietary patterns also, in the present study we did not collect dietary records expansive enough to validly assess and track changes in dietary habits across the intervention. Finally, we only assessed outcomes following four weeks of exercise training and therefore whether this intervention resulted in clinically meaningful long-term changes is yet to be determined.

The present study is the first to compare the differential effects of moderate and high intensity exercise in any cancer survivorship population. Our results demonstrate HIE to be superior to MIE in promoting improvements in VO$_2$peak, PPO and in body composition, which were not evident in response to MIE. Current guidelines for cancer survivors recommend exercise in accordance with general physical activity guidelines (i.e. 150 minutes of moderate intensity or 75 minutes of vigorous intensity exercise per week) [182]. Our results indicate that HIE appears to be significantly more efficacious than MIE to facilitate improvements in cardiopulmonary fitness and body composition for colorectal cancer survivors in the short term. Given the clinical importance of improving cardiopulmonary fitness and body composition in oncology, our results present HIE as a time-effective therapeutic utility, which may lead to clinically meaningful improvements in colorectal cancer prognosis.

**Compliance with Ethical Standards**

This study was funded by Queensland Health (Remserv) (project number: 2013001802); no other conflicts of interest exist for this study. All procedures performed in the present study involving human participants were in accordance with the ethical standards of the Human Ethics Committee of the University of Queensland and with the 1964 Helsinki declaration and its later amendments. Written and informed consent was obtained from all individual participants included in the study.
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5.3 Review of Molecular Mechanisms linking IL-8 to Mitogenesis and Angiogenesis

Interleukin-8 and Mitogenesis in Colorectal Cancer

At the cellular level, cancer is characterised by cell proliferation exceeding that of cellular apoptosis. As such, in normal cellular functioning a delicate balance between proliferative and apoptotic factors exists and an imbalance towards the former can result in tumorigenesis. As previously described, IL-8 is a cytokine with proliferative qualities whose overexpression has been found to correlate with CRC and its progression[3, 64, 66]. Cancer cells, which have both CXCR1 and CXCR2 receptors, receive IL-8 via autocrine, paracrine, and endocrine avenues; cancer cells have been reported to express IL-8 which can then act in a autocrine fashion[3]. It has been documented that this transcription of IL-8 within cancer cells is derived from activation of binding factors including nuclear factor (NF)-kappa-B, activating protein (AP)-1, and polyomavirus enhancer A binding protein (PEA)-3, however the primary source of constitutive IL-8 production is via NF-kB and AP-1 binding[208-210]. In a review of the mitogenic effects of IL-8 on cancer cells, Zhu and Woll[3] illustrated this pathway in Figure 8.

![Diagram of IL-8 signaling pathway](image)

Sourced from Zhu and Woll[3]. Fig. 8. Signal Pathway of IL-8 and cross-talk between IL-8 and EGFR. Cancer cells constitutively transcribe IL-8 mainly through NF-kB and AP-1 binding in IL-8 promotor. Secreted IL-8 exerts its multiple functions on cells binding to its receptors CXCR1 and 2. IL-8 activates EGFR via ADAM and HB-EGF.[3]

This IL-8 can then bind to the CXCR1 and CXCR2 receptors on its parent cell or adjacent CRC cells, constituting autocrine or paracrine functioning. In addition to this source of IL-8, cancer cells
are able to exploit IL-8 in circulation that has been produced in remote cell bodies and deployed to the cancer site as a part of the immune response[3].

The binding of this IL-8 to CRC cells triggers a number of intracellular pathways, ultimately leading to proliferation[95]. The exact mechanisms underlying this phenomenon are not comprehensively understood, however, there has been significant research into the mitogen activated protein kinase (MAPK) signalling pathway (flow of this pathway is depicted down the left hand-side of Figure 8) [3]. Basis for this claim arises from the fact that within cancer cells including those of CRC, it has been found that magnitude of MAPK pathway activation correlates well with rates of cell proliferation[1, 67].

Further to this correlation, much research has been conducted into the mechanistic avenues at the crux of this observation[1, 3]. Initial CXCR1 and CXCR2 activation, and indeed activation of other G protein coupled receptors, is accompanied by transactivation of epidermal growth factor receptors (EGFR)[211, 212]. EGFR transactivation is facilitated by a disintegrin and metalloproteinase (ADAM)[95]. ADAMs are molecules located on the cell membrane enacting ectodomain shedding to prime numerous proteins and receptors by creating bioactive versions[213]. The ectodomain, domain portion of a protein that protrudes into the extracellular space allowing for binding to receptors on other cell membranes, is cleaved from the molecule leaving a soluble ligand capable of receptor activation on the host or adjacent cells[214]. This process of ectodomain shedding is required for EGFR activation (reviewed by Blobel[215]). In CRC cells, IL-8 activation of ADAM triggers the release heparin-binding EGF-like growth factor (HB-EGF), capable of causing EGFR phosphorylation[95].

Following activation of an EGFR, primes Ras for function by the exchange of its guanosine diphosphate for a guanosinetriphosphate molecule[96]. This bioactive Ras molecule can activate a kinase cascade that chronologically includes RAF proto-oncogene serine/threonine protein kinase, MAPK kinase, MAPK, and extracellular signal-regulated kinases (ERK) 1 and ERK2, triggering a variety of transcription factors, including the E2F family and activator protein (AP)-1, with the principal task of transcribing cell proliferation genes[1, 3, 96].

In the field of CRC, this is by far the most promising pathway with Itoh and colleagues [95] having examined the cascade of events in vitro in CRC cell lines with great success, confirming the ability for IL-8 to induce cell proliferation via the EGFR-MAPK pathway. This was done through using factors that would inhibitor various parts of this pathway and examining how it effected IL-8-induced cell proliferation in CRC cell lines[95]. The inhibitors used acted on the ADAM, EGFR,
and MAPK portions of the cascade and were all found to significantly inhibit cell proliferation, indicating the involvement of these factors in the pathway [95]. It was then the task of this experiment to examine certain processes within the postulated pathway. Confirmation of transactivation of EGFR arose as a transient increase of 4.5-fold in EGFR phosphorylation was observed in direct response to a treatment of 20ng/ml of IL-8[95]. To determine whether ADAM-cleavage was involved in this process, an ADAM inhibitor was applied to the experiment, negating all EGFR phosphorylation in response to IL-8 treatment[95]. To investigate whether HB-EGF is indeed the messenger from ADAM-cleavage to EGFR activation, HB-EGF neutralising antibodies were imparted upon the microenvironment and found to significantly hinder IL-8-induce cell proliferation[95]. The results of this study provide strong evidence for an important involvement of ADAM and EGFR which inturn leads to MAPK signalling in IL-8-induced mitogenesis. Another interesting finding of this study was that in trials without the treatment of an inhibitor there existed a dose-response relationship between IL-8 treatment and CRC cell proliferation[95]. Thus, as IL-8 expression increases there is a concomitant increase in cell proliferation, suggesting that IL-8 is an important mediating factor in this process[95].

Another postulated method for IL-8-induced mitogenesis is via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway[3]. Interestingly, cell proliferation is not a result of NF-κB, rather the Rho off-branch of the pathway, as seen in middle portion of Figure 8. Once IL-8 binds to either CXCR1 or CXCR2, the Rho-GTPase pathway is activated[81]. The binding of IL-8 to CXCR1 will cause rapid Rho-GTPase activation whilst CXCR2 binding results in delayed activation, either of which affect enzymatic cascades promoting the formation of phospholipase D (PLD)[81, 216]. PLD is an enzyme of the phospholipase family with proliferative qualities, thus playing a role in cancer[216]. PLD has two isoforms, PLD1 and PLD2, the former of which can be activated by the Rho-GTPase pathway[217]. Whilst there is some suggestion that Rho-GTPase activation can induce proliferation via this pathway there remains conjecture with no studies being conducted in CRC cell lines[216, 218, 219]. Foster and Xu[216] have reviewed this area extensively including further discussion of interactions PLD may have with other GTPase families and growth factors, other than IL-8, and how that can aid in cell proliferation.

IL-8 appears to be intrinsically linked with mitogenesis in CRC via transactivation of EGFR[1, 3, 95]. Through ADAM-cleavage, HB-EGF binds to EGFR eliciting a cascade of enzymes eventuating with the up regulation of many transcription factors, namely AP-1 and E2F, which induce cellular propagation[1, 3]. There also exists some evidence to support the notion that IL-8 could also induce PLD activation and thus mitogenesis however this is yet to be extensively
investigated[3, 216].

Interleukin-8 and Angiogenesis in Colorectal Cancer

One of the major mechanisms of cellular growth, in normal biological functioning and in cancerous tissue, is angiogenesis[97]. With the formation of new blood vessels comes a greater ability for delivery of nutrients, oxygen, growth factors, and other blood-borne elements such that supply equals demand[97]. Initiated very early in tumour life, angiogenesis is essential to the progression and growth of any tumour, including that of CRC[97-99]. CRC-induced angiogenesis is stimulated by angiogenic factors, such as IL-8, binding to receptors on endothelial cells in the microenvironment [99, 100]. The ability of IL-8 to exert its angiogenic potential on endothelial cells has been demonstrated both in vitro and in vivo[89, 101, 102]. Interestingly, the effectiveness of IL-8 to induce angiogenesis in endothelial cells is more pronounced in microvasculature than macrovasculature[103].

Endothelial cells have been documented to exhibit both CXCR1 and CXCR2 however there is suggestion that only the CXCR2 receptor that possess the ability to initiate angiogenic process[104, 105]. Any chemokine displaying the ELR motif has the potential to bind to CXCR2 and initiate this process, however the abundance of IL-8 produced by the surrounding CRC cells means that it is a potent angiogenic factor[104, 105].

The intracellular process driving angiogenesis is the same as that of proliferation previously outlined with the additionally property of control and direction[106]. Rather than proliferating in a random fashion as is the case in cancer cells, angiogenesis involves the systematic breakdown of the extracellular matrix to direct the proliferation of endothelial cells in certain directions and structures necessary for neovessel formation[107]. The extracellular matrix is broken down by a family of proteinases, matrix metalloproteinase (MMP), with the newly proliferated endothelial cells filling the void created[107, 220]. Of important interest is that MMPs, specifically MMP-2 and MMP-9, are up regulated in in vitro endothelial cells incubated with IL-8 further implicating IL-8 as an angiogenic promoter[107]. This phenomenon has been demonstrated in vivo in melanoma, prostate cancer, lung cancer, and Kaposi’s sarcoma[108-111]. Whilst no data for CRC exists, it is reasonable to assume that CRC-produced IL-8 would up regulate MMPs given that this occurs within endothelial cells in the microenvironment which would not differ between cancer types. Moreover, Ning et al[112] demonstrated in vivo that CRC cells transfected with IL-8 induced angiogenesis in the microenvironment however MMP expression was not measured.
In addition to tumour-produced IL-8 acting as a paracrine agent, there is also evidence to suggest that endothelial cells can produce IL-8 and work in an autocrine manner[221]. Endothelial cells, which exhibit toll-like receptors, have been observed to produce IL-8 thus creating the possibility for autocrine activation[222, 223]. Li, et al[221] examined this in vitro and observed that endothelial-produced IL-8 could perform in an autocrine manner and in turn mediate cell proliferation and angiogenesis.

As such, IL-8 is abundantly expressed in the CRC microenvironment and is a potent angiogenic stimulator. This, in conjunction with its integral role in facilitating proliferation of CRC cells, demands the investigation for interventions with the ability to manipulate this variable. One such method previously discussed in this review is exercise and physical activity.
References


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119. Croft, L., et al., *High-intensity interval training attenuates the exercise-induced increase


160. Galvao, D.A., et al., Compliance to exercise-oncology guidelines in prostate cancer


175. Lynch, B.M., et al., Modes of presentation and pathways to diagnosis of colorectal cancer


### BOX 5.2 Indications for Terminating Exercise Testing

**ABSOLUTE INDICATIONS**
- Drop in systolic blood pressure of $>10$ mm Hg from baseline\(^a\) blood pressure despite an increase in workload when accompanied by other evidence of ischemia.
- Moderately severe angina (defined as 3 on standard scale).
- Increasing nervous system symptoms (e.g., ataxia, dizziness, or near syncope).
- Signs of poor perfusion (cyanosis or pallor).
- Technical difficulties monitoring the ECG or systolic blood pressure.
- Subject's desire to stop.
- Sustained ventricular tachycardia.
- ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V\(_1\) or aVR).

**RELATIVE INDICATIONS**
- Drop in systolic blood pressure of $>10$ mm Hg from baseline\(^a\) blood pressure despite an increase in workload in the absence of other evidence of ischemia.
- ST or QRS changes such as excessive ST depression ($>2$ mm horizontal or downsloping ST-segment depression) or marked axis shift.
- Arrhythmias other than sustained ventricular tachycardia, including multifocal PVCs, triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias.
- Fatigue, shortness of breath, wheezing, leg cramps, or claudication.
- Development of bundle-branch block or intraventricular conduction delay that cannot be distinguished from ventricular tachycardia.
- Increasing chest pain.
- Hypertensive response (systolic blood pressure of $>250$ mm Hg and/or a diastolic blood pressure of $>115$ mm Hg).

ECG, electrocardiogram; PVC, premature ventricular contraction.

\(^a\)Baseline refers to a measurement obtained immediately before the test and in the same posture as the test is being performed.

### 5.5 Three Day Food Diary

*Please provide as much detail as possible and include all drinks.*  
*Keep in mind you will be required to follow this diet at 3 other time points during the study.*

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<thead>
<tr>
<th>Day</th>
<th>Breakfast</th>
<th>Snack</th>
<th>Lunch</th>
<th>Snack</th>
<th>Dinner</th>
<th>Snack</th>
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</table>
### 7 DAY ACTIVITY DIARY

*Record duration (in mins) that activity was completed for.
*Add any activity not already listed to ‘other’.

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5.7 Example of Heart Rate Graphs

Heart rate graphs from a single participant completing a single HIIT session (top panel) and a single participant completing a single MIT session (bottom panel) as recorded by Suunto Ambit2 S heart rate monitor (Suunto Oy, Vantaa, Finland)
### 5.8 Changes in \( \dot{V}O_2 \text{peak} \) in CRC survivors following an exercise intervention – A comparison of the present study and Sellar et al.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Present Study * (mL.kg(^{-1}).min(^{-1}))</th>
<th>Sellar et al. (^\wedge) (mL.kg(^{-1}).min(^{-1}))</th>
<th>4 week Change</th>
<th>Endpoint Absolute Change(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>21.1 (3.6)</td>
<td>22.5 (6.7)</td>
<td>-</td>
<td>3.0 (1.4 to 4.6)</td>
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<tr>
<td>4 weeks</td>
<td>26.7 (9.9)</td>
<td>-</td>
<td>6.1 (7.0)</td>
<td>8.0 (6.5)</td>
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<tr>
<td>8 weeks</td>
<td>27.0 (7.5)</td>
<td>25.5 (7.0)</td>
<td>-</td>
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<tr>
<td>12 weeks</td>
<td>-</td>
<td>25.5 (7.0)</td>
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</tbody>
</table>

*Values expressed as medians (IQR)

\(^\wedge\)Values expressed as means (95% confidence intervals)

\(^*\)Endpoint for present study = 8 weeks; endpoint for Sellar et al. = 12 weeks.
The Effects of High Intensity Interval Training on Interleukin-8 in Colorectal Cancer Survivors

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Co-Investigators:
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PhD Candidate, School of Human Movement Studies
Kate Bolam, BScApp (Hons)
PhD Candidate, School of Human Movement Studies
A/Prof David Jenkins, PhD, MSc, BA
Lecturer, School of Human Movement Studies
Dr Tina Skinner, PhD, BScApp (Hons)
Lecturer, School of Human Movement Studies
Professor Suzanne Chambers, PhD
Australian Research Council Future Fellow, Griffith University
Professor Jeff Dunn
CEO, Cancer Council Queensland
Dr Joanne Aitken
Director of Research, Cancer Council Queensland

Why are we conducting this study?
Research has found that people who have previously had Colorectal Cancer are at heightened risk of disease recurrence. Pleasingly, physical activity has been associated with a decrease in this risk, especially that of higher intensities. However researchers are yet to investigate how exercise training at different intensities can influence important biological markers of Colorectal Cancer. We wish to assess this in an effort to develop guidelines for the best type and intensity of exercise for the health of colorectal cancer survivors.

Are you eligible to participate in this study?
We require 15 males and females who have been diagnosed with Colorectal Cancer.
Participants must not have any musculoskeletal, neurological, respiratory, or cardiovascular conditions that prevent them from safely completing the exercise demands of the study. Participants will also be excluded if they are receiving pharmacological treatment to increase their insulin sensitivity and/or exogenous insulin therapy. In addition, volunteers will need to obtain consent from their doctor (GP) before participating in the study.

**What does the exercise program involve?**

Participants will be randomly assigned to one of two groups: (1) high intensity aerobic interval training or (2) moderate intensity aerobic continuous training. Both groups will exercise three times per week for 8 weeks. All sessions will be completed on stationary bicycles.

High intensity groups will exercise at 90% of their maximum capacity for 4x4 minute intervals, interspersed by 3 min periods of recovery at a lower intensity. Moderate intensity groups will exercise at 70% of their maximum capacity for the duration of the session (50 minutes). All high intensity interval exercise sessions will include a 10 min warm-up and 5 min cool-down. With warm-ups and cool-downs, each exercise session will last approximately 60 minutes in total.

All exercise sessions will be undertaken at the School of Human Movement Studies on The University of Queensland’s St Lucia campus. All sessions will be conducted in small groups of 3-4 participants under direct supervision of a qualified exercise physiologist.

The type of exercise training used in this study has been previously investigated in clinical populations including patients with heart failure and type 2 diabetes. No adverse events were reported in these trials; high intensity exercise training is safe to use in this population. Blood pressure, heart rate and perceived exertion will be monitored during exercise testing and training sessions to further ensure the safety of all participants.

**What will testing involve?**

*We will conduct a testing session before you start training, half way through your training (4 weeks), and at the end of your training (8 weeks) to track any differences. All testing will be completed by an accredited exercise physiologist trained to conduct each measure. Testing sessions take approximately 1.5 hours to complete and you will need to be 12-hours fasted prior to each session (water is fine).*

**Body composition**

- Height and body weight.

- Muscle and fat mass of the whole body will be measured by dual energy x-ray absorptiometry (DXA), a routine technique for the measurement of body composition. You will lie on a specially designed table (*as shown in the adjacent photo*) for approximately 7 minutes and a scanning arm will move above your entire body.

There is no pain or discomfort associated with these measures.
Cardiorespiratory (aerobic) fitness

- Aerobic fitness will be measured via a maximal cardiorespiratory fitness test. The test will involve cycling on an exercise bike for 10-15 minutes whilst you breathe through an apparatus that measures your oxygen consumption (as shown in the adjacent photo).

Blood Analysis: Biomarkers of colorectal cancer growth

- Blood will be sampled for us to assess biological markers that have been linked to Colorectal Cancer growth. All blood will be sampled and be analysed at The University of Queensland’s School of Human Movement Studies biochemistry laboratory by a qualified phlebotomist. You will be required to avoid food and drink (except water) for twelve hours before those sessions where your blood will be sampled.

Questionnaires

- Levels of self-reported physical activity will be assessed by the leisure score index from the Godin Leisure-Time Exercise Questionnaire that you can complete at home. Dietary information will be collected using a 3-day food diary.

Are there any risks associated with being involved in this study?

DXA uses very low energy x-rays to determine body composition and bone mass. The total dose associated with the scans you will undergo in this study is approximately 12 µSv. In comparison, an individual receives approximately 7 µSv for daily natural background exposure, 80 µSv for a return trans-Pacific flight, 100 µSv for a chest x-ray, and 2000 µSv for a lumbar spine x-ray. Therefore, although radiation is used in the scan, the amount of radiation is very small and the corresponding risk from participating in this study is extremely low.

It is possible that some initial muscle soreness may result from testing and training; however, all participants will undertake a warm-up prior to, and cool-down immediately following each session. In the event that an emergency occurs, medical assistance will be available from the university health service according to our established emergency procedures.

Lastly, the discomfort associated with the blood drawing procedures is minimal. There is a risk that bruising and infection may occur and that the arm might become sore. Risk of bruising or infection from the blood draws will be minimized because all blood will be sampled by a trained phlebotomist. The total amount of blood drawn during each testing session will not exceed 6mL, is equivalent to approximately 1 teaspoon. No syringes, lancets, needles or other devices capable of transmitting infection from one person to another shall be reused. All of these items, which are disposable, will be destroyed after each use. As an additional safeguard in preventing contamination, new disposable gloves will be worn for all blood draws. All contaminated items will be disposed of promptly in sharps containers.

All information will be strictly confidential and kept safely locked in a filing cabinet in the principal investigator’s office. Should publications result from this study, no
reference will be made to any individuals. There is no financial reimbursement for participating in this study. On completion of the intervention and measurements, a summary of study findings and individual results will be made available to all participants.

**PARTICIPATION IS VOLUNTARY AND SUBJECTS ARE FREE TO WITHDRAW FROM THIS STUDY AT ANY TIME, FOR ANY REASON.**

This study has been cleared by one of the human ethics committees of the University of Queensland in accordance with the National Health and Medical Research Council's guidelines. You are of course; free to discuss your participation in this study with project staff *(Gareth Hughes contactable on 0407 141 555)*. If you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924.

**Thank you for your interest in this study.**