THE UNIVERSITY OF HULL

Exercise Prehabilitation in Colorectal Cancer Surgery Patients: The Effects on Physical Functioning, Health Related Quality of Life and Markers of Cellular Protection

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List of Abbreviations, Acronyms and Symbols

1RM	1 repetition max
6MWT	6 minute walk test
95% CI	95% confidence intervals
95% LOA	95% limits of agreement
ACCP	American College of Chest Physicians
AET	Aerobic exercise training
APAF-1	Apoptosis protease-activating factor-1
AT	Anaerobic threshold
ATS	American Thoracic Society
BPM	Beats per minute
CABG	Coronary artery bypass graft
CAR	Cortisol awakening response
CAT	Catalase
cJNK	c-jun N-terminal kinase
cm	centimetres
CPET	Cardiopulmonary exercise test
CR	Cardiac rehabilitation
CRUK	Cancer Research UK
CuZnSOD	Copper zinc superoxide dismutase.
CWT	Circuit weight training.
ELISA	Enzyme linked immunosorbent assay
ERAS	Enhanced recovery after surgery
ES	Effect size
FBS	Fetal bovine serum
FTSTS	Five times sit to stand test
g	gravity
GHS	Global health status
$_{g}$Hsp72/32	Granulocyte Hsp72/32
GP	General Practitioner
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidised glutathione

H_2O_2	Hydrogen peroxide
HADS	Hospital and anxiety scale
HGD	Handgrip dynamometry
HIT	High-intensity interval training
HO	Haem-oxygenase
HO-1	Haem-oxygenase-1
HO-2	Haem-oxygenase-2
HRP	Horseradish peroxidase
HRQOL	Health related quality of life
HRR	Heart rate reserve
HSCIC	Health & Social Care Information Centre
Hsf	Heat shock factor
Hsps	Heat shock proteins
Hsp27	Heat shock protein 27
Hsp32	Heat shock protein 32
Hsp72	Heat shock protein 72
HUVEC	Human umbilical vein endothelial cells
IBD	Inflammatory bowel diseases
ICC	Intra-class correlation coefficient
IL-1	Interleukin-1
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
IMT	Inspiratory muscle training
IQR	Interquartile range
IRI	Ischaemia reperfusion injury
kg	kilograms
$_l$Hsp72/32	Lymphocyte expressed Hsp72/32
LOS	Length of hospital stay
m	metres
MDC_{95}	Minimum detectable change with 95% confidence intervals
MDT	Multidisciplinary team
MFR	Median fluorescence ratio
$_m$Hsp72/32	Monocyte expressed Hsp72/32
ml.beat	millilitres per beat

ml.kg.min	millilitres per kilogram per minute
MnSOD	Manganese superoxide dismutase
MOFS	Multiple organ failure syndrome
NACRT	Neoadjuvant chemo radiotherapy
NFκB	Nuclear factor kappa B
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
nm	Nanometre
$NO^{\cdot}$	Nitric oxide
NSCLC	Non-small cell lung cancer
O_2	Oxygen
O_2^{-}	Superoxide
$OH^{\cdot}$	Hydroxyl radical
$ONOO^{-}$	Peroxynitrite
p38 MAPK	p38 mitogen-activated protein kinase
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
Pmol	Picomole
PREHAB	Prehabilitation
QOL	Quality of life
RCT	Randomised controlled trial
RNS	Reactive nitrogen species
ROM	Range of motion
ROS	Reactive oxygen species
RR	Relative risk
SaO_2	Oxygen saturation
SCT	Stair climb test
SEM	Standard error of measurement
SIRS	Systemic inflammatory response syndrome
SOD	Superoxide dismutase
T-spine	Thoracic spinal
TGSH	Total glutathione
TBARS	Thiobarbituric acid reactive substances
TNF-α	Tumour necrosis factor-α
TNM	Tumour-nodes-metastases

TUG	Timed up and go test
UK	United Kingdom
VO_2	Oxygen consumption
VO_{2peak}	Peak oxygen consumption
WR_{peak}	Peak work rate
XO	Xanthine oxidase
µl	Microlitre

Abstract

Since being introduced in the late 1990's, enhancing recovery after surgery (ERAS) protocols have been promoted for inclusion in the care pathway for colorectal cancer patients scheduled for resection surgery (Gustaffson et al., 2012). This multimodal approach to stress management is thought to reduce the amount of surgical stress encountered; attenuating the debilitating effect surgery has on the patient and aiding subsequent recovery with a reduction in hospital length of stay reported (Lv et al., 2012). Although not currently included in ERAS, a period of pre-operative exercise training, known as PREHAB, has been proposed as a potential mechanism of improving the patient's pre-operative fitness ahead of surgery (Carli & Zavorsky, 2005). As no research currently exists into how feasible it would be to incorporate a period of PREHAB into the current NHS colorectal cancer care pathway in the United Kingdom, this thesis aimed to address this gap in the literature as well as investigate whether participation in PREHAB would alter physical functioning and health related quality of life (HRQOL) prior to surgery and improve post-operative recovery. The impact of PREHAB on upregulating the body's heat shock protein (Hsp) and glutathione defence systems was also explored.

The purpose of the first experimental chapter was to investigate through a questionnaire-based approach whether an interest in the potential use of PREHAB existed in the Hull and East Riding area and what were the perceived benefits and barriers to participation. Over 75% of respondents indicated they would be interested in PREHAB if awaiting surgery although a lack of time (62% of respondents), cost (46%) and work responsibilities (43%) were identified as the main barriers to participation. Having established an interest existed in PREHAB, the test-retest reliability and measurement error of the five tests of physical functioning (Timed up and go [TUG], five times sit to stand [FTSTS], 5 step stair climb [SCT], handgrip dynamometry [HGD] and 6 minute walk test [6MWT]) that would be used to assess the effectiveness of the PREHAB intervention was investigated. All five tests displayed excellent test-retest reliability (all ICCs: >0.90) with the standard error of measurement and minimum detectable changes at 95% as a percentage of the mean ranging from 2.3% to 5.2% and 6.3% to 16.1% respectively.

In the third experimental chapter, a randomised controlled pilot trial investigating a novel PREHAB intervention based on the joint-by-joint approach to training was conducted in colorectal cancer patients. The ineligibility of 43% (84 out of 198) of patients due to insufficient time to scheduled surgery (< 2 weeks) and subsequent poor consent rate of eligible patients (18.4%; 21 out of 114 patients) indicates implementing PREHAB into the current colorectal care pathway would be difficult. However, improvements in TUG, SCT and 6MWT performance were observed in all nine patients randomised to PREHAB (all $p < 0.05$), a result not replicated in the control group (improved performance at reassessment: TUG: 2 out of 9; SCT: 3 out of 9; 6MWT: 4 out of 9). This suggested that despite the limited time from recruitment to surgery (median PREHAB period: 23 [IQR: 14] days), the PREHAB programme was sufficient to improve physical functioning in these patients. There was however no significant difference in length of hospital between the two group (Control: 8 [5] days; PREHAB: 10 [7] days).

In the final two experimental chapters, the effects of the PREHAB intervention on basal Hsp72 and Hsp32 expression and the glutathione defence system was explored although the low recruitment rates previously described limited the results. No changes were evident in Hsp32 or Hsp72 expression; or in total glutathione or GSH/GSSH ratio for either group during the pre-operative period. There was a potential time of day effect for monocyte Hsp72 as expression decreased in 13 out of 16 at pre-operative reassessment ($p < 0.05$) thus potentially masking any adaptations to have taken place. Furthermore, attempts to establish whether PREHAB altered Hsp72 inducibility were not possible due to inadequate viable samples being available. Given the limited sample size, definite conclusions were difficult to make although it was plausible the absence of change in Hsp72, Hsp32 and glutathione following PREHAB was due to insufficient stimulus being present given the often low to moderate intensity of the intervention.

The findings of this thesis highlighted the issues regarding the limited time available in the pre-operative period that would need to be overcome in order to practically implement a PREHAB intervention into the current NHS colorectal cancer care pathway. Despite this, the improvements observed in physical functioning following PREHAB suggests if the intervention could be adapted to a cost-effective home-based programme it may be a viable addition to the ERAS programme.

Chapter 1: Introduction

In 2013, 41,112 new cases of colorectal cancer were diagnosed in the United Kingdom (UK) making it the fourth most prevalent cancer (Cancer Research UK [CRUK], 2016). Statistics released within the National Bowel Cancer Audit Report 2015 indicated that during the previous five years, around two thirds of the patients diagnosed with colorectal cancer received surgical intervention to remove the tumour (Health & Social Care Information Centre [HSCIC], 2015). Whilst the surgical removal of the tumour is recognised as the most effective curative form of treatment, especially in locally confined cancers (HSCIC, 2015), undergoing major surgery presents its own challenges to the patient. From a metabolic standpoint, the trauma associated with major surgery can trigger a cascade of endocrinological, immunological and haematological responses, all of which can have a detrimental effect on the patient's recovery (Carli, 2015; Desborough, 2000). Following elective abdominal surgery, an elevated level of surgically induced inflammation (as measured by pro-inflammatory cytokine interleukin-6; IL-6) has been associated with both a rapid decline in muscular endurance and increased self-reported fatigue 2-4 days post-surgery (Bautmans, Njemini, De Backer, De Waele, & Mets, 2010). The detrimental effects of surgery (e.g. increased inflammation and fatigue, decreased muscular endurance) are further accentuated in those patients of an older age (Bautmans et al., 2010). Additionally, the psychological stresses caused by both the diagnosis of cancer and the prospect of imminent surgery can impact on the patients' health related quality of life (HRQOL) and mental well-being both prior to and during the subsequent recovery from the procedure (Alacacioglu et al., 2010; Carlsson, Berndtsson, Hallen, Lindholm, & Persson, 2010; Gray et al., 2014).

In an attempt to reduce the patient's surgical stress response, in the late 1990's, Kehlet and Colleagues pioneered an approach known as fast track surgery in colorectal patients (Kehlet & Wilmore, 2002; Wilmore & Kehlet, 2001). This multimodal approach to stress management, also known as 'Enhanced Recovery after Surgery' (ERAS), has now been widely accepted, with modified guidelines introduced for use within other surgical specialties (Cerantola et al., 2013; Lassen et al., 2012; Mortensen et al., 2014). ERAS incorporates a number of pre-, peri and post-operative strategies, each of which contribute to reducing the physiological, physical and/or psychological burden on the patient (Fearon et al., 2005; Kehlet & Wilmore, 2002), and has been reported to reduce post-operative length of hospital stay (LOS) without increased risk of patient morbidity and mortality (Lv, Shao, & Zhou, 2012; Walter, Collin, Dumville,

Drew, & Monson, 2009). Given these reported benefits, in the UK, adoption of the principles of ERAS is promoted within the National Health Service (NHS) although variations in the protocols adopted are likely to exist between centres. Recent colorectal ERAS guidelines have identified at least 20 different elements for potential inclusion within ERAS pathways (Fearon et al., 2005; Gustafsson et al., 2012; Steenhagen, 2016), however one strategy not currently included is the use of pre-operative exercise training, also known as prehabilitation or PREHAB (Carli & Zavorsky, 2005).

There is growing research interest in the potential role PREHAB may play in preparing patients for colorectal resection surgery (Carli et al., 2010; Li et al., 2013; West et al., 2015), as well as in other surgical procedures including lung resection surgery (Jones et al., 2007) and coronary artery bypass graft surgery (Arthur, Daniels, McKelvie, Hirsh, & Rush, 2000; Sawatzky et al., 2014). Low pre-operative fitness has been inversely associated with poorer post-operative outcomes in major elective abdominal surgery, with an anaerobic threshold (AT) of less the 11 ml.kg.min indicative of patients being at high risk of post-operative morbidity (Snowden et al., 2013) and mortality (Older, Hall, & Hader, 1999; Wilson, Davies, Yates, Redman, & Stone, 2010). It stands to reason therefore that strategies to improve pre-operative physical fitness have the potential to improve post-operative outcomes.

Whilst studies by Gillis et al. (2014); Li et al. (2013) (both colorectal surgery), and Sawatzky et al. (2014) (CABG surgery) have reported both improved pre-operative functional capacity (as measured using the 6 minute walk test; 6MWT) following PREHAB as well as a quicker return of functioning post-surgery, whether this results in fewer post-operative complications or reduced LOS remains unclear. Moreover, the majority of literature to date relating to PREHAB in colorectal cancer patients has used interventions lasting at least 4 weeks (Carli et al., 2010; Kim do, Mayo, Carli, Montgomery, & Zavorsky, 2009; Li et al., 2013). In the UK however, NHS requirements dictate that the initiation of cancer treatment should commence within a maximum of 31 days from diagnosis (Baker & Nakatudde, 2015). It is therefore likely the time available to apply a PREHAB intervention prior to surgery would be less than previous observed (Carli et al., 2010; Kim do et al., 2009; Li et al., 2013). This raises questions as to how practically PREHAB could be applied into a UK secondary care setting.

If a PREHAB intervention could be adopted within the colorectal care pathway, the benefits achieved may not be limited to those on a physical and psychological level. During major intra-abdominal surgery, the generation of oxidative stress represents a major challenge (Pappas-Gogos et al., 2013). A number of complex protective mechanisms exist against oxidative stress, including the antioxidant defence system (Valko et al., 2007) and a series of stress proteins known as heat shock proteins (Hsps) (Benjamin & McMillan, 1998). Glutathione, the predominant endogenous non-enzymatic antioxidant in the body, scavenges free radicals and other reactive oxygen species (ROS) (Maher, 2005) and has been found to be elevated following six weeks of aerobic and/or resistance training (Elokda & Nielsen, 2007). Furthermore, Hsps which are induced in response to a number of stressors including heat exposure (Lovell, Madden, Carroll, & McNaughton, 2007), oxidative stress (Khassaf et al., 2003) and ischaemia (Richard, Kaeffer, & Thuillez, 1996), function as molecular chaperones, assisting with protein degradation, and regulating cell death (Kiang & Tsokos, 1998). The prior induction of heat shock protein 72 (Hsp72), the most highly inducible and researched member of the Hsp family, as a result of exposure to a non-lethal stressor such as heat and/or exercise is proposed to provide cross-tolerance when subjected to a subsequent more severe stressor (McClung et al., 2008; Taylor et al., 2012). Although less researched in exercise, similar protective properties also exist for small Hsps, heat shock proteins 27 (Hsp27) and heat shock protein 32 (Hsp32) (Gozzelino, Jeney, & Soares, 2010; Mymrikov, Seit-Nebi, & Gusev, 2011). If these systems could be upregulated prior to major surgery through the use of PREHAB, this may represent additional protection against surgically induced inflammation and oxidative stress.

The purpose of this thesis therefore was to examine the implementation of an inclusive PREHAB intervention within the current colorectal resection surgery pathway of a local NHS trust. This was with the aim of investigating both the feasibility of implementing such a programme and exploring the potential effects it may have on the patient from a physical, physiological and psychological perspective. The primary objectives of the research therefore were as follows:

- To explore the feasibility of delivering a supervised PREHAB intervention within the standard care pathway of patients awaiting major colorectal surgery in a local NHS trust (chapters 3 and 5).

- To investigate the effects of PREHAB on pre-operative physical functioning, HRQOL and clinical outcomes in colorectal cancer patients (chapters 4 and 5).
- To examine the effect of PREHAB on circulatory markers of stress with a particular focus on leukocyte Hsp72 (chapters 6 and 7).
- To examine the influence of basal Hsp72 expression on further induction in response to subsequent stress response (chapter 7).

Chapter 2: Literature Review

2.1. Overview of colorectal cancer in the United Kingdom

Within the UK, colorectal cancer is the fourth most prevalent cancer; accounting for over 12% of all new cases in 2013 (CRUK, 2016a). Furthermore, unlike prostate cancer (male only) and breast cancer (predominantly females), colorectal cancer is not gender specific, as emphasised by its position as the third most common cancer in both males and females (CRUK, 2016a). Encompassed within the general definition of colorectal cancer are malignancies of both the colon and rectum. This can be further refined according to the region of the colon (caecum, appendix, ascending colon, transverse colon, descending colon and sigmoid colon) or rectum (rectosigmoid junction and rectum) where the tumour is located. Survival from colorectal cancer is related to stage of tumour development at the time of diagnosis. In the UK, the staging of the tumour is now based on the tumour-nodes-metastases (TNM) classification (Edge, Byrd, Compton, Fritz, Greene, & Trotti, 2010; Taylor, Garcia-Aguilar, & Goldberg, 2002). This classification displayed in Table 2.1 identifies the size of the primary tumour, whether any of the surrounding lymph nodes are affected and whether any distant metastatic spread has occurred (Taylor et al., 2002). The stage of cancer development at the time of diagnosis can therefore influence the likely five year survival rate following surgery. Patients diagnosed with a stage 1 tumour (e.g. T1 or 2;N0;M0) have a 93% chance of living for five years or more, however this declines as the stage of cancer develops with just 6% expected to live five years or more with stage 4 (Any T; Any N; M1) cancers (CRUK, 2016b).

Table 2.1: Definitions of TNM classification (adapted from AJCC Cancer Staging Manual, Seventh Edition, by Edge et al, 2010)

Primary Tumour		**Regional Lymph Nodes**		**Distant Metastasis**	
TX	Primary tumour cannot be assessed	NX	Regional lymph nodes cannot be assessed	MX	Distant metastasis cannot be assessed
T0	No evidence of primary tumour	N0	No regional node metastasis	M0	No distant metastasis
Tis	Carcinoma *in situ*	N1 - N3	Increasing involvement of regional lymph nodes	M1	Distant metastasis
T1 - T4	Increasing size and/or local extent of the primary tumour				

2.1.1. Non-modifiable risk factors

The risk of developing colorectal cancer is more prevalent within an ageing population. In 2012, 83% of new UK cases of colorectal cancer were diagnosed in individuals aged over 60 years of age (CRUK, 2016a). Overall incidences of colorectal cancer within the UK tend to have a slight male predominance with 56% of the 41,112 new cases reported in 2013 occurring in males (CRUK, 2016a). However when assessed according to age, of the new cases diagnosed in individuals aged 60-75 years, over 60% of new cases were diagnosed in males (60-64 years: males 62%; 65-69 years: males 62% 70-74 years: males 60%). Moreover, it is only after 85 years that colorectal cancer begins to have a female predominance (Figure 2.1; CRUK, 2016a). Gender also appears to influence the location of colorectal cancer with a higher percentage of rectum (31.5% vs. 23.1%) and sigmoid colon (23.1% vs. 20.4%) cancer cases reported in males than females and cases of caecum (17.2% vs. 12.2%) and ascending colon cancer (9.8% vs. 7.3%) more prevalent in females (CRUK, 2016a).

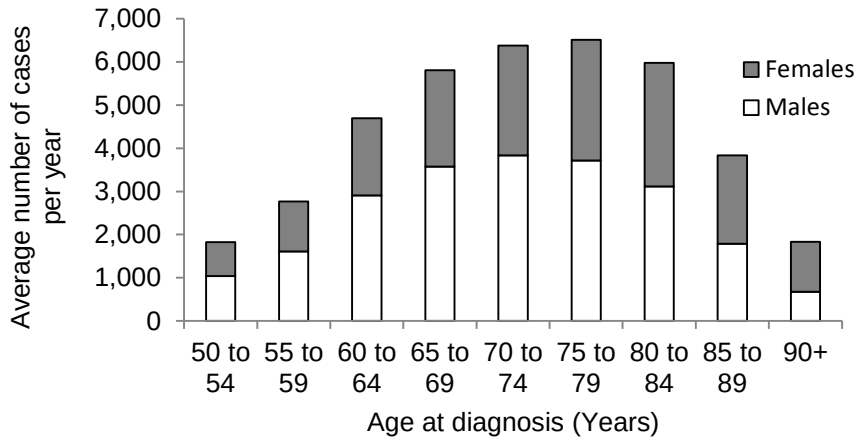


Figure 2.1: Mean number of incidences of colorectal cancer in 2011 - 2013 according to age and gender. Adapted from data presented by Cancer Research UK (CRUK, 2016a).

The presence of pre-existing medical conditions can also increase the risk of colorectal cancer developing. Individuals with a history of inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease are at increased risk of developing colorectal cancer (Lutgens et al., 2013). A recent meta-analysis suggested individuals' with IBD are 70% more at risk of developing colorectal cancer than the general population (Lutgens et al., 2013). Additionally, the risk is increased based on the longer the individual has suffered from the disease, the extent of the condition and in those

with a diagnosis at a young age (Lutgens et al., 2013). Meta-analyses by Jiang et al. (2011) and Luo, Cao, Liao, and Gao (2012) reported a 27% increase in the relative risk (RR) of developing colorectal cancer in individuals with diabetes mellitus. Moreover, the association was stronger in males (RR: 1.47; 95% confidence intervals [95% CI]: 1.15 to 1.86) than in females (RR: 1.08; 95% CI: 1.00 to 1.17) (Luo et al., 2012). The formation of adenomatous polyps in the colon increases the risk of developing colorectal cancer as although they are initially benign growths, they can act as precursor lesions for the development of colorectal cancer (Botteri, Iodice, Bagnardi, Raimondi, Lowenfel and Maisonneuve (2008). Between 5-10% of colorectal cancer cases are related to familial syndromes with ademomatous polyposis and hereditary non-polyposis colon cancer the most common (Hardy, Meltzer, & Jankowski, 2000).

2.1.2. Modifiable risk factors

Several factors related to lifestyle choices are associated with the risk of developing colorectal cancer. Meta-analyses by Botteri et al., (2008) and Liang, Chen, and Giovannucci (2009) supported the association between smoking and the development of colorectal cancer (RR: 1.15-1.20) compared to non-smokers. Furthermore, smoking has been reported to increase the risk of adenomatous polyps developing, a known colorectal cancer precursor (Botteri et al., 2008; Shrubsole et al., 2008). Excessive alcohol consumption has been associated with up to a 41% increased risk of developing colorectal cancer (Cho et al., 2004; Huxley et al., 2009) with acetaldehyde, a carcinogenic by-product of alcohol metabolism thought to be a causative agent (Haas, Ye, & Lohr, 2012). In addition, both cigarette smoking and excessive alcohol consumption are associated with not just an increased risk but also the earlier onset of colorectal cancer development (Acott, Theus, Marchant-Miros, & Mancino, 2008).

A high dietary intake of red meat and processed meat is reported to increase the risk of colorectal cancer by up to 22% compared to individuals with a low intake (RR: 1.22; 95% CI: 1.11 to 1.34 (Chan et al., 2011) although the risk is more pronounced for rectal cancer (Larsson & Wolk, 2006). A potential mechanism for this increased risk is due to increased carcinogenesis induced by elevated haem iron levels (Santarelli, Pierre, & Corpet, 2008). In contrast, it has been suggested that individuals' with high levels of dietary fibre intake have a 10% reduced risk (RR: 0.90; 95% CI: 0.86 to 0.94) of

developing colorectal cancer (Aune et al., 2011) with increased faecal bulk and reduced colonic transit time both linked as potential contributing factors (Swan, 2006).

There appears to be an inverse relationship between lifelong physical activity and colorectal cancer although due to study heterogeneity, results remain inconclusive. Meta-analyses by Boyle, Keegel, Bull, Heyworth, and Fritschi (2012); Harriss et al. (2009); Huxley et al. (2009) and Wolin, Yan, Colditz, and Lee (2009) all reported the risk of colon cancer reduced by up to 24% with increased levels of lifelong physical activity, however the same association was less clear between lifelong physical activity and rectal cancer (Harriss et al., 2009).

2.1.3. Treatment options

The primary treatment for colorectal cancer is surgery. For colon cancer, a procedure known as a colectomy is performed whereby the relevant section of the colon containing the tumour is removed along with an adequate margin of the surrounding tissue and the lymphatic vessels (NHS, 2014). Following the excision of the cancer, either an anastomosis connecting the proximal and distal ends of the colon is constructed or the colon is redirected out of an opening in the abdomen forming a stoma, which allows the colon time to recover (NHS, 2014). Depending of the staging of the cancer, the individual may then undergo adjuvant chemotherapy post-surgery (National Institute for Health and Care Excellence [NICE], 2011).

In rectal cancer, whether the patient is classified as being low, medium or high risk based on factors such as the stage of the tumour and lymph node status can determine the course of treatment the individual receives. Current guidelines from NICE (2011) recommend that individuals with low-risk operable rectal cancer have the tumour surgically removed immediately. As with colon cancer, surgery involves the removal of the tumour along with a margin of rectal tissue that is free from cancerous cells. The surrounding mesentery which contains the lymphatic vessels that supply the rectum is also removed in a procedure known as a total mesenteric excision. Those classified with moderate to high risk operable rectal cancer however may be offered neoadjuvant chemoradiotherapy (NACRT) prior to surgery in an attempt to shrink the tumour prior to excision (NICE, 2011).

2.1.4. Side effects to chemotherapy/chemoradiotherapy

Neoadjuvant chemoradiotherapy can play a positive role in the treatment of colorectal cancer pre-operatively by restricting the growth and potentially even shrinking the tumour thus improving its operability (NICE, 2011). Post-operatively, the risk of reoccurrence can be reduced in curable cancer whilst in advanced cases, the tumour may be shrunk and symptoms reduced, potentially extending life expectancy. Despite these benefits, NACRT can often also prove to have a debilitating effect on the patient. Cancer-related fatigue is a consequence of the disease, reportedly affecting over 70 % of patients, with NACRT a leading contributory cause (Mock, 2001; Morrow, Andrews, Hickok, Roscoe, & Matteson, 2002). This can often impact on the patient, resulting in impaired physical functioning and a subsequent decline in HRQOL (Ahlberg, Ekman, Gaston-Johansson, & Mock, 2003). The symptoms can continue to persist for a long time after treatment has finished, with symptoms of fatigue reported in approximately one third of cancer survivors (breast, colorectal and prostate) up to 6 years post treatment (Jones et al., 2016). This can impact on the patient physically and mentally as well as emotionally (Ahlberg et al., 2003). The effects of cancer related fatigue however have been reported to be attenuated through the implementation of exercise training during treatment (Cramp & Byron-Daniel, 2012; Meneses-Echavez, Gonzalez-Jimenez, & Ramirez-Velez, 2015), highlighting the potential beneficial role physical activity and exercise may play during cancer treatment.

More recently the detrimental effect NACRT can have on cardiorespiratory fitness has been reported. West et al. (2014a) reported patients undergoing NACRT prior to surgery for rectal cancer experienced a clinically significant reduction in cardiorespiratory fitness. Performing a cardiopulmonary exercise test (CPET) two weeks prior to and seven weeks post a 5 week course of NACRT, decreases in peak oxygen uptake (VO_{2peak}) (18.1 vs. 16.7 ml.kg.min), VO_2 at estimated lactate threshold (12.1 vs. 10.6 ml.kg.min), oxygen pulse at peak (11.2 vs. 10.1 ml.beat) and oxygen pulse at estimated lactate threshold (8.7 vs. 8.1 ml.beat) were observed. Similar results have been found in patients four weeks post neoadjuvant chemotherapy for oesophagogastric cancer by the same research group (Jack et al., 2014). Observational studies by West et al. (2014b; 2014c) have demonstrated an association between increased postoperative morbidity and individuals with lower VO_2 at estimated lactate threshold, VO_{2peak} or oxygen pulse at estimated lactate threshold in patients undergoing

either major colonic or rectal surgery. This potentially demonstrates the clinical implications that the reduced cardiorespiratory fitness associated with NACRT may have on subsequent post-operative outcomes.

2.1.5. Complications

The time spent in hospital following major surgery is often influenced by the occurrence of post-surgical medical and/or surgical complications (Table 2.2). Surgical complications refer to complications whose origins potentially relate to the surgical technique and procedure whereas medical complications may be more reflective of patient factors (Lee et al., 2013). It does however need noting the surgical and medical complications are not necessarily exclusively separate with surgical complications having the potential to trigger subsequent medical complications (Lee et al., 2013). A number of factors can influence the likelihood of complications occurring. For example, surgical complications have been associated with factors such as the surgeon's experience and duration of surgical procedure meanwhile individuals with impaired aerobic fitness are potentially more predisposed to medical complications (Older et al., 1999; Wilson et al., 2010). Certain specific risk factors are more predictive of certain complications than others (Kirchhoff, Clavien, & Hahnloser, 2010). For the purpose of this thesis, only a brief overview of the main complications associated with colorectal surgery is included. Detailed reviews covering post-operative complications have previously been published (Kehlet, 1997; Kirchhoff et al., 2010) on specific complications including post-operative ileus (Holte & Kehlet, 2000), anastomotic leaks (Fujita, Torashima, Kuroki, & Eguchi, 2014; Pommergaard et al., 2014) and sepsis (Monkhouse, 2006).

Table 2.2: Examples of surgical and medical complications

Medical complications	Surgical complications
Cardiac complications	Ileus
Pulmonary complications	Anastomotic leak
Thromboembolic complications	Surgical site infection
	Wound dishension
	Systemic Inflammatory Response syndrome
	Sepsis

2.1.6. Anastomotic leakage

As described in section 2.1.3, following the excision of the tumour, the remaining ends of the colon and/or rectum may be re-joined by forming an anastomosis. If the anastomosis fails an anastomotic leak may occur. Anastomotic leakage, which has been reported to occur in between 1.5% to 16% of colorectal surgery patients (Akasu et al., 2010; Damrauer, Bordeianou, & Berger, 2009; Khan et al., 2008; Smith, King, Lane, & Thompson, 2003; Sorensen et al., 1999), is potentially one of the most serious complications encountered, with mortality rates ranging from 0.8% up to as high as 27% having previously been reported (Akasu et al., 2010; Buchs et al., 2008; Damrauer et al., 2009; Thornton et al., 2011). Considered to be of multi-factorial origin, numerous risk factors have been linked with the occurrence anastomotic leakage and subsequent patient mortality, including site of tumour (colon/rectum), male gender, surgeons experience and prior chemo radiotherapy (Fujita et al., 2014; Thornton et al., 2011). In the event of an anastomotic leak, the potential consequences can be grave. Colorectal cancer patients experiencing anastomotic leakages are at increased risk of reoperation, the need for a permanent stoma and local reoccurrence of the disease (Daams, Luyer, & Lange, 2013; Pommergaard et al., 2014).

2.1.7. Ileus

Post-operative ileus is defined as a transient non-mechanical blockage in the intestine as a result of impaired gastrointestinal tract peristalsis that can occur following major surgery (Holte & Kehlet, 2000). In major gastrointestinal surgery it is considered an unavoidable consequence of surgery occurring in up to 25% of patients (Su'a et al., 2015). The duration of post-operative ileus is considered dependent on the segment of the gastrointestinal tract affected. The colon is most susceptible to post-operative ileus with an average paralytic duration of 48 to 72 hours compared to 0 to 24 hours for the small intestine and 24 to 48 hours for the stomach. The development of post-operative ileus has been associated with a number of factors including duration of surgery, degree of intestinal handling and severity of the inflammatory response encountered (Holte & Kehlet, 2000; Kirchhoff et al., 2010).

2.1.8. Surgical site infection

Given the function of the colon, colorectal surgery is often considered as a clean-contaminated procedure at best (Kirchhoff et al., 2010). Despite precautions being in place, major resection surgery of the colorectal tract can expose the peritoneal cavity and surrounding wound surfaces to the unclean microenvironment within thus increasing the risk of contamination and subsequent infection (Kirchhoff et al., 2010). Incidence rates ranging from as low 4% up to 25% for surgical site infection have been reported following colorectal surgery (Blumetti et al., 2007; Kurz et al., 2015; Tang et al., 2001) with open surgery, rectal surgery, male gender, the individual surgeon and need for a blood transfusion all identified as potential risk factors (Boni et al., 2006; Tang et al., 2001).

2.1.9 Systemic inflammatory response syndrome and sepsis

Systemic inflammatory response syndrome (SIRS) and sepsis represent serious complications to patients in general surgery (Moore, Moore, Jones, Xu, & Bass, 2009) . Systemic inflammatory response syndrome is defined as the pathophysiological response to a non-specific insult that can be of either infectious or non-infectious origin (Bone et al., 1992). At least two of the criteria displayed in Table 2.3 need to be identified at the same time for a diagnosis of SIRS to be given (Bone et al., 1992). Sepsis has been defined as SIRS when a known infectious source is confirmed (Bone et al., 1992; Levy et al., 2003). The chances of SIRS or sepsis developing are increased in the presence of other surgical complications such as anastomotic leakage and surgical site infections (Monkhouse, 2006). In its most severe form, sepsis can cause mental confusion, multiple organ failure syndrome (MOFS) and death (Bone et al., 1992).

Table 2.3: Criteria for defining systemic inflammatory response syndrome

Temperature	>38 °C or <36 °C
Heart rate	>90 beats per minute
Respiratory rate	>20 breaths per minute or $PaCO_2$ < 4.27 kPa
White cell count	>12,000//mm^3 or <4000/mm^3 or > 10% immature neutrophils

Adapted from Bone et al. (1992)

2.1.10. Medical complications

The main medical complications following major colorectal surgery can be broken down into three categories: cardiac complications, pulmonary complications and thromboembolic complications. Myocardial infarction represents the major cardiac complication, with individuals with pre-existing cardiac risk factors prior to surgery at greater risk (Arora, Velanovich, & Alarcon, 2011). An impairment of pulmonary function has been associated with the development of pulmonary complications such as atelectasis and pneumonia following surgery. Contributing factors attributed to pulmonary complications include prolonged periods in the supine position both during surgery and in the post-operative recovery period as well as the use of general anaesthesia during surgery (Kehlet, 1997). Thromboembolic complications can manifest after surgery as a result of increased hypercoagulation, impaired fibrinolysis and reduced lower extremity blood flow culminating in the development of deep vein thrombosis or pulmonary embolisms (Kehlet, 1997).

2.2. Surgical stress response

As a result of the acute trauma that major surgery places on the body, a number of endocrinological, immunological and haematological effects are stimulated (Desborough, 2000) with the severity of the surgical stress response related to the magnitude of the surgical trauma that occurred (Carli, 2015; Giannoudis, Dinopoulos, Chalidis, & Hall, 2006). An upregulation in the release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6 occurs activating an immune response known as the systemic inflammatory response (Carli, 2015). Whilst this stress response is the body's natural defence against the trauma caused by the surgery, the effects can have a detrimental effect on the patient, manifesting in a number of forms; including pain, tissue damage, post-operative complications and cognitive dysfunction (Carli, 2015).

The development of acute post-operative insulin resistance is now recognised as a potential consequence of undergoing a major surgical procedure (Thorell et al, 1999b), representing a significant physiological challenge to post-operative recovery (Carli, 2015). Reduced insulin sensitivity impacts on the metabolism of carbohydrates as peripheral glucose uptake, primarily by muscles, is inhibited by altered insulin

stimulated glucose transporter type-4 translocation (Thorell et al., 1999a). This impaired glucose uptake can result in the catabolic breakdown of muscle protein, causing weight loss and muscle wasting within a few days of undergoing surgery especially in individuals who remain bed bound (Gustafsson et al., 2012). Another important component to surgical stress is the potential occurrence of oxidative stress following surgery, with the gastrointestinal tract, in particular, susceptible to cellular damage as a result of oxidative stress (Anup & Balasubramanian, 2000).

2.2.1. Oxidative stress, free radicals and reactive oxygen and nitrogen species

Oxidative stress has been defined as a change in the balance between oxidants and antioxidants resulting in the potential for cellular damage (Halliwell & Gutteridge, 2007). At rest, the body maintains a delicate homeostatic balance between oxidants and antioxidants however when subjected to a variety of stresses including physical trauma, excessive exercise or exposure to heat/cold this balance can be disturbed (Valko et al., 2007). This can be as a result of a decline in the antioxidant defence system and/or through an increased production of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Halliwell & Gutteridge, 2007). A free radical has been defined as a molecule containing one or more unpaired electrons that is capable of independent existence (Halliwell & Gutteridge, 2007). Reactive oxygen species and RNS are molecules that promote oxidation reactions with other molecules such as lipids, proteins and DNA as a result of their molecular instability (Gomes, Silva, & de Oliveira, 2012). These ROS and RNS react with each other, forming other reactive species and these interactions are depicted in Figure 2.2.

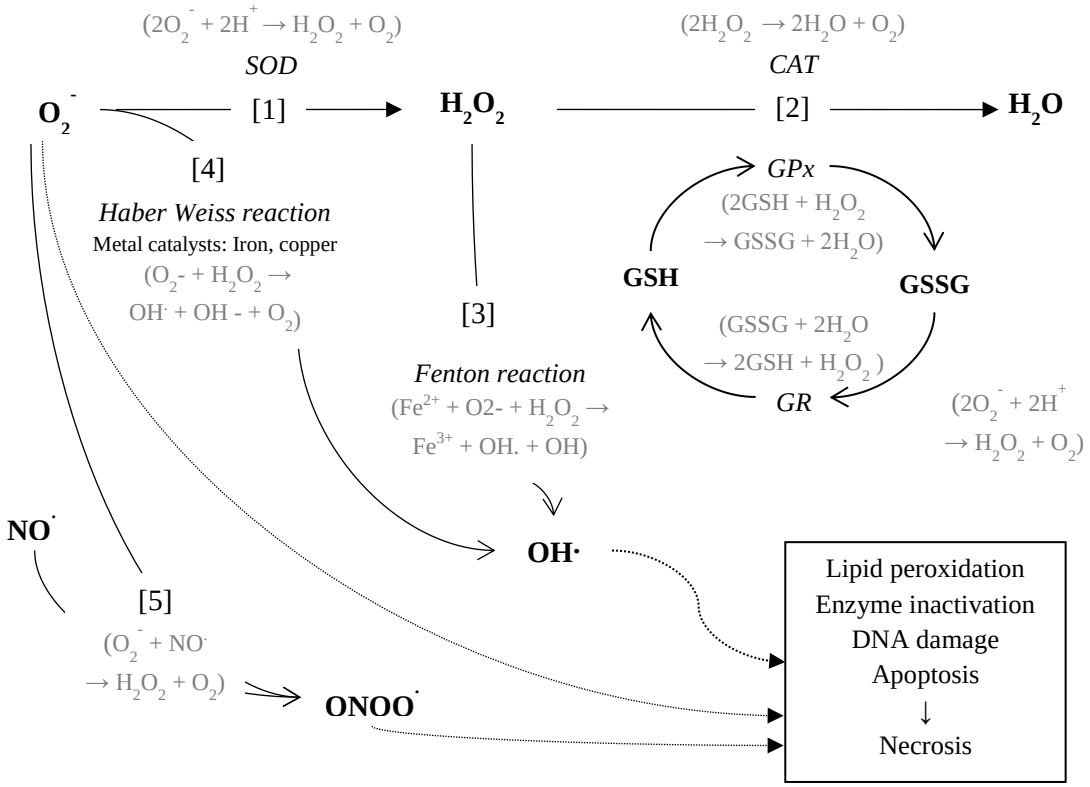


Figure 2.2: Production of reactive oxygen and nitrogen species. [1] Superoxide (O_2^-), produced as a by-product of a number of metabolic processes, is catalysed by antioxidant enzyme superoxide dismutase (SOD) forming hydrogen peroxide (H_2O_2). [2] The removal of H_2O_2 is facilitated by enzymatic enzymes glutathione peroxidase (GPx) or catalase (CAT). [3] If not neutralised, H_2O_2 can form the highly reactive hydroxyl radical ($OH^\cdot$) through the metal catalysed Fenton reaction, whilst O_2^- can form both [4] $OH^\cdot$ via the Haber-Weiss reaction or [5] peroxynitrite ($ONOO^-$) through its interaction with nitric oxide ($NO^\cdot$); [6] a consequence of which can include lipid peroxidation, and DNA damage. Adapted from Newsholme et al. (2011)

In low to moderate concentrations, the production of ROS and RNS may act in a number of cellular signalling pathways including stimulating the upregulation of important protective systems such as the antioxidant defence system and a family of protective stress proteins known as heat shock proteins (Hsps) (Valko et al., 2007). As the level of oxidation increases however, the effects on the cell may become deleterious potentially resulting in cellular damage to DNA, proteins and lipids, senescence or even cell death (Halliwell & Gutteridge, 2007). Elevated levels of oxidative stress have often been observed in a number of pathological conditions such as cardiovascular disease (Lee, Margaritis, Channon, & Antoniades, 2012), Alzheimer's disease (Wang et al., 2014) and cancer (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). Moreover, an individual's lifestyle can also contribute to the presence of circulatory oxidative stress with levels of habitual physical activity (Karolkiewicz et al, 2003), smoking (Mons,

Muscat, Modesto, Richie Jr, & Brenner, 2016; Yanbaeva, Dentener, Creutzberg, Wesseling, & Wouters, 2007) and alcohol consumption (Kahraman, Cakar, & Koken, 2012) all reported as contributory factors.

2.2.2. Oxidative stress in intra-abdominal surgery

Undergoing major intra-abdominal surgery is associated with the generation of oxidative stress with activation of reduced xanthine oxidases, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and ROS leakage from the mitochondria all implicated (Rosenfeldt et al, 2013). One of the proposed primary sources of reactive species generation in the intestine is via the xanthine oxidase (XO) pathway (Thomas & Balasubramanian, 2004); which has been implicated in the production of ROS during ischaemia reperfusion. During intra-abdominal resection surgery, a restriction of oxygenated blood to the bowel creates a hypoxic environment in which initial energy demands are met through increased rates of glycolysis. This results in the depletion of cellular stores of adenosine triphosphate (ATP) as it is systematically degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine. Adenosine deaminase converts adenosine to inosine which culminates in an accumulation of hypoxanthine. Concurrently, the ischaemic conditions trigger the degradation of xanthine dehydrogenase to xanthine oxidase. As oxygen supply is returned to the now ischaemic tissue following the reperfusion of the blood, hypoxanthine is degraded into xanthine by XO resulting in the increased production of $O_2^{\cdot}$ and hydrogen peroxide (H_2O_2), (Halliwell & Gutteridge, 2007) increasing the risk of subsequent tissue injury.

Under normal conditions, the intestinal mucosa of the gastrointestinal tract performs an important barrier function in preventing the translocation of bacterial pathogens into the systemic circulation (Anup & Balasubramanian, 2000). When placed under a stressor such as surgical stress, increased intestinal permeability has been reported placing the individual at increased risk of potentially developing sepsis, SIRS or MOFS (Anup, Aparna, Pulimood, & Balasubramanian, 1999). Laparotomy and mild intestinal manipulation have been demonstrated to be sufficient to induce a transient increase in enterocyte xanthine oxidase activity and decrease the activity of antioxidant enzyme, catalase (CAT) (Anup, Susama, & Balasubramanian, 2000; Thomas, Pulimood, & Balasubramanian, 2003). This has been associated with causing damage to

the mucosal function and structure resulting in increased intestinal permeability and the widening of intercellular spaces (Anup et al., 1999) as well as with mitochondrial dysfunction occurring (Ramachandran, Patra, & Balasubramanian, 2001).

Mitochondria are central to cellular energy production; they also however are reported to be a source of ROS and RNS production (Gomes et al., 2012). It is suggested that the leakage of up to 3% of all electrons as they pass along the electron transport chain, in particular complex I and complex III, may generate $O_2^{\cdot}$ instead of contributing to the reduction of oxygen to water (Valko et al., 2007). At complex I, it is reported $O_2^{\cdot}$ is exclusively released into the mitochondrial matrix therefore when the mitochondria is undamaged, leaked $O_2^{\cdot}$ is undetectable (Muller, Liu and Van Remmen., 2004). In contrast, at complex III, both extra mitochondrial and mitochondrial matrix $O_2^{\cdot}$ was been observed (Muller et al., 2004). The development of mitochondrial dysfunction therefore can contribute to increased leaked electrons, resulting in an increased production of $O_2^{\cdot}$ once the oxygen supply is returned (Solaini & Harris, 2005).

Finally, the introduction of surgery-induced bacteria and inflammation can initiate a phagocytic response by professional phagocytes such as neutrophils and monocytes and macrophages (Redmond, Watson, Houghton, Condron, Watson and Bouchier-Hayes., 1994), although surgery has also been suggested to have an immunosuppressive effect in the immediate post-operative period (Hogan, Peter, Shenoy, Horgan and Hughes., 2011). Whilst the process of phagocytosis facilitates the ingestion of the organism, there is an intense period of oxygen consumption, known as 'the respiratory burst' which results in large amounts of $O_2^{\cdot}$ being produced (Wientjes and Segal., 1995). The reaction is catalysed by NADPH oxidase according to the reaction below (Babior., 2004):

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^{\cdot}$$

2.3. Defence mechanisms against oxidative stress

The excessive generation of oxidative stress following major surgery therefore represents a challenge to the patient following gastrointestinal surgery. In protection against the potential damage oxidative stress can cause, the body has developed complex defence mechanisms which function to alleviate and protect against the

destructive effects of excessive oxidative stress. Included in these defence mechanisms are the body's antioxidant defence system (Valko et al., 2007) as well as a family of stress proteins known as Hsps (Benjamin & McMillan, 1998). As both of these defence systems have been found to be altered by ageing, physical activity and exercise in humans, in the following sections, an overview of the functions of both systems shall be covered, before discussing how PREHAB may result in beneficial adaptations that increase protection against surgically induced oxidative stress.

2.3.1. Enzymatic antioxidant defence systems

Within the body exists a series of different enzymatic and non-enzymatic antioxidant defences, each of which functions in protecting against cellular damage in the presence of oxidative stress. The key enzymatic antioxidants are SOD, CAT and glutathione peroxidase (GPx). SOD provides the primary defence system against O_2^- radicals, facilitating its dismutation into H_2O_2 and water (Powers & Jackson, 2008) (Figure 2.2). Three isoforms of SOD exist within humans, two intracellular isoforms and one extracellular isoform (Powers & Jackson, 2008). Of the intracellular isoforms, manganese superoxide dismutase (MnSOD) is located exclusively in the mitochondrial matrix and is thought to act as one of the main mitochondrial defence system against oxidative stress and ischaemia reperfusion injury (Powers, Quindry, & Kavazis, 2008). The second intracellular isoform is copper zinc superoxide dismutase (CuZnSOD) which is primarily located in the cytosol and the mitochondrial intermembrane space (Powers & Jackson, 2008). The final SOD isoform also requires copper-zinc as a cofactor but is located in the extracellular space (Powers & Jackson, 2008). Whilst serving several biochemical functions, the principle purpose of the antioxidant enzyme CAT is to catalyse the breakdown of H_2O_2 produced by the dismutation of $O_2^{\cdot}$ by SOD into H_2O and O_2 (Halliwell & Gutteridge, 2007). Although situated predominantly in the peroxisomes, CAT is also present in the other organelles such as the mitochondria and the endoplasmic reticulum.

GPx functions in catalysing the reduction of H_2O_2 into H_2O through the oxidation of the non-enzymatic antioxidant, reduced glutathione (GSH) into oxidised glutathione (GSSG). A minimum of five different glutathione peroxidases (GPx1-GPx5) have been identified in mammals with each one having potential cell and tissue specific functions (Powers & Jackson, 2008). A prolonged accumulation of GSSG can

have a detrimental effect on cellular function therefore in the presence of NADPH the enzyme glutathione reductase can regenerate GSH from GSSG (Maher, 2005). The role of the GSH and GPx antioxidant system will be covered in greater detail in Section 2.3.3.

2.3.2. Non-enzymatic antioxidant defence systems

The non-enzymatic antioxidant defence systems include glutathione, uric acid, lipoic acid and bilirubin, in addition to dietary antioxidants such as vitamin A (carotenoids), vitamin C (ascorbic acid) and vitamin E (tocopherols), all of which provide additional protection (Valko et al., 2007). However as the predominant endogenous non-enzymatic antioxidant in the body, glutathione shall be the main antioxidant defence system focused on within this thesis.

2.3.3. Glutathione antioxidant defence system

Glutathione, a water soluble low-molecular-weight tripeptide, is the most abundant non protein thiol in the body (Dickinson & Forman, 2002). Providing multiple defence mechanisms against the cellular damage caused by oxidative stress, glutathione is particularly effective again the highly toxic hydroxyl radicals ($OH^{\cdot}$) for which no other enzymatic defence is known (Maher, 2005; Wu, Fang, Yang, Lupton, & Turner, 2004). At rest, glutathione is predominantly found in its reduced state within the cell. GSH however is not distributed equally within the cell (Maher, 2005); with the majority of cellular GSH (85-90%) present in the cytosol where it is synthesised (Wu et al., 2004). Separate pools of GSH are also located in both the mitochondria and nuclei, however to a certain extent these are considered independent from the cytosol pool as changes in mitochondrial or nucleic GSH are not always reflected with changes in cytosol GSH or vice versa (Maher, 2005).

Although glutathione can be directly obtained exogenously from food sources, the majority is synthesised from the amino acids glutamate, cystine and glycine within the cytosol; a process which requires the consecutive actions of the enzymes glutamate-cysteine ligase and glutathione synthase (Maher, 2005). In its reduced state, GSH acts as an electron donor during periods of oxidative stress, forming GSSG in the reduction of H_2O_2 by the reaction catalysed by the antioxidant enzyme GPx (Maher, 2005; Valko

et al., 2007). An accumulation of GSSG however can have a detrimental effect on cellular function therefore GSH can be regenerated from GSSG via the activity of the enzyme glutathione reductase (GR) (Maher, 2005). In addition to its role in the reduction of H_2O_2, glutathione has other protective roles against oxidative stress (Valko et al., 2007). These include: (i) assisting amino acid transport through the plasma membrane; (ii) scavenging $OH^{\cdot}$ and singlet oxygen directly and (iii) regenerating important antioxidant vitamins C and E back to their active forms (Valko et al., 2007), highlighting the important role it plays within human physiology.

2.3.4. Glutathione and ageing

During the process of ageing, it has been proposed that an accumulation of oxidative damage as a result of increased ROS and RNS production (e.g. mitochondrial dysfunction) and/or a decline of the body's protective defence mechanisms (e.g. antioxidant defence system), is the primary cause of declines in cellular function (Beckman & Ames, 1998; Maher, 2005). This decline has been implicated in potentially increasing the likelihood of conditions such as cardiovascular disease (Dhalla, Temsah, & Netticadan, 2000), cancer (Valko et al., 2006) and certain neurodegenerative diseases (Halliwell, 2001) developing. Age-dependent decreases in TGSH and/or GSH have been reported in the brain, heart, kidney and livers of mice and rats (Liu & Dickinson, 2003; Mosoni, Breuille, Buffiere, Obled, & Mirand, 2004; Sandhu & Kaur, 2002), although the evidence is not conclusive with species-dependent changes potentially existing (Maher, 2005). In humans, given the difficulties that exist in obtaining direct samples from specific organs, determination of both blood GSH and GSSG is suggested to provide an essential index of whole body GSH status, thus potentially reflecting the GSH status in less accessible tissues and organs (Douris et al., 2009). Of note however is the fact that the actual resting concentrations of glutathione reported differs across studies, a consequence of the component of blood (e.g. whole blood, erythrocytes, plasma) from which the sample was measured (Serru et al., 2001).

Despite this, age-related decreases in GSH, increases in GSSG and altered GSH/GSSG ratio have all consistently been reported in human blood although in many cases the relationship between age and these parameters does not always appear linear (Erden-Inal, Sunal, & Kanbak, 2002; Gil et al., 2006; Jones, Mody, Carlson, Lynn, & Sternberg, 2002). Erden-Inal et al. (2002) and Gil et al. (2006) reported relatively

consistent levels of GSH up to the age of 40 years in whole blood and erythrocytes respectively after which point levels declined. Similarly, elevations in GSSG levels were reported as starting after 40 years (Erden-Inal et al., 2002) and 50 years (Gil et al., 2006) of age respectively. In Jones et al. (2002), whilst plasma GSH decreased linearly with age, there was no association found between age and GSSG concentration in participants until they reached approximately 45 years of age from which point the increase was associated linearly. Furthermore, GSH/GSSG ratio remained constant until a similar time point before declining linearly. Similar findings were also reported in whole blood (Erden-Inal et al., 2002) and erythrocytes (Gil et al., 2006). It has been postulated that these results suggest that from late middle age onwards, GSH metabolism is unable to maintain its efficiency at matching oxidising events (Maher, 2005). Whilst the ageing process has been associated with changes in GSH and GSSG, no such relationship appears present in terms of gender effects (Erden-Inal et al., 2002; Jones et al., 2002; Yang, Chou, Liu, Tsai, & Kuo, 1995). Yang et al. (1995) reported no gender difference in plasma GSH and GSSG levels in Chinese males and females regardless of age, a result replicated in whole blood by Erden-Inal et al. (2002) whilst Jones et al. (2002) also reported no gender difference for plasma GSH/GSSG ratio.

2.3.5. Glutathione and physical activity

Whilst there appears to be a decline in the antioxidant defence system as a consequence of ageing, it has been suggested that the level of decline may be associated to the individuals' physical fitness status (Douris et al., 2009; Karolkiewicz et al., 2003). More specifically, habitual and prolonged periods of physical activity appear to maintain or elevate circulating GSH levels whilst maintaining the GSH/GSSG ratio (Douris et al., 2009), indicating lower levels of oxidative stress, especially in ageing populations (Karolkiewicz et al., 2003). Douris et al. (2009) reported individuals participating in martial arts at least twice a week for the past four years had higher resting erythrocyte GSH concentrations (+16%) and lower erythrocyte GSSG concentrations (-18%) compared to the aged and sex matched sedentary control group. When exposed to an acute bout of exercise induced oxidative stress, the martial arts group also displayed a more effective response to the stress than the sedentary controls. Comparing healthy elderly males separated based on estimated energy expenditure per week; Karolkiewicz et al. (2003) reported higher resting erythrocyte GSH concentrations (+18%) and attenuated oxidative stress (-31%; as measured via

thiobarbituric acid reactive substances (TBARS) assay) in highly active males compared to the less active individuals. Interestingly, no significant difference was seen between the two groups for their respective total antioxidant systems. This suggests adaptations to the glutathione antioxidant system rather than the overall total antioxidant system may be responsible for improved resistance against oxidative stress in more physically active individuals.

Research is currently limited regarding the potential use of short term exercise protocols in humans to upregulate the glutathione and associated enzymatic antioxidant systems. Using either 40 minutes of aerobic exercise training (65-75% HR_{max}; AET), circuit weight training (at least two sets of 15 repetitions of chest press, latissimus dorsi pull down, knee extension, abdominal flexion and leg press; CWT) or a combination of both (20 minutes of both AET+CWT), Elokda and Nielsen (2007) aimed to replicate current cardiac rehabilitation (CR) recommendations (40 minutes, 3 days a week for 6 weeks) in the United States of America. At the end of the 6 week training period, the authors reported elevated resting erythrocyte GSH (+11% to +19%), decreased GSSG (-16% to -22%) and an improved GSH/GSSG ratio (+33% to +48%) for all groups compared to the control group. Furthermore, the improvement in GSH/GSSG ratio was significantly greater for the AET+CWT group compared to AET and CWT groups alone. In an observational study, Karolkiewicz et al. (2009) studied the effects of an 8 week aerobic exercise programme (40 minutes x 3 days at 70-80% ventilatory threshold) on postmenopausal women, reporting a significant increase in erythrocyte GSH (+24%) post-intervention. Additionally, levels of oxidative stress (determined by TBARS assay) had decreased (-20%) post-exercise programme. Both increased and decreased GPx activity has been reported following a period of exercise training. Using 12 week exercise programmes of contrasting intensities, both Miyazaki et al. (2001) and Takahashi et al. (2013) reported increased GPx activity at the end of the study period. Miyazaki et al. (2001) found 60 minutes of running at 80% HR_{max}, 5 times a week for 12 weeks in young healthy males increased GPx activity by 11.5% and SOD activity by 17.1%, whilst also decreasing the level of exercise induced lipid oxidation post intervention compared to pre-intervention levels. In comparison, following a low intensity and volume exercise programme in older adults consisting of walking for 30-60 minutes, twice a week, Takahashi et al. (2013) reported an 8% increase in GPx activity in the exercise group compared to the age matched control group.

2.3.6. Heat shock proteins

Hsps are a ubiquitous family of stress response proteins found in both prokaryotic and eukaryotic cells (Daugaard, Rohde, & Jaattela, 2007). Initially discovered by Ritossa (1962) in the salivary glands of the Drosophila following an acute heat exposure, subsequent research has identified a number of different families of Hsps, each of which have been grouped based upon their molecular weight. These have traditionally been grouped into the following families: small Hsps (8-32 kDa), the 40- to 60-kDa Hsps, the 70-kDa Hsps, the 90-kDa Hsps and the 100-kDa Hsps (Powers, Locke, & Demirel, 2001). For the purpose of this thesis, the focus will be on Hsp72, the main inducible member of the 70-kDa family and small Hsps, Hsp32, otherwise known as haem-oxygenase 1(HO-1) and Hsp27. Hsp72 is the most heavily researched Hsp in terms of its response to exercise and ageing. Although less work exists on the effects of exercise on Hsp32 and Hsp27, Hsp32 is considered an crucial mediators of both antioxidant and tissue protective activities (Otaka, Odashima, & Watanabe, 2006) whilst Hsp27 is reported to have important functions in stabilising the cytoskeleton in response to cellular stress (Mymrikov, Seit-Nebi, & Gusev, 2011).

2.3.7 Heat shock protein 72

In humans, the 70-kDa family of Hsps consists of at least eight different members (Daugaard et al., 2007). These include the constitutively expressed heat shock cognate (Hsp70/Hsp73), the endoplasmic reticulum located glucose regulated protein (Grp78) and the mitochondrial localised Hsp75 (Daugaard et al., 2007). Hsp72, also known as Hsp70 and Hsp70-1, is the highly inducible member of the 70-kDa heat shock family. Initially credited with providing cytoprotection following heat exposure, a number of stressors including hypoxia (Taylor et al., 2011; Taylor et al., 2010b), oxidative stress (Khassaf et al., 2003), strenuous exercise (Peart, Kirk, Hillman, et al., 2012), and psychological distress (Fleshner, Campisi, Amiri, & Diamond, 2004) have been shown to elevate Hsp72 expression. Located in the cytosol and nucleus, Hsp72 is expressed at low levels in unstressed conditions (Locke, Noble, & Atkinson, 1991), however when exposed to cellular stress Hsp72 expression becomes elevated. This is thought to evoke a number of protective functions including the prevention of protein aggregation (Benjamin & McMillan, 1998), assisting with the correct refolding of damaged proteins (Beckmann, Mizzen, & Welch, 1990), aiding in the removal of

damaged proteins and providing cellular protection against apoptosis (Mosser, Caron, Bourget, Denis-Larose, & Massie, 1997). The transcription and translation of Hsp72, along with other Hsps, is primarily mediated by stress inducible transcription factor, heat shock factor 1 (Hsf-1) (Kiang & Tsokos, 1998) and is depicted in Figure 2.3.

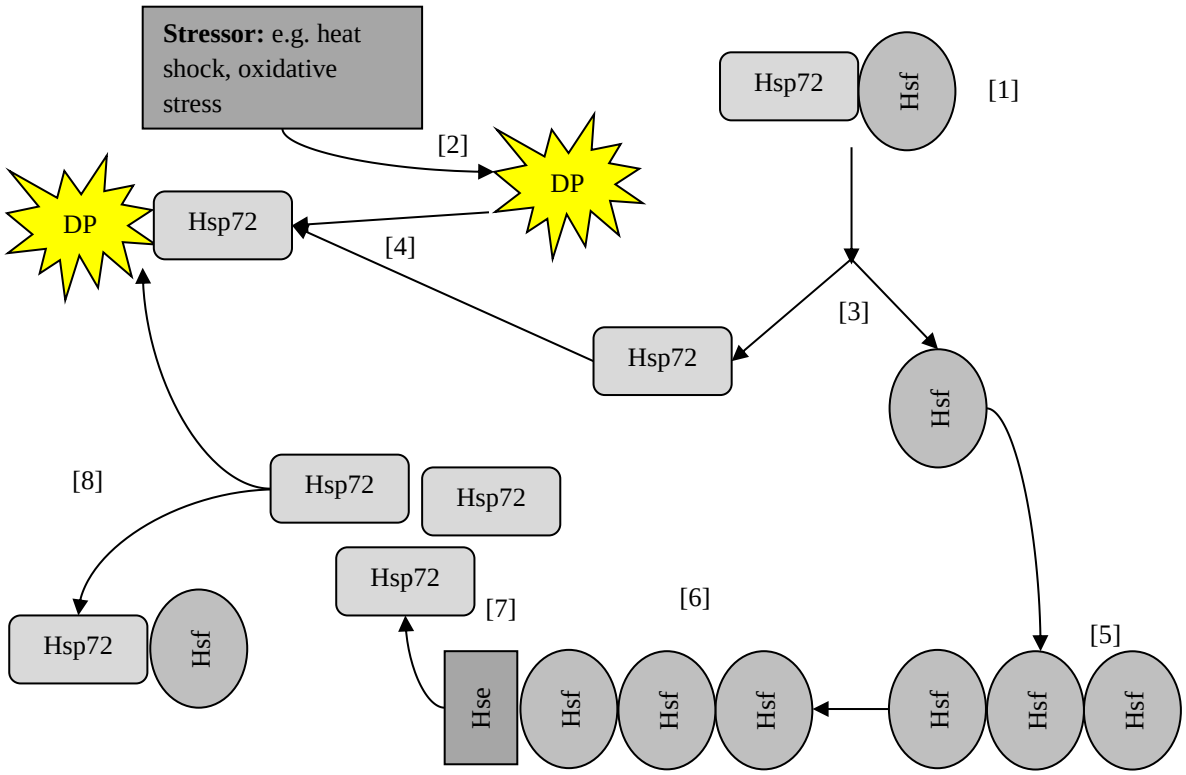
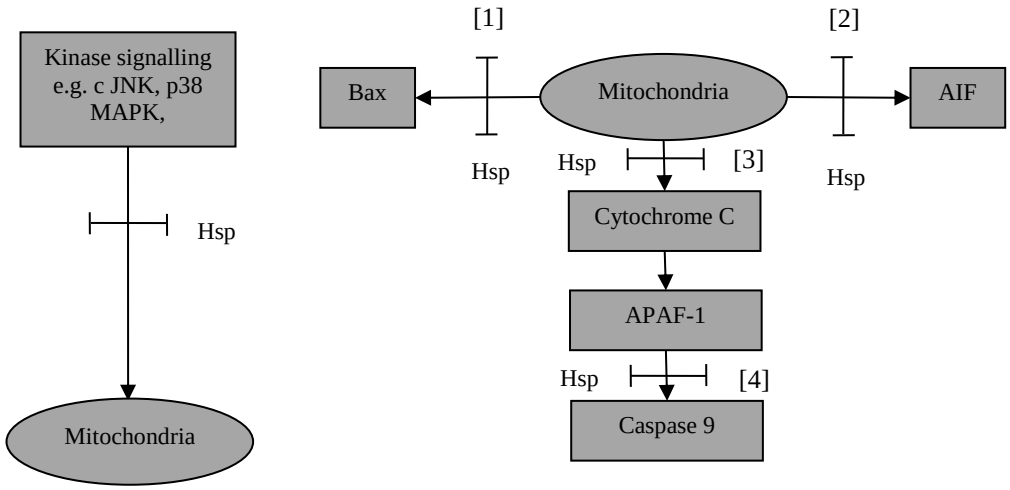


Figure 2.3: Transcription and translocation of Hsp72. [1] In unstressed conditions, Hsf-1 is a monomer bound to the Hsp72 in the cytosol of the cell. [2] In the presence of a stressor, proteins become denatured [3] stimulating the disassociation of Hsp72 and Hsf-1. [4] Hsp72 binds with denatured protein (DP). [5] Activated Hsf-1 undergoes phosphorylation via protein kinases (e.g. protein kinase C) resulting in trimerisation. [6] Hsf-1 trimer undergoes further phosphorylation via Hsf kinases; binding with heat shock element (Hse). [7]. This results in transcription of Hsp messenger RNA resulting in the synthesis of Hsp. [8] Newly synthesised Hsp either binds with denatured proteins or re-binds with Hsf-1 in preparation for any subsequent stress. Adapted from Kiang and Tsokos (1998) and Khalil, Kabapy, Deraz, and Smith (2011) .

An important function of Hsp72 in protecting cells from cellular stress is its ability to block pathways of programmed cell death (Mosser et al., 1997; Stankiewicz, Lachapelle, Foo, Radicioni, & Mosser, 2005; Zhang et al., 2015). Multiple pathways of apoptosis inhibition by Hsp72 have been identified which have previously been reviewed in detail (Garrido, Brunet, Didelot, Zermati, Schmitt,& Kroemer, 2006; Mosser & Morimoto, 2004; Murphy, 2013). At a pre-mitochondrial level (Figure 2.4

(a)), Hsp72 is reported to convey an anti-apoptotic effect through binding and blocking several stress-induced kinase signalling pathways including c-jun N-terminal kinase (cJNK) and p38 mitogen-activated protein kinase (p38 MAPK) (Mosser et al., 1997). At the mitochondria, expression of Hsp72 blocks the translocation of pro-apoptotic protein Bax to the mitochondrial membrane, reducing membrane permeability and limiting the release of pro-apoptotic factors (Stankiewicz et al., 2005).



(a) **Pre-mitochondrial** (b) **Post-mitochondrial**

Figure 2.4: Potential anti-apoptotic pathways of Hsp72. (a) Pre-mitochondrial blocking of kinase signalling pathways (c JNK, p38 MAPK) through Hsp72 binding prevents apoptosis inducing signalling. (b) Post-mitochondrial Hsp72 binding inhibits [1] Bax translocation, [2] apoptosis-inducing factor (AIF) and [3] cytochrome C release from the mitochondria and [4] recruitment of procaspase 9 by blocking oligmerisation of apoptotic protease activating factor 1(APAF-1), all of which inhibit apoptotic pathways. Adapted from Garrido et al. (2006) and Murphy (2013).

At the post mitochondrial level, Hsp72 has been reported to inhibit apoptosis via both the caspase dependent and caspase independent pathways (Khalil et al., 2011). In the caspase dependent pathway, different points have been identified at which apoptosis is inhibited. Generally Hsp72 is thought to inhibit apoptosis downstream of the mitochondrial release of cytochrome c and prior to the activation of caspase 3 (Jaattela, Wissing, Kokholm, Kallunki, & Egeblad, 1998; Li, Lee, Ko, Kim, & Seo, 2000). Binding of Hsp72 to apoptosis protease-activating factor-1 (APAF-1) has been reported to block the oligomerisation of APAF-1, thus preventing the recruitment of procaspase 9 and the formation of the apoptosome (Saleh, Srinivasula, Balkir, Robbins, & Alnemri, 2000). Alternatively though, Steel et al. (2004) reported Hsp72 inhibition of apoptosis

was the result of blocked cytochrome c release from the mitochondria rather than relating to the apoptosome formation. In the caspase independent pathway, Hsp72 binds to apoptosis-inducing factor (AIF) inhibiting mitochondrial release and nuclear accumulation (Ruchalski et al., 2003).

2.3.8. Hsp72, ageing, physical activity and exercise

A number of factors including age, gender and levels of physical activity have been associated with changes in the expression of Hsp72. It has been generally accepted that an age-related decline in the inducibility of Hsp72 in peripheral blood mononuclear cells (PBMC) of normal human subjects exists (Jin et al., 2004; Njemini et al., 2002; Singh et al., 2006). Exposing PBMC obtained from a population aged between 21 and 81 years to an in vitro 42°C heat shock for 1 hour, Njemini et al. (2002) demonstrated an age-related decline in the Hsp72 inducibility in monocytes and lymphocytes. This was supported in studies by Jin et al. (2004) and Singh et al. (2006), with Singh et al. (2006) in particular demonstrating a more pronounced age-related decay in Hsp72 synthesis existed in monocytes compared to lymphocytes.

As the human body is rarely exposed to the extreme conditions that cells are subjected to during in vitro studies, Njemini et al. (2007) suggested that basal levels of Hsp expression may be of greater relevance in ageing populations than their actual ability to induce Hsp expression in response to external stressors. Njemini et al. (2007) reported an age-related upregulation in basal expression of Hsp72 as well as Hsp32 and Hsp90 in both monocytes and lymphocytes from an independently living elderly population (age 75.0 ± 6.3 years) compared to healthy young volunteers (age 36.4 ± 8.1 years). This age-related difference however was only evident in apparently healthy individuals as basal Hsp72, Hsp32 and Hsp90 expression was elevated in all patients who were hospitalised for acute infection (identified through a rise in serum C-reactive protein), eliminating the age-related difference that previously existed (Njemini et al., 2007). This association between inflammation and Hsp72 expression is highlighted by the positive relationship that has been found to exist between Hsp72 and levels of both C-reactive protein and, IL-6 (Njemini et al., 2007). These age and inflammation-related elevations in basal Hsp72 expression raises the question whether the associated cells are better prepared for further exposure to a secondary stressor or in fact simply represent

the fact the cells are simply under a prolonged period of age-related or inflammatory stress.

Comparing an elderly population (60-90 years) based on their level of self-reported physical activity over the previous year, Simar, Malatesta, Badiou, Dupuy, and Caillaud (2007) reported the high physical activity group (defined as a mean score of 20.7 ± 7.4 on the Modified Baecke Questionnaire for Older Adults and regular engagement in sporting activities [> 4 hrs/wk] for the previous 5 years) were observed to have lower basal leukocyte Hsp72 expression in comparison to the low-moderate physical activity group (mean Modified Baecke Questionnaire score:13.6 ± 3.9, and not engaged in any sporting activity). This was in conjunction with a higher antioxidant capacity (plasma Trolex equivalent antioxidant capacity and vitamin E) and lower levels of circulating oxidative damage (as determined by plasma TBARS and advanced oxidation protein products), supporting the age-related association between Hsp72 expression and oxidative stress. Within younger trained and sedentary populations, similar findings have previously been reported. Both Shastry, Toft, and Joyner (2002) and Shin et al. (2004) reported elevated basal leukocyte Hsp72 levels (+100% and +23% respectively) in untrained age-matched controls (Shastry: 25 ± 1.3 years; Shin: 21.3 ± 1.1 years) compared to trained individuals (Shastry: 29 ± 1.9 years; Shin: 22.4 ± 1.5 years). Furthermore, although the sedentary control group where significantly older (Trained: 32.3 ± 9.3 years; Control: 45.4 ± 11.4 years), Fehrenbachet al. (2000a) reported lower basal levels of Hsp72 expressed in monocytes and granulocytes in trained half marathon runners compared to untrained controls. This down-regulation in basal Hsp72 expression potentially is related to an upregulation of the body's antioxidant defence system (Fehrenbach et al., 2000a; Simar et al., 2007).

It has been postulated that preconditioning strategies to promote the upregulation of Hsp72 prior to a secondary stressor may convey additional cellular protection (Madden, Sandstrom, Lovell, & McNaughton, 2008). Following a period of heat acclimation, basal levels of Hsp72 have been reported to be elevated in both human PBMCs (McClung et al., 2008) and serum (Sandstrom, Siegler, Lovell, Madden, & McNaughton, 2008) as well as in the left ventricle of rats (Horowitz, Maloyan, & Shlaier, 1997; Maloyan, Palmon, & Horowitz, 1999). Similar results have been reported within the skeletal muscle in humans following a period of exercise training. Willoughby, Priest, and Nelson (2002) demonstrated both elevated Hsp72 mRNA

(33.7% increase) and protein expression (30.2% increase) within the vastus lateralis following 12 weeks of cycling (60 ± 15.7 minutes, twice a week at 75% % age predicted HR_{max}) in individuals with spinal injuries. Gjovaag and Dahl (2006) reported 5-8 weeks of both low (30% 1 Repetition Max; 1RM) and high intensity (60% 1RM) elbow extension exercises matched for volume (total weight lifted) elevated both Hsp72 and Hsp27 levels within the triceps brachii of healthy males and females (mean age: 22.0 ± 0.6 years). The greatest increases however were seen within the high intensity exercise group compared to the low intensity exercise group potentially indicating the importance of training stimulus on adaptive responses occurring.

From a clinical perspective, there appears to be potential benefits from inducing Hsp72 expression prior to a secondary stressor (Fujimori et al., 1997; Odashima et al., 2006; Wischmeyer et al., 2001). A period of short term exercise preconditioning (3-5 days, 60 minutes running at ~60-80%VO_{2max}) has been reported to be sufficient to instigate an elevation in myocardial basal Hsp72 content providing cardioprotection against myocardial stunning and IRI in rats (Demirel et al., 2001; Lennon et al., 2004). However, conflicting results have since shown an elevation in Hsp72 however may not be essential for cardioprotection to be gained (Quindry et al., 2007). The prior induction of Hsp72 has been reported to play an important role in providing cytoprotection in various organs when subjected to an array of harmful stressors and toxins. In Sprague-Dawley rats, the induction of Hsp72 expression by either prior hyperthermic exposure or zinc L-carnosine supplementation has been reported to have a protective effect against acetic acid induced colonic mucosa damage (Odashima et al., 2006; Otani et al., 1997). Similar cytoprotective effects have been reported in Sprague-Dawley rats following zinc L-carnosine induced Hsp72 expression of the gastric mucosa (Wada et al., 2006) as well as following hyperthermia induced expression of Hsp72 in the liver (Fujimori et al., 1997) and oesophageal tract (Izumi et al., 2009).

The dietary induction of Hsp72 with glutamine has also been reported to play an essential role in protecting against models of endotoxin shock, sepsis and tissue damage in the lungs, heart, colon and kidneys of mice and rats (Singleton & Wischmeyer, 2007; Wischmeyer et al., 2001). The prior induction of Hsp72 by either zinc L-carnosine or glutamine supplementation has been reported to attenuate the activation of transcription factor nuclear factor kappa B (NFκB) (Odashima et al., 2006; Singleton & Wischmeyer, 2007), resulting in a suppression of the subsequent pro-inflammatory response. This is

further supported by the suppressed levels of pro-inflammatory cytokines TNF-α and IL-1β in rat oesophageal tissue (Izumi et al., 2009), TNF-α and IL-6 in rat lung tissue (Singleton & Wischmeyer, 2007) and TNF-α in human PBMC (Wischmeyer et al., 2003) that have been observed following prior Hsp72 induction.

2.3.9. Heat shock protein 32

Hsp32 (also known as HO-1) is a stress inducible isoform of the haem oxygenase (HO) family (Halliwell & Gutteridge, 2007). Along with the constitutive isoform (HO-2), HO acts as the rate-limiting enzyme in the catabolism of free haem into biliverdin, carbon monoxide and ferritin (Gozzelino, Jeney, & Soares, 2010). This reaction is important within human physiology as if left, free haem has a pro-oxidant capacity making it cytotoxic, acting as a catalyst in the production of free radicals as a result of the Fenton reaction (Gozzelino et al., 2010). This makes the HO family of proteins crucial mediators of both antioxidant and tissue protective activities (Otaka, Odashima, & Watanabe, 2006). Furthermore, all three end products of the HO catalysed reaction offer cytoprotective properties against a variety of diseases (Gozzelino et al., 2010) as well as helping to re-establish homeostasis after a period of oxidative insult (Soares & Bach, 2009). Biliverdin and its product bilirubin, which is produced via the enzyme biliverdin reductase, act as potent antioxidants against products of oxidative stress (Gozzelino et al., 2010) whilst carbon monoxide serves both as an important intracellular signalling molecule and a potent vasodilator (Guo, Shin, & Cho, 2001). Whilst the free iron released as part of the reaction can contribute to the Fenton reaction thus increasing the production of ROS (Liao, Zhu, Li, & Zhu, 2013), it can also regulate the expression of iron storing proteins such as ferritin making it cytoprotective (Guo et al., 2001).

As mentioned in section 2.3.8, an age-related upregulation in basal expression of Hsp32 has been observed in both monocytes and lymphocytes from an independently living elderly population (age 75.0 ± 6.3 years) compared to healthy young volunteers (age 36.4 ± 8.1 years) (Njemini, Lambert, Demanet, Kooijman, & Mets, 2007). This reaffirmed the earlier findings by the same researchers (Njemini, Lambert, Demanet, & Mets, 2005). In contrast to Hsp72 stress response however the same study reported Hsp32 was only induced in monocytes with expression in lymphocytes actually attenuated in response to heat shock (1 hour at 42°C followed by 15 hours recovery)

(Njemini et al., 2005). Compared to Hsp72, less research exists into Hsp32 expression in response to both short and long term exercise. Acutely, increased Hsp32 expression in leukocytes has been observed post-exercise, although the response appears to be duration dependent (Fehrenbach et al., 2003; Peart, Kirk, Madden, Siegler, & Vince, 2013). Fehrenbach et al (2003) reported higher levels for Hsp32 expression in 12 well trained male athletes (age: 32.3 ± 9.0) following an official half marathon lasting approximately 90 minutes compared to 15 males (age: 26.5 ± 3.2 years) completing an exhaustive run (run duration: 10.8 ± 0.7 minutes) at 110% AT. Furthermore, eccentric exercises of the quadriceps femoris muscle group (six sets of 10 eccentric contractions) failed to induce Hsp32 expression in 12 endurance trained athletes (age: 29.2 ± 5.7 years) (Fehrenbach et al., 2003). Given the ageing status of colorectal cancer patients however, the Hsp32 stress response potentially will differ in to that of trained populations. Thirty minutes of treadmill walking at 50% VO_{2max} in 12 sedentary males (age: 54 ± 4 years) had no effect on monocytes or lymphocytes Hsp32 expression either immediately post-exercise or during the subsequent 7 days period (Markovitch, Tyrrell, & Thompson, 2008). To the author's knowledge, no training studies in to the effects of short term training exist. Niess et al. (1999) observed higher basal monocyte and granulocyte Hsp32 expression in 12 untrained individuals (< 2 hours of physical activity per week) compared to 10 endurance trained runners (> 4 hours running per week for a minimum of 2 years). This suggests a downregulation in basal Hsp32 may occur following prolonged training.

2.3.10. Heat shock protein 27

Hsp27 is a member of the small Hsp family (Powers, Lock, & Demirel, 2001), ubiquitously expressed throughout the body. Primarily located in the cytosol, exposure to heat shock along with other stressors, stimulates the translocation of Hsp27 to the nucleus and cytoskeleton (Mymrikov et al., 2011), which it is thought to stabilise through its ability to bind to the actin filament (Wieske et al., 2001). Hsp27 also has an important role in providing protection against the production of ROS, inhibiting apoptosis (Kennedy, Jager, Mosser, & Samali, 2014) and displaying the ability to increase cellular GSH concentration (Preville et al., 1999).

Research relating to changes in Hsp27 expression following exercise training is limited. Acutely, repeated eccentric resistance exercise (30 sets of 10 repetitions of

maximal, isokinetic eccentric quadriceps contractions; Paulsen et al., 2007; two sets of 25 repetitions of maximal eccentric resistance during bicep curl; Thompson, Scordilis, Clarkson, & Lohrer, 2001) but not 45 minutes of non-damaging treadmill running (Morton et al., 2006; Morton et al., 2008) is reported to increase the expression of Hsp27 in skeletal muscle. This is a likely reflection of Hsp27 role in stabilising the cytoskeleton (Paulsen et al., 2007). Furthermore following both maximal cycling exercise (Jammes, Steinberg, & Delliaux, 2012) and maximal static handgrip to exhaustion (Brerro-Saby et al., 2010) plasma Hsp27 levels are reported to be elevated. In response to more prolonged training, Beltran Valls et al. (2014) reported a ~50% decrease in leukocyte Hsp27 expression (as measured by Western blot) following 12 weeks of explosive resistance training (leg extension, leg curl, low row, and chest press; Weeks 1 and 2: 4 sets of 15 repetitions at 40-50% of baseline 1RM; Week 3 onwards: 3-4 sets of 10-12 repetitions at 70% of baseline 1RM). Beltran Valls et al. (2014) attributed this decreased basal expression to repeated exposure to exercise stress facilitating physiological adaptations.

2.2.11. Hsp72 and Hsp27 in cancer

Whilst Hsp72 and Hsp27 provide an important role in protecting healthy cells and tissue against cellular stress through their ability to act as molecular chaperones and inhibit apoptosis, their overexpression has been implicated in negatively affecting the effectiveness of various anti-cancer therapies (Garrido et al., 2006; Grivicich et al., 2007; Rashmi, Kumar, & Karunagaran, 2004). Elevated levels of Hsp72, Hsp27 and main heat shock transcription mediator, Hsf-1 have previously been observed in various tumours including colorectal cancer (Milicevic, Petkovic, Drndarevic, Pavlovic, & Todorovic, 2007; Shotar, 2005), breast cancer (Ciocca et al., 1993) and leukaemia (Dempsey, Leoni, Ireland, Hoyle, & Williams, 2010; Madden et al., 2012). This overexpression has been reported to promote tumourigenesis and elevate cell resistance to anti-cancer therapy making the suppression of Hsps/Hsf-1 an area of interest in cancer treatment research (Murphy, 2013).

Many of the same anti-apoptotic pathways identified through which Hsp72 and Hsp27 protect normal cells from stressors such as oxidative stress can also function to attenuate the effectiveness of anti-cancer therapies (Ciocca, Arrigo, & Calderwood, 2013). The ability of Hsp72 and Hsp27 to block apoptosis at both a pre-mitochondrial

and post mitochondrial level has been reported. In addition to the pathways discussed for Hsp72 in section 2.3.7, tumour cell senescence is suppressed in the presence of Hsp72 as both the tumour suppressor protein p53 dependent pathway and the p53 independent pathway are impaired (Yaglom, Gabai, & Sherman, 2007). Hsp27 is suggested to mainly function in the post-mitochondrial inhibition of the caspase activation, although regulation of pre-mitochondrial signalling pathways has also been implicated for its anti-apoptotic ability (Garrido et al., 2006).

The clinical significance of Hsp72 expression in colorectal tumour cells however remains unclear. Conflicting findings have previously been reported regarding the prognostic value of Hsp72 expression. Studies by Hwang et al. (2003), Milicevic et al. (2007) and Wang, Qiu, Liu, and Chen (2005) all reported elevated Hsp72 expression was associated with lymph node involvement and advanced tumour stage but not tumour cell differentiation. In contrast to these however, no association was seen by Ozdemirler Erata et al. (2005), Shotar (2005), or Kanazawa et al. (2003) between Hsp72 and tumour grade or stage. Bauer et al (2012) reported high levels of either tumoural Hsp72 or Hsp27 expression in colorectal cancer patients was an independent prognostic factor for poorer survival rates, even in those with lymph node or distant metastases. Similar results relating to Hsp27 and poorer prognosis and outcomes have also been seen by Wang, Zhang, Shi, Yang, and Qin (2012) and Yu, Zhi, Peng, Zhong, and Xu (2010) although in colorectal patients with a poor prognosis, Tweedle et al. (2010) reported the association between poor survival and elevated Hsp27 was only evident in rectal cancer and colon cancer.

2.3.12. Potential benefits of manipulating antioxidant and Hsp defence systems prior major surgery

As discussed in sections 2.3.1 to 2.3.10, both the antioxidant and Hsp defence systems have the potential to play important roles in protecting against cellular stress. As colorectal cancer patients are a predominantly ageing population, a decline in the glutathione and Hsp72 defence systems as discussed in sections 2.3.4 and 2.3.8 would represent a reduced capacity to protect against excessive oxidative stress generated during surgery. In the same context, the ability to upregulate either systems through participation in a short period of PREHAB would therefore potentially increase the protection to available against any surgery induced oxidative assault. Adaptive changes

in Hsp72 basal content and stress response as well glutathione content and enzymatic activity have previously been observed following both short term and long term exercise training (Elokda and Nielsen., 2007; McClung et al., 2008; Miyazaki et al., 2001; Simar et al., 2007). It is unclear however whether sufficient time would exist in the pre-operative period for colorectal cancer patients to elicit such an adaptive response or if the exercise delivered as part of a PREHAB functional programme would be of sufficient duration or intensity. Having discussed surgical stress and the potential protective role the antioxidant and Hsp defence systems may play in protecting against it, in the next section current strategies in the management of it will be reviewed.

2.4. Enhanced Recovery after Surgery (ERAS) Pathways

As a greater understanding of surgical stress has been gained over the years, strategies to reduce the impact the procedure can have on a patient both during and following surgery have been researched and developed. Many of strategies now form part of the Enhanced Recovery after Surgery (ERAS) approach adopted within most modern surgery. Up until the end of the 1990s, the traditional approach to major gastrointestinal resection surgery used to result in patient hospital stays of up to two weeks following surgery (Schoetz et al., 1997). This approach often incorporated limiting patient mobilisation which in conjunction with inadequate pain management and intestinal dysfunction, have been proposed as key factors in delaying post-surgery recovery (Kehlet, 1997). Since just before the turn of the century however, a number of investigators pioneered the introduction of a multimodal approach to stress management both during and following surgery with the Kehlet group from Denmark at the forefront (Kehlet & Wilmore, 2002; Wilmore & Kehlet, 2001). This multimodal approach to surgery is now generally referred to as the ERAS pathway and is implemented into modern medicine with the aim of reducing the physical, physiological and psychological stresses that surgery brings (Fearon et al., 2005; Kehlet & Wilmore, 2005).

Through incorporating a range of peri, pre and post-operative components (Figure 2.5), the application of ERAS pathways to colorectal surgery in particular has been suggested to reduce surgical stress, attenuate the decline in physical functioning and ultimately reduce post-operative LOS (Anderson et al., 2003). Meta-analyses by Lv, Shao, and Zhou (2012) (weighted mean difference: -1.88 days [95% CI: -2.91 to -0.86

days]); Walter, Collin, Dumville, Drew, and Monson (2009) (weighted mean difference: -3.75 days [95% CI: -5.11 to -2.40 days]) and Zhuang, Ye, Zhang, Chen, and Yu (2013) (weighted mean difference: -2.44 days [95% CI: -3.06 to -1.83 days]) have all reported a significant reduction in LOS in colorectal cancer patients receiving treatment through the ERAS pathway compared to traditional treatment. Furthermore, this reduction in LOS has been achieved without compromising patient safety (Lv et al., 2012).

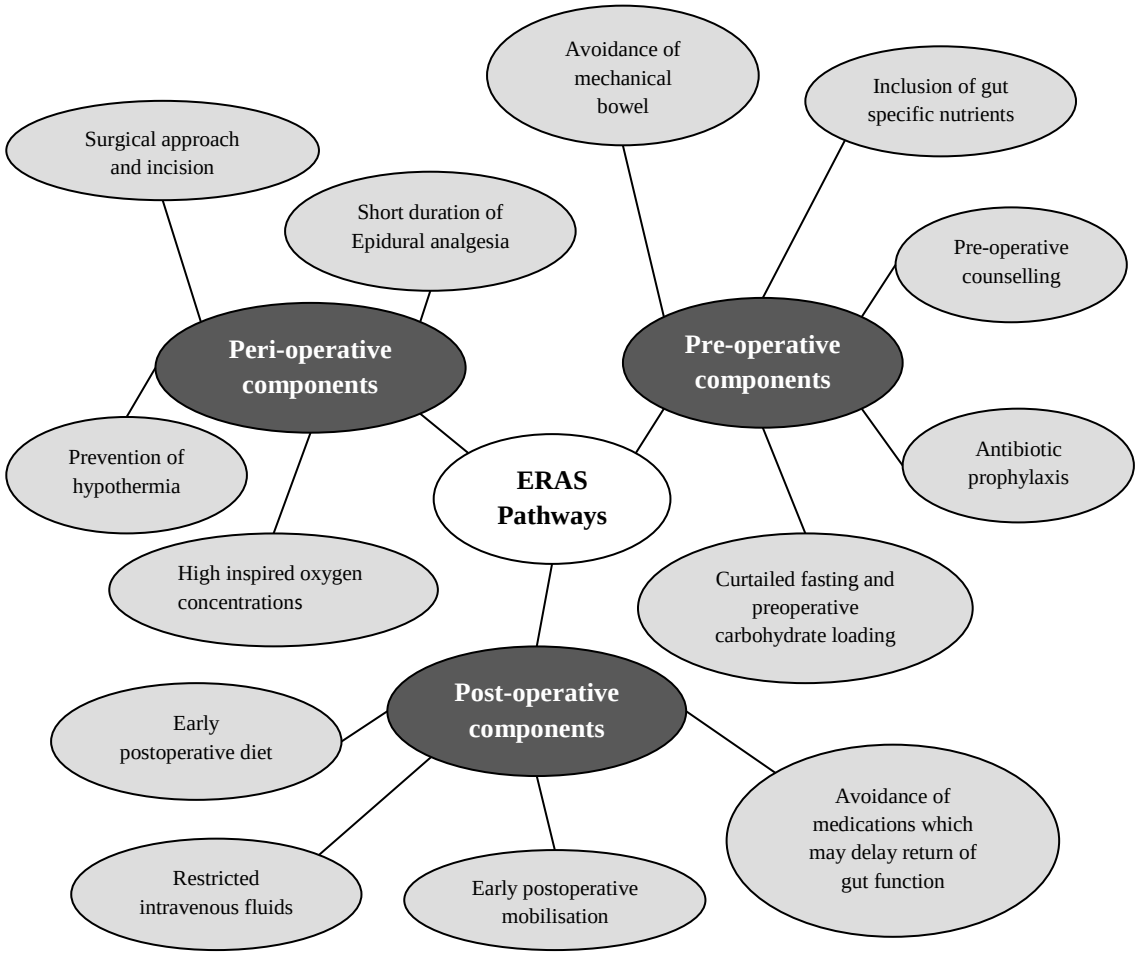


Figure 2.5: Schematic of different peri-, pre and post-operative components of Enhanced Recovery After Surgery (ERAS) pathways.

With over 20 components identified for potential inclusion within ERAS protocols, it should be noted the evidence for the effectiveness of some of these specific elements individually appears weak (Gustafsson et al., 2012). The multimodal approach of ERAS protocols however argues the combined effect of multiple strategies is greater than the individual role of specific components (Kehlet & Wilmore, 2002). For the purpose of this thesis, the following section will briefly discuss the role of some the

more predominant pre-operative, peri-operative and post-operative components of ERAS in improving post-surgical outcomes following colorectal surgery. Comprehensive reviews and ERAS recommendations are covered in greater detail in a number of previously published review articles and guidelines (Fearon et al., 2005; Gustafsson et al., 2012; Khan, Gatt, Horgan, Anderson, & MacFie, 2009; Steenhagen, 2016).

2.4.1. Pre-operative components

The pre-operative components of the ERAS pathway are focused on the physical and mental preparation of the patient for surgery. Finding out that you require major surgery or have cancer can obviously place considerable stress on the patient potentially leading to anxiety, depression and in some cases denial (Kiecolt-Glaser, Page, Marucha, MacCallum, & Glaser, 1998). Detailed written and verbal pre-operative information and counselling outlines what the patient is to expect during their hospital stay. This can entail meetings with different members of the multidisciplinary team (MDT)(e.g. surgeons, dieticians, nutritionists, stoma nurses) who are involved in their treatment both prior to and after surgery as well as setting targets for post-operative mobilisation and nutritional intake (Steenhagen, 2016). This is reported to potentially reduce the anxiety caused by uncertainty and aid subsequent post-surgical recovery (Aarts et al., 2012; Kiecolt-Glaser et al., 1998).

As discussed in Section 2.2, the development of acute insulin resistance is a key characteristic of the inflammatory response following major surgery (Carli, 2015). Traditionally prior to surgery, patients would undertake a period of fasting from midnight on the night before surgery to ensure an empty stomach prior to surgery however scientific evidence in support of this practice is lacking (Ljungqvist & Soreide, 2003). Furthermore, subsequent recommendations of allowing the consumption of carbohydrates up to 6 hours prior to surgery and clear fluids up to 2 hours prior to anaesthesia is reported to attenuate the development of insulin resistance post-surgery (Ljungqvist & Soreide, 2003).

The other pre-operative components aim to reduce the risk of a number of post-surgical complications occurring. Colorectal surgery by its nature involves working in an environment where bacteria are prevalent, therefore the administration of antibiotic

prophylaxis 30-60 minutes prior to surgery has been recommended as a preventative measure against surgical site infection (Gustafsson et al., 2012; Khan et al., 2009). Initiation of prophylaxis 2-12 hours prior to surgery with low molecular-weight heparin can reduce the risk of thromboembolism, especially when combined with wearing compression stockings post-surgery (Sachdeva, Dalton, Amaragiri, & Lees, 2014). Although traditionally believed to be necessary, ERAS guidelines now recommend the clearance of colonic contents by mechanical bowel preparation (MBP; e.g. laxatives) is not routinely used in patients awaiting colon resection surgery (Gustafsson et al., 2012; Khan et al., 2009). Associations have been made between MBP and a number of adverse physiological effects which include severe dehydration (Holte, Nielsen, Madsen, & Kehlet, 2004), prolonged illeus (Jung et al., 2007) and potentially an increased risk of anastomotic leak developing (Wille-Jorgensen, Guenaga, Castro, & Matos, 2003). The evidence however is less clear in rectal resection patients (Khan et al., 2009).

2.4.2. Peri-operative components

During the peri-operative stage of treatment, the anaesthetist has an important role during surgery; managing the patients stress reaction to surgery, administrating the anaesthetic protocol used whilst controlling fluid replacement (Gustafsson et al., 2012). Effective control of the surgical stress response can attenuate the development of insulin resistance (Kehlet & Wilmore, 2002), whilst effective analgesia during surgery can facilitate early mobilisation during the surgery recovery phase (Gustafsson et al., 2012).

The amount of surgical stress encountered can also be heavily influenced by the surgical approach utilised. Whilst both open and laparoscopic surgery approaches can be taken, laparoscopic surgery is associated with reduced levels of oxidative stress during surgery than an open approach (Pappas-Gogos et al., 2013), therefore a laparoscopic approach to colonic resection surgery is recommended where the surgical expertise is available (Gustafsson et al., 2012). The direction of surgical incision is proposed to effect recovery with transverse incisions potentially resulting in reduced incidences of wound dehiscence, less pulmonary impairment and lower pain compared to vertical incisions (Brown & Tiernan, 2005).

2.4.3. Post-operative components

Post-operatively, ERAS aims to accelerate the patient back to normal functioning. Despite it being acknowledged that the evidence from randomised controlled trials (RCT) to date is weak (Gustafsson et al., 2012), a key element to post-operative care is the promotion of early patient mobilisation. Periods of immobilisation have been associated with a number of deleterious consequences, including increased insulin resistance, loss of muscle strength, reduction in pulmonary function and increased risk of developing pneumonia (Gustafsson et al., 2012; Kehlet & Wilmore, 2002). Furthermore, periods of immobilisation may expose patients to an increased risk of developing thromboembolism (Fearon et al., 2005). Current recommendations suggest care plans should be in place to facilitate patients being out of bed for 2 hours on the day of surgery and for 6 hours a day there after until they are discharged (Lassen et al., 2009). The ability to facilitate early mobilisation therefore can be influenced by a number of other post-operative factors including achieving adequate pain management as a result of appropriate analgesia strategies, the need for intravenous fluids and the avoidance of urinary drains (Gustafsson et al., 2012).

As well as physical functioning, a return of normal gut function is an important objective in subsequent patient care recovery following colorectal surgery (Fearon et al., 2005). The traditional approach to post-operative feeding was to limit oral intake following surgery, however more recently early feeding strategies (<24 hours post-surgery) have been promoted (Gustafsson et al., 2012; Lassen et al., 2009). A systematic review by Lewis, Egger, Sylvester, and Thomas (2001) reported there appeared to be no advantage in keeping patients nil by mouth following surgery; in fact early feeding may reduce the risk of anastomotic leakage and infections developing post-surgery. In malnourished patients or those unable to tolerate early feeding due to postoperative nausea and vomiting, incorporating oral nutritional supplements may provide a viable option both prior to and after surgery (Lassen et al., 2009).

As acceptance of the multimodal ERAS approach continues to grow, research is now directed towards identifying further components for inclusion within ERAS which may further aid the patient in the pre-, peri and post-operative period. One such area that is not currently included within ERAS protocols, although it is a growing field of

interest, is the use of pre-operative exercise interventions or PREHAB as a means of preparing patients for the stresses of major surgery.

2.5. Exercise prehabilitation in humans undergoing surgery.

In 1981, an observational study by Asoh and Tsuji (1981) assessed the physical fitness of 29 cardiac patients scheduled for major abdominal surgery, identifying 11 who were deemed to have inadequate physical fitness levels for surgery. Those with inadequate physical fitness (defined as being unable to achieve a steady state heart rate at a treadmill speed of 50 metres/minute and 7 degree incline) were assigned to a 1 to 3 week exercise intervention consisting of cycling twice a day for 20 minutes. Upon reassessment prior to surgery, seven of the 11 patients had improved to a level where physical fitness was deemed to be adequate for surgery. In the four patients deemed to have inadequate fitness, all experienced post-operative complications following surgery with two dying within 30 days of surgery due to cardiac failure. In comparison, of the 25 patients deemed to have adequate fitness prior surgery, only three experienced complications with no deaths in the 30 days following surgery. Whilst difficult to draw definitive conclusions given a number of methodological limitations (observational design, small sample) and additional influencing factors (pre-existing cardiac condition) the results showed promise for the potential benefits PREHAB could offer.

Subsequently, the role of PREHAB prior to surgery has been investigated in a number of different clinical populations; including CABG patients (Arthur et al., 2000; Sawatzky et al., 2014), patients scheduled for resection surgery for either lung cancer or colorectal cancer (Carli et al., 2010; Li et al., 2013), patients awaiting abdominal aortic aneurysm repairs (Kothmann et al., 2009) and orthopaedic surgeries such as total hip or knee arthroplasties (Wang, Gilbey, & Ackland, 2002). Recently a number of systematic reviews (Lemanu, Singh, MacCormick, Arroll, & Hill, 2013; O'Doherty, West, Jack, & Grocott, 2013; Pouwels et al., 2014; Santa Mina et al., 2014) have been published, each including different studies as a result of differing search criteria, clinical populations, modalities of PREHAB and outcome measures of interest. The reviews have generally concluded PREHAB to have a positive effect however, a general consensus remains that further evidence is required before definitive conclusions can be reached.

In individuals scheduled for major elective resection surgery, especially for cancer, typically only a short period of time exists during which patients could undergo a period of PREHAB. The current NHS cancer waiting time standards dictate treatment should be started within 31 days of the decision to treat (Baker & Nakatudde, 2015). In patients receiving NACRT prior to surgery this time may be extended to 6 -12 weeks in order to facilitate recovery from the treatment. This review of the literature has therefore focused on the use of PREHAB interventions lasting between approximately 1 to 12 weeks in individuals undergoing major elective resection surgery for non-orthopaedic conditions. The predominant use of either aerobic and/or resistance exercise training was required for inclusion with studies solely focused on the use of inspiratory muscle training (IMT) not included. The following sections will therefore aim to provide a summary of the current literature relating to PREHAB in patients scheduled for different major elective non-orthopaedic surgeries before covering important issues such as the feasibility, efficacy and effectiveness of PREHAB interventions. An assessment of study quality is included for each study according to the modified Delphi list (Verhagen et al., 1998) as used in Singh, Newton, Galvao, Spry, and Baker (2013).

2.5.1. PREHAB in coronary artery bypass graft surgery

Three RCTs investigating PREHAB in coronary bypass graft surgery (CABG) patients are included in Tables 2.4 and 2.5, one of which was a pilot study. In the only RCT conducted to date that was powered to detect a change in hospital LOS, 246 patients awaiting elective surgery for CABG were randomised to receive either a multi-dimensional pre-operative intervention or usual care (Arthur et al., 2000). The intervention consisted of twice weekly, 90 minute group exercise sessions for approximately 8 weeks in addition to a pre-operative educational content and psychological support. Each exercise session involved a minimum of 30 minutes aerobic exercise at 40-70% of the individual's functional capacity in addition to a variety of stretching and range of motion exercises. Arthur et al. (2000) reported a significant reduction in LOS (PREHAB: 5 [5-6] days; Control: 6 [5-7] days) as well as reduced time spent in the intensive care unit (ICU; PREHAB: 24.67 hours; Control: 26.71 hours) in the PREHAB group compared to control.

Table 2.4: Methodological quality of PREHAB studies in coronary artery bypass surgery.

Author / Study deign	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Arthur et al (2000) RCT	Y	Y	Y	Y	N	N	Y	N	Y	Y	NR	Y	Y	Y	10
Rosenfeldt et al (2011) RCT	Y	Y	Y	N	N	N	Y	N	Y	Y	NR	Y	Y	Y	9
Sawatzky et al (2014) RCT	Y	Y	Y	N	N	N	Y	Y	Y	Y	NR	Y	Y	Y	10

1: Treatment allocation concealed; 2: Groups/subjects similar at baseline regarding important prognostic values; 3: Eligibility criteria specified; 4: Blinded outcome assessor; 5: Was care provider blinded; 6: Was patient blinded; 7: Were point estimates and measures of variability presented for the primary outcome measures; 8: Did the analysis include an intension-to-treat analysis; 9: Between-group statistical comparison; 10: Dropout reported; 11: Adherence reported; 12: Adequate description of control/comparison group; 13: Supervision of exercise session; 14: Power calculation; RCT: randomised controlled trial; CT: controlled trial; Y: Yes; N: No; NR: not reported.

Table 2.5: Exercise Prehabilitation studies in patients awaiting coronary artery bypass surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measure Measure used	Results		
Arthur et al. (2001) Canada RCT	N = 246 (15%) PREHAB: N = 123 (13%) 62* years Control: N = 123 (17%) 64* years	90 min, twice a week (hospital) • 10-15 min warm up and stretching • At least 30 min aerobic interval training at 40-70% functional capacity • 10 min cool down and stretching	8.3	VO_{2peak} (ml.min) (Baseline only) LOS (days) Time in ICU (hrs)	PREHAB: Control: PREHAB: Control: PREHAB: Control:	1327.6 ± 320 1201.2 ± 288 5 [5-6]** 6 [5-7] 24.7** 26.7	
Rosenfeldt et al. (2011) Australia RCT	N = 117 PREHAB: N = 60 (22%) 62.5* years Control: N = 57 (30%) 68* years	*First 2 weeks:* 60 min, twice weekly (hospital) • 10 min gentle stretching • 40 min aerobic exercise (cycle & arm ergometry, & walking) at up to 60%HR_{max} • 30-60 min, twice weekly home based aerobic exercise *Week 3 onwards* • 30-60 min home based aerobic exercise	10	SF 36	No difference between groups		
Sawatzky et al. (2014) Canada RCT	N = 15 PREHAB: N = 8 (25%) 63 ± 9 years Control: N = 7 (14%) 64 ± 7 years	60 min, twice a week (hospital) + additional voluntary sessions. • Aerobic exercise at 85%VO_{2max} • Light resistance exercise (not defined) • Stretching • Educational sessions (diet, exercise, stress, medication use & risk factor management).	8.2 ± 2.2	6MWT (m) Baseline to post-op 5 m gait (s)	PREHAB: Control: PREHAB: Control: PREHAB: Control:	342 ±79 337 ± 52 342 ±79 337 ± 52 5.5 ± 1.7 5.3 ± 0.9	474 ± 101** 332 ± 27 487 ± 106** 357 ± 27 4.0 ± 0.7** 5.3 ± 1.0

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; P: Prehab group; C: Control group; LOS: Length of stay; ICU: Intensive care unit; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; 6MWT: 6 minute walk test; *Significantly different to baseline ($p < 0.05$); $^{+}$Significantly different to Control group ($p < 0.05$).

In a small pilot RCT using 6 minute walk test (6MWT) distance as the primary outcome, Sawatzky et al(2014) reported both improved 6MWT distance and 5-metre gait speed at the pre-operative (between group difference: 6MWT: + 136 metres; 5 metre gait speed: -1.6 sec) and 3 month post-operative (between group difference: 6MWT: + 123 metres; 5 metre gait speed: -1.2 sec) reassessments following a four to sixteen week (mean: 8.2 ± 2.2 weeks), twice a week PREHAB programme compared to standard care. The intervention lasted 60 minutes (duration of specific components not known) and was encompassed within a pre-existing CR programme consisting of different modalities of aerobic exercise (walking and cycling at 85% VO2max), resistance exercises (body weight and resistance bands) and stretching. As to be expected, given the trial was not powered to do so, no reduction was reported in ICU LOS or hospital LOS between groups however the improvement in post-operative walking distance in the PREHAB group suggests PREHAB may aid subsequent recovery following surgery.

In the final RCT comparing approximately 10 weeks of holistic treatment (mental stress reduction and 40 minutes aerobic exercise at up to 60% HR_{max}, twice weekly for two weeks followed by encouragement to perform 30 minutes walking, four times a weeks from week three onwards) with usual care, Rosenfeldt et al. (2011) failed to find a significant between group improvement in either LOS (PREHAB: 6 [5-8] days; Usual care: 6 [5-8] days) or HRQOL (SF-36 physical or mental composite scores). The absence of an objective measure of physical fitness means it is unknown whether PREHAB improved patients' fitness prior to surgery. However, the low intensity of the exercise prescribed was probably insufficient to elicit physiological change. Rosenfeldt et al. (2011) rationalised the exercise intensity used based on the potential of low intensity aerobic exercise (~40-50% HRR) to induce fitness improvements in individuals with low starting fitness (American College of Sports Medicine, 2013), however the exercise intensity actually prescribed (60% HR_{max}) would have been below that threshold (e.g. PREHAB group mean age: 62 years; 60% HR_{max}: 95 beats per minute (bpm); estimated %HRR based on resting hr of 70 bpm: ~30%).

In summary, although limited research exists into PREHAB in CABG patients, the results from Arthur et al. (2000) support the potential use of PREHAB as a means of reducing post-operative LOS. Sawatzky et al. (2014) suggested improvements in physical functioning both pre and post-surgery may be achieved following PREHAB

although the small sample mean the results need interpreting with caution. Importantly, it needs noting the mean duration (> 8 weeks) of the PREHAB intervention used in all three studies would exceed the time available in colorectal patients awaiting surgery who had not received NACRT.

2.5.2. PREHAB in lung resection surgery

The quality of evidence for PREHAB in lung resection surgery is poor (Table 2.6) with only two small RCT and a further four observational studies identified all of which were limited by small sample sizes (all n < 20). Descriptions of all studies are detailed in Table 2.7. In the only RCTs conducted, Benzo et al. (2011) reported the results of two separate trials recruiting cancer patients scheduled for lung resection surgery. Study one intended to compare 4 weeks of preoperative pulmonary rehabilitation (PR) vs. usual care, however, poor recruitment (9 patients in 18 months) led to the early termination of the trial. In study two, recruitment improved (19 patients in 12 months) to a one week PR intervention (twice daily for 5 days + weekend) consisting of upper and lower extremity endurance training (20 minutes, intensity not specified), theraband exercises (2 sets of 10-12 reps - specific details not defined) and IMT (15-20 minutes per day) reported a near significant decrease in LOS (Control: 11 ± 6.3 days; PR: 6.3 ± 3.0 days; $p = 0.058$) and a significant reduction in days with chest tubes (Control: 8.8 ± 5.3 days; PR: 4.3 ± 2.1 days; $p = 0.004$) in the PR group. Given Hulzebos et al. (2006) reported reduced pulmonary complications (IMT: 25 out of 139 [18%] patients; Control: 48 out of 137 patients [35%]) and LOS (IMT: 7 days [range: 5 to 41 days]; Control: 8 days [range: 5 to 41 days]) in high risk CABG patients following a minimum of two weeks (mean number of days: 29.7 days; [range 14-90 days]) of IMT training (20 minutes daily) only, the reported changes in Benzo et al. (2011) are potentially more likely related to the IMT component than the aerobic and resistance exercise elements of the intervention. Furthermore, given analysis was based on the results of just 17 patients (2 were excluded due to not undergoing surgery), the potential of a type 1 error having occurred cannot be ignored.

Table 2.6: Methodological quality of PREHAB studies in lung resection surgery.

Author / Study deign	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Benzo et al. (2011) Study 1 RCT	NR	NR	Y	NR	NR	NR	N	N	N	NR	NR	N	NR	N	1
Benzo et al. (2011) Study 2 RCT	Y	Y	Y	Y	N	N	Y	N	Y	Y	NR	N	Y	N	8
Bobbio et al. (2008) Observational	-	-	Y	-	-	-	Y	N	-	NR	Y	-	Y	N	4
Coats et al. (2013) Observational	-	-	Y	-	-	-	Y	N	-	Y	Y	-	N	N	4
Cesario et al. (2008) Observational	-	-	N	-	-	-	Y	N	-	NR	NR	-	Y	N	2
Jones et al. (2007) Observational	-	-	Y	-	-	-	Y	Y	-	Y	Y	-	Y	N	6

1: Treatment allocation concealed; 2: Groups/subjects similar at baseline regarding important prognostic values; 3: Eligibility criteria specified; 4: Blinded outcome assessor; 5: Was care provider blinded; 6: Was patient blinded; 7: Were point estimates and measures of variability presented for the primary outcome measures; 8: Did the analysis include an intension-to-treat analysis; 9: Between-group statistical comparison; 10: Dropout reported; 11: Adherence reported; 12: Adequate description of control/comparison group; 13: Supervision of exercise session; 14: Power calculation; RCT: randomised controlled trial; CT: controlled trial; Y: Yes; N: No; NR: not reported; -: not applicable.

Table 2.7: Exercise prehabilitation studies in patients awaiting lung resection surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measure		
				Measure used	**Results**	
Benzo et al. (2011) USA RCT (Study 1)	N = 9 (N/R) Age N/R PREHAB: N = 5 (N/R) Control: N = 4 (N/R	4 weeks of preoperative pulmonary rehabilitation using current guidelines for exercise prescription (Nici et al., 2006).	4	N/R due to poor recruitment		
Benzo et al. (2011) USA RCT (Study 2)	N = 19 (53%) Age N/R PREHAB: N = 10 (50%) Control: N = 9 (56%)	Twice daily for 5 days • 20 min lower extremity endurance exercise • 2 x 10-12 reps of resistance exercise alternating between upper and lower extremities • IMT and breathing exercises	1	Shuttle Walk test LOS (days) Complications reported	No change PREHAB: Control: PREHAB: Control:	 6.3 ± 3.0 11.1 ± 6.3 3 5
Bobbio et al. (2008) Italy Observational	N = 12 (17%) 71 ± 4 years	5 days a week 30 min at 50% WR_{peak} (progressing to 80% by week 4): • 10 min stretching • Upper extremity and core free weight resistance exercises • Breathing exercises	4	VO_{2peak} (ml.kg.min) AT (ml.kg.min) WR_{peak} (Watts) LOS (days)	Baseline: Pre-op: Baseline: Pre-op: Baseline: Pre-op:	13.5 ± 1.3 16.3 ± 1.9* 10.1 ± 1.9 13.4 ± 3.3* 65 ± 14 79 ± 19* 17.5 ± 14.8

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; P: Prehab group; C: Control group; LOS: Length of stay; ICU: Intensive care unit; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; 6MWT: 6 minute walk test; *Significantly different to baseline ($p < 0.05$); $^{+}$Significantly different to Control group ($p < 0.05$).

Table 2.7 (Continued): Exercise prehabilitation studies in patients awaiting lung resection surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measures used and results Measure used	 Results	
Cesario et al. (2008) Canada Observational	N = 8 (N/R) Age N/R	5 days a week 180 min sessions, each consisting of: • Aerobic exercise (Cycling/treadmill) at 80% WR_{peak} (Duration not stated) • Abdominal muscle activities • Educational sessions	4	6MWT (m)	Baseline: Pre-op:	261 ± 96.5 340 ± 66.5*
Coats et al. (2013) Canada Observational	N = 13 (62%) 59 ± 9 years	3-5 days a week • Cycling at 60-80% peak workload for a cumulative time of 30 min • Up to 2 x 15 repetitions of free weights and gravity assisted resistance exercises (bicep curl, wall push-up, lateral shoulder raises, wall squat, hip raise and sit ups	4	6MWT (m) VO_{2peak} (ml.kg.min) Baseline: Pre-op	Baseline: Pre-op: Baseline: Pre-op: 	261 ± 96.5 340 ± 66.5* 21.6 ± 7.8 23.3 ± 7.5
Jones et al. (2007) & Peddle et al. (2009) Canada Observational	N = 20 (70%) 65 ± 10 years	5 consecutive sessions per week until surgery • Week 1 – 60% VO_{2peak} for 20 min progressing to 65% VO_{2peak} for 30 min. • Week 2 & 3 – Four sessions at 60-65% VO_{2peak} for 25-30 min + one at VT based on VE/VO_2 ratio for 25-30 min. • Week 4 onwards – Three sessions at 60-65% VO_{2peak} and one at VT all lasting 20-30 min + one interval session. The interval session was 30 s at VO_{2peak} followed by 60 s active recovery for 10-15 reps.	4-6	VO_{2peak} (ml.kg.min) 6MWT (m) LOS (days) No change in HRQOL or fatigue	Baseline: Pre-op: Baseline: Pre-op: 	15.7 ± 3.7 18.0 ± 3.4* 438 ± 77 478 ± 75* 10 ± 8

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; P: Prehab group; C: Control group; LOS: Length of stay; ICU: Intensive care unit; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; 6MWT: 6 minute walk test; *Significantly different to baseline ($p < 0.05$); $^{+}$Significantly different to Control group ($p < 0.05$).

The remaining four studies (Bobbio et al., 2008; Cesario et al., 2007; Coats et al., 2013; Jones et al., 2007), for which changes in physical fitness (e.g. CPET variables, 6MWT) were the primary outcomes, were all single group observational designs thus limited by the absence of a control group. All four reported improved functional capacity following their respective PREHAB interventions with only one study home-based (Coats et al., 2013) and the remaining three hospital-based. Jones et al. (2007) reported an improved mean VO_{2peak} of 2.9 ml.kg.min and 6MWT distance of 40 metres post-intervention following a progressive aerobic endurance and HIT exercise intervention (5 consecutive days/week until surgery; mean no. sessions 30 ± 27 [Range 0-75 sessions]) in 20 patients scheduled for resection surgery for malignant lung lesions. No change in HRQOL or fatigue was found between baseline and pre-operative reassessment following PREHAB (Peddle et al., 2009) however given the data was based on only nine patients, it is unlikely sufficient power would have existed to detect a true significant change.

An improvement in mean VO_{2peak} (+2.8 ml.kg.min) and AT (+3.3 ml.kg.min) was also reported by Bobbio et al. (2008) following a 4 week pulmonary rehabilitation programme in 12 unfit (defined as VO_{2peak} less than 15 ml.kg.min) chronic obstructive patients with non-small cell lung cancer (NSCLC). The intervention involved five 90 minute hospital based sessions per week, consisting of incentive spirometry and controlled breathing exercises, 30 minutes of aerobic exercise at 50-80% of the workload peak obtained during the pre-intervention CPET and upper extremity and core resistance exercises (specific details not defined). No change in pulmonary function (Forced vital capacity [FVC], Forced expiratory volume in 1 second [FEV1]) was reported. In contrast, Cesario et al. (2007) reported a significant increase in FVC in following a similar four week, five sessions a week pulmonary rehabilitation programme in eight lung cancer patients. This coincided with a mean increase in 6MWT distance of 79 ± 30.4 metres.

In the only home-based study, Coats et al. (2013) investigated the effects of a 4 week exercise intervention in NSCLC patients scheduled for lung resection surgery. The intervention included 30 minutes of aerobic exercise at 60-80% of a predetermined workload peak, 3-5 times a week and resistance exercise (specific detail not defined) three times a week. Unlike in Jones et al. (2007) and Bobbio et al. (2008), no significant improvement was seen in the VO_{2peak} of the 13 patients to complete the intervention,

with a number of factors (e.g. baseline fitness, number of sessions, training intensity) potentially contributing to this. The mean baseline VO_{2peak} in Coats et al. (2013) was higher (21.6 ± 7.8 ml.kg.min) that either Jones et al. (2007) (15.7 ± 3.7 ml.kg.min) or Bobbio et al. (2008) (13.5 ± 1.3 ml.kg.min), therefore a ceiling effect may have existed on the improvements that could be achieved. Furthermore, in Jones et al. (2007), the greater mean number of sessions (30 ± 27 sessions) compared to 15 ± 5 sessions in Coats et al. (2013) as well as the inclusion of higher intensity training (HIT and exercise at ventilatory threshold) were also likely to be contributory factors for the difference. In Coats et al. (2013), significant improvements in duration of cycling at a constant workload (+157 ± 195 seconds) and 6MWT distance walked (+28 ± 29 metres), as well as deltoid (+1.8 ± 2.8 kg), triceps (+1.3 ± 1.8 kg) and hamstring (+3.4 ± 3.7 kg muscle strength were demonstrated post intervention. Achieving improved muscular endurance and strength prior to surgery may therefore play an important role in post-surgical recovery given the debilitating effects of undergoing surgery. There was no change in any HRQOL parameter as measured by the SF-36 or the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) post-intervention, however a clinically significant reduction in depression according to the hospital and anxiety scale (HADS) was reported. Again as the study was not powered to detect a change using the HADS, the limited sample size means the potential of a type 1 error having occurred cannot be ignored.

In summary, although the quality of the research is limited, there does appear to be evidence that PREHAB can improve physical fitness prior to surgery in lung resection patients. It needs noting however, with the exception of Coats et al. (2013), all interventions consisted of at least five hospital-based supervised exercise sessions a week therefore a considerable time and resource (money, facility and staffing availability) burden would be placed on both the exercise provider and patient in order to participate in the intervention.

2.5.3. PREHAB in gastrointestinal and colorectal surgery

In addition to Asoh and Tsuji (1981), seven further studies (four RCT, two controlled trials and one observational trial) have examined the effect of exercise PREHAB in patients undergoing gastrointestinal/colorectal surgery (Tables 2.8 and 2.9). None of these however were powered to detect changes in hospital LOS or

complications with measures of physical fitness (6MWT, VO_{2peak}, predicted VO_{2peak}) the preferred primary outcome measures. Four of these studies (Carli et al., 2010; Gillis et al., 2014; Kim do et al., 2009; Li et al., 2013) were conducted by the same research group based at MacGill University, Canada. In their initial study (Carli et al., 2010), a randomised sham controlled trial (n = 133) compared bike and strengthening exercises (20-30 minutes daily aerobic exercise starting at 50% HR_{max} and progressing by 10% each week as tolerated in addition to weight training of the major groups – e.g. biceps, quadriceps, hamstrings, three times a week) with a sham walking group (30 minutes walking daily and breathing exercises). Over a four week period, patients in the bike/strengthening group were expected to have completed approximately 14 hours of exercise/physical activity, a figure only 16% fully adhered to. Carli et al. (2010) attributed this to the intensive nature of the intervention potentially proving intimidating to patients with a low baseline fitness levels. No improvement in 6MWT distance was observed in either group at pre-operative reassessment or during the post-operative follow-up period. It is important to note, the study proved underpowered to detect a change at post-operative reassessment based on the power analysis that was performed for the primary outcome (6MWT). A total sample of 128 patients (64 per group) was identified as being required despite randomising 133 patients, issues with patients not receiving surgery (n = 21) and attrition meant complete data was only available for 68 patients; a figure increased to 112 following multiple imputation of missing data. Interestingly, a greater percentage of the walking/breathing group displayed improved 6MWT performance compared to baseline following the preoperative (47% vs. 22%) and post-operative (41% vs. 11%) periods although task specificity (e.g. walking intervention and walking based outcome measure) may have contributed to this.

Table 2.8: Methodological quality of PREHAB studies in gastrointestinal and colorectal surgery.

Author / Study deign	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Carli et al. (2010) RCT	N	Y	Y	N	N	N	Y	Y	Y	Y	NR	Y	N	Y	8
Donkers et al. (2010) RCT	Y	Y	Y	Y	N	N	Y	Y	Y	NR	Y	N	Y	N	9
Gillis et al. (2014) RCT	Y	Y	Y	Y	N	N	Y	N	Y	Y	Y	Y	N	Y	10
Kim et al. (2009) RCT	N	Y	Y	N	N	N	Y	Y	N	Y	Y	Y	N	N	7
Li et al. (2013) CT	-	-	Y	-	-	-	Y	Y	Y	Y	N	Y	N	N	5
West et al. (2014) CT	-	-	Y	-	-	-	Y	N	Y	Y	Y	-	Y	Y	7
Asoh & Tsuji (1981) Obs	-	-	N	-	-	-	Y	N	-	Y	N	-	NR	N	2
Timmerman et al. (2010) Obs	-	-	Y	-	-	-	Y	N	-	Y	Y	-	Y	N	5

1: Treatment allocation concealed; 2: Groups/subjects similar at baseline regarding important prognostic values; 3: Eligibility criteria specified; 4: Blinded outcome assessor; 5: Was care provider blinded; 6: Was patient blinded; 7: Were point estimates and measures of variability presented for the primary outcome measures; 8: Did the analysis include an intension-to-treat analysis; 9: Between-group statistical comparison; 10: Dropout reported; 11: Adherence reported; 12: Adequate description of control/comparison group; 13: Supervision of exercise session; 14: Power calculation; RCT: randomised controlled trial; CT: controlled trial; Obs: observational trial; Y: Yes; N: No; NR: not reported; -: not applicable.

Table 2.9: Exercise prehabilitation studies in patients awaiting gastrointestinal and colorectal resection surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measure Measure used	Results	
Asoh and Tsuji (1981) Japan Observational	N = 29 68 years Range: 57 to 82 years	20 min cycling twice daily • Work rate not exceeding 130 bpm • Classified as unfit if patient unable to achieve steady state HR walking at 3 km/hr	1-3	Unfit for surgery: *Unable to achieve steady state HR walking at 3 km/hr* In hospital mortality	Baseline: Pre-op: Fit: Unfit:	11/29 unfit 4/29 unfit 0 of 25 2 of 4
Carli et al. (2010) Canada RCT	61 ± 16 years Control: N = 54 (43%) 60 ± 15 years	Bike/strengthening group • 20-30 min daily cycling starting at 50%HR_{max} (increasing by 10% per week if tolerable) • Weight training for 10-15 min, 3 times a week (sit-ups, push-ups & lunges – up to 12 repetitions and bicep curls, deltoid lateral lifts and squats to volitional fatigue. Walk/breathing group • Walking daily for at least 30 min • Breathing exercises 5 min per day • 5-10 min of circulation exercises	7.7 ± 7.6	6MWT (m) Base to Pre-op Base to 2-4 months Post-op VO_{2peak} (ml.min) LOS (days) HADS (Depression)	Bike: Walk: Bike: Walk: Bike: Walk: Bike: Walk: Bike: Walk: No change in HADS anxiety in either group	474 ± 15 to 464 ± 19 494 ± 16 to 503 ± 16 474 ± 15 to 440 ± 19 494 ± 16 to 482 ± 19 1395 ± 76 to 1529 ± 88* 1400 ± 71 to 1511 ± 84 11.9 ± 34.6 6.6 ± 3.6 4.0 ± 0.5 to 3.2 ± 0.4* 3.6 ± 0.4 to 3.4 ± 0.5

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; Observational: Observational trial; P: Prehab group; C: Control group; LOS: Length of stay; IMT:Inspiratory muscle training; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; AT: Anaerobic threshold; WR_{peak}: Peak work rate; 6MWT: 6 minute walk test; bpm: beats per minute; TUG: timed up and go test; *Significantly different to baseline ($p < 0.05$); *Significantly different to baseline ($p < 0.05$).

Table 2.9 (continued): Exercise prehabilitation studies in patients awaiting gastrointestinal and colorectal resection surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measure Measure used	Results
Dronkers et al. (2010) Holland RCT	N = 42 (11) PREHAB: N = 22 (32%) 71 ± 6 years Control: N = 20 (20%) 69 ± 6 years	60 mins, twice weekly consisting of: • Lower limb extensor muscle resistance training • 15 min IMT • 20-30 mins aerobic training at 55-75% HR_{max} (11-13 on Borg scale) • Functional activities training • 30 min daily walking/cycling	2-4	Predicted VO_{2peak} (ml.kg.min) TUG (s) LOS (days) Complications HRQOL	PREHAB: 29.4 ± 9.5 to 27.6 ± 6.5 Control: 31.6 ± 6.5 to 32.9 ± 6.9 PREHAB: 8.0 ± 3.6 to 7.8 ± 3.3 Control: 6.4 ± 1.3 to 6.6 ± 1.2 PREHAB: 16.2 ± 11.5 Control: 21.6 ± 23.7 PREHAB: 9 Control: 8 No change in EORTC QLQ-C30 in either group
Gillis et al. (2014) Canada RCT Versus 8-week post-op rehab only	N = 77 PREHAB: N = 38 (45%) 66 ± 14 years Control: N = 39 (31%) 66 ± 9 years	50 min sessions, 3 times a week including: • 20 min aerobic exercise at 40% HRR • 10-12 reps of the following resistance exercises (~20 min): Push ups, Bicep curls, Triceps extension, Deltoid lateral lifts, Squats, Hamstring curls, Calf raises, Abdominal curls Nutrition • Whey protein 1.2 g/kg BW per day Anxiety reduction • 90 min psychologist visit providing anxiety reduction techniques	4 (+ 8 weeks post-op rehab)	6MWT (m) *Base to Pre-op* *Base to 2 months Post-op* LOS (days) HRQOL	PREHAB: 421 to 446* Control: 425 to 409 PREHAB: 421 to 444* Control: 425 to 403 PREHAB: 4 [3-5] Control: 4 [3-7] No change in SF-36 or HADS in either group

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; P: Prehab group; C: Control group; LOS: Length of stay; ICU: Intensive care unit; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; 6MWT: 6 minute walk test; *Significantly different to baseline ($p < 0.05$); $^{+}$Significantly different to Control group ($p < 0.05$).

Table 2.9 (continued): Exercise prehabilitation studies in patients awaiting gastrointestinal and colorectal resection surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measure Measure used	Results	
Kim et al. (2009) Canada RCT	N = 21 PREHAB: N = 14 (36%) 55 ± 15 years Control: N = 7 (43%) 65 ± 9 years	Progressive daily programme • 20-30 min cycling at 40 to 65% HRR (11-12 to 16 RPE)	4	VO_{2peak} (ml.min) 6MWT (m)	PREHAB: Control: PREHAB: Control:	21.5 ± 10.1 to 20.9 ± 8.7 20.3 ± 4.6 to 19.9 ± 5.6 436 ± 64 to 467 ± 80 478 ± 99 to 504 ± 103
Li et al. (2013) Canada CT	N = 87 PREHAB: N = 42 (48%) 67.4 ± 11 years Control: N = 45 (36%) 66.4 ± 12 years	Exercise • 30 minutes aerobic exercise 3 times a week at 50% predicted HR_{max} • Resistance exercise as in Gillis et al. (2014) Nutrition • As in Gillis et al. (2014) Anxiety reduction • As in Gillis et al. (2014)	6	6MWT (m) *Base to Pre-op* *Pre-op to 2 months Post-op* LOS (days) HADS anxiety HADS depression	PREHAB: Control: PREHAB: Control: PREHAB: Control: PREHAB: Control:	422 ± 87 to 464 ± 92 m* NR to 402 ± 57 464 ± 92 to 459 ± 101 402 ± 57 to 375 ± 58 4 (3-6) days 4 (3-6) days 5 (2.3-8.8) to 4 (2-6)$^{+}$ N/R 2.5 (1-4) to 1(0-2)$^{+}$ N/R

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; P: Prehab group; C: Control group; LOS: Length of stay; ICU: Intensive care unit; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; 6MWT: 6 minute walk test; *Significantly different to baseline ($p < 0.05$); $^{+}$Significantly different to Control group ($p < 0.05$).

Table 2.9 (continued): Exercise prehabilitation studies in patients awaiting gastrointestinal and colorectal resection surgery.

Authors Country Design	Total patients (% Female) Mean age ± SD	Intervention	Duration (weeks)	Outcome measure Measure used		
Timmerman et al. (2011) Holland Observational	N = 15 (20%) 59 ± 8 years	Two times a week for 2 hours • 30-50 minutes aerobic exercise at 65-85% HRR • Machine based resistance exercises targeting, arms, shoulders, chest, abdomen, back, hips, and legs. 3 sets of 12-20 reps at 60-80% 1RM	5	Predicted VO_{2peak} (ml.kg.min) Grip strength (N)	Base: Pre-op: Base: Pre-op:	25 ± 0.5 33 ± 0.9* 894 ± 158 961 ± 166*
West et al. (2015) UK CT	N = 35 PREHAB: N = 22 (36%) 64 years Range: 45-82 years Control: N = 13 (31%) 72 years Range: 62-84 years	Three times a week starting immediately post NACRT • Interval training • 6 x 3 minutes at 80% WR at VO_2 at AT (moderate intensity) • 6 x 2 minutes at 50% of difference in WR between VO_{2peak} and VO_2 at AT (severe intensity 5 minutes warm up and cool down	6	VO_{2peak} (ml.kg.min) VO_2 at AT (ml.kg.min)	PREHAB: Control: PREHAB: Control:	16.0 (4.3) to 18.7 (4.3)* 15.7 (5.0) to 14.4 (4.5) 10.3 (2.6) to 12.4 (2.7)* 10.1 (3.4) to 9.5 (2.9)

SD: Standard deviation; N/R: Not reported; RCT: Randomised controlled trial; P: Prehab group; C: Control group; LOS: Length of stay; ICU: Intensive care unit; HR_{max}: Maximum heart rate; VO_{2peak}: Peak oxygen uptake; 6MWT: 6 minute walk test; *Significantly different to baseline ($p < 0.05$); $^{+}$Significantly different to Control group ($p < 0.05$).

Two subsequent follow-up studies (Gillis et al., 2014; Li et al., 2013) implemented a trimodal PREHAB programme incorporating a home based exercise intervention consisting of aerobic (30 minutes, three times a week at 50% HR_{max}) and resistance exercises (eight exercises, three times a week to volitional exhaustion), whey protein supplementation (increasing daily protein intake of 1.2g/kg body weight) and a 90 minute session with a psychologist learning anxiety reduction techniques. In a observational controlled pilot study (PREHAB: n = 42; Control: n = 45), Li et al. (2013) reported a significant improvement in 6MWT distance of 42 ± 41 metres between baseline and preoperative assessment following 3 to 7 weeks (median duration: 33 days) of PREHAB. The absence of a baseline assessment for the control group (the first assessment took place one week prior to surgery) however means the true meaning of the improvement is difficult to interpret as a learning effect has previously been found for the 6MWT in the absence of sufficient familiarisation (Kervio, Carre, & Ville, 2003). Potentially of more importance though, at 8 weeks post-surgery a significantly greater percentage of the intervention group (81% vs. 40%) had returned to or above baseline levels for functional capacity. This coincided with greater levels of self-reported physical activity in the PREHAB group at both 4 (PREHAB: 18 kcal/kg/wk; Control: 3 kcal/kg/wk) and 8 weeks (PREHAB: 23 kcal/kg/wk; Control: 8 kcal/kg/wk) post-surgery. No significant differences for hospital LOS, complications or HRQOL were reported between groups however anxiety and depression for the PREHAB group decreased by one point each on the HADS scale ($p < 0.05$). The clinical significance of such a small change is hard to interpret, especially with the absence of corresponding data from the control group.

In an RCT, Gillis et al (2014) reported the same trimodal PREHAB intervention (4 weeks followed by 8 weeks post-operative rehabilitation against 8 weeks post-operative rehabilitation only) resulted in a greater number of patients (32 out of 38; 82%) returning to baseline 6MWT distance by 8 weeks post-surgery than with rehabilitation alone (24 out of 39; 62%). Bordes, Cardinal, and Kaiser (2015) highlighted the greater compliance to post-operative rehabilitation in the PREHAB group (53%) compared to rehabilitation alone (31%) as a likely explanation for the difference. It may however be argued that the benefits of PREHAB go beyond the pre-operative period, serving as an ideal opportunity to educate and prepare patients prior to post-surgery rehabilitation (Gillis et al., 2014).

Two further pilot RCTs were conducted by Kim do et al. (2009) and Dronkers et al. (2010). Kim do et al. (2009) (n = 21) reported peak power output (+26 ± 27%; Effect size (ES): 0.24) was the most responsive maximal variable whilst oxygen consumption (-13 ± 15%; ES: -0.24) and heart rate (-7 ± 6%; ES: -0.40) at a given submaximal workload were the most responsive submaximal variables following a four week, daily progressive home-based aerobic exercise programme (20-30 minutes of daily progressive cycling at 40-65% HRR) in patients scheduled for bowel resection surgery. An 8% increase (ES: 0.48) in 6MWT distance was also reported for the intervention group; however this was almost matched by a 6% increase (ES: 0.27) in distance in the control group. A learning effect potentially accounted for the improvement in both groups (Kervio et al., 2003) although it was noted during the PREHAB period, the self-reported amount of walking performed by the control group also increased, therefore contributing to the improvements observed (Kim do et al., 2009). Dronkers et al. (2010) reported no significant difference in functional capacity (predicted VO_{2peak} , timed up and go (TUG) & chair rise tests), HRQOL (EORTC QLQ-C30), post-operative complications or LOS between groups following a 2-4 week PREHAB intervention (two 60 minute supervised sessions a week involving lower limb resistance exercises, 20-30 minutes aerobic training at 55-75% HRR and 15 minutes IMT) prior to colon surgery. Given the low number of sessions (5.1 ± 1.9) performed by the PREHAB group, it is possible the training stimulus was insufficient to evoke adaptive changes, although the different measures of functional capacity used makes comparisons with the other studies difficult. Dronkers et al. (2010) did report increased respiratory muscle endurance (as measured by energy expenditure) in the PREHAB group (+146 ± 160 joules; $p < 0.001$) but not the control group (-44 ± 279 joules; $p > 0.05$) with the inclusion of IMT the most likely explanation.

In the most recent study published to date, West et al. (2015) implemented a slightly different approach to PREHAB, enrolling rectal cancer patients (PREHAB: n = 22; Control: n = 13) at the conclusion of NACRT in a six week PREHAB programme followed by an 8 week rest period prior to surgery. The same research group had previously reported the detrimental effect of NACRT on VO_2 at AT and VO_{2peak} (Jack et al., 2014; West et al., 2014). Using a 40 minute interval training protocol (which alternated between moderate (6 x 3 minutes at 80% of workload at AT) and severe (50% of difference in workload between VO_{2peak} and VO_2 at AT) intensity workloads, three times a week) at the end of the six week period, the PREHAB group had exceeded

their pre-NACRT levels for both VO_{2peak} and VO_2 at AT, whilst the control group had experienced worsening fitness levels (Table 2.9). Furthermore, by the time of surgery (14 weeks post-NACRT), VO_2 at AT had continued to decline in the control group whilst the PREHAB maintained VO_2 at AT at pre-NACRT levels. The authors acknowledged the use of a non-randomised design (controls were individuals unable to commit to the intervention) weakens the strength of the study to a certain degree as does the significantly older mean age of the control group (PREHAB: 64 years; Control: 72 years) however the results do highlight the extended time window for PREHAB that may exist in patients receiving NACRT prior to surgery.

2.5.4. A role for resistance training in PREHAB

In the general population, due to sedentary living, as well as in ageing populations a number of common movement deficits can develop all of which can impact on the individual's ability to perform activities of daily living (Sahrmann, 2002). For example, a reduction in ankle range of motion is typically seen in ageing populations (Menz, Morris, & Lord, 2005; Schultz, 1992), resulting in decreased gait speed (Daley & Spinks, 2000), impaired balance and increased risk of falls (Menz et al., 2005; Menz, Morris, & Lord, 2006). Similarly, a reduction in hip extension has been observed in older populations when walking (Kerrigan, Lee, Collins, Riley, & Lipsitz, 2001), a problem potentially further exacerbated by sedentary behaviour (e.g. prolonged periods of sitting) with an increase in hip flexor tightness and decreased gluteal activation identified as likely causes (Kerrigan et al., 2001). A consequence of such reductions in ankle dorsiflexion and hip flexion is an increased risk of falls in ageing as a result of reduced ground clearance during gait (Alcock, Vanicek, & O'Brien, 2013). The presence of these aforementioned movement deficits can affect other joints situated higher or lower up the kinetic chain as they attempt to compensate for the deficiencies (Boyle, 2010). A reduction in ankle mobility is likely to result in compensatory movement from the knee, resulting in decreased stability and the potential development of knee pain (Boyle, 2010). This in turn can impact on gluteal (medial and maximus) activation resulting in reduced hip extension. As a compensatory mechanism for reduced hip extension, increased anterior pelvic tilt can be experienced potentially predisposes the individual to low back pain (Kerrigan et al., 2001). The presence of these deficiencies therefore interact along the kinetic chain, impacting on the performance of many simple everyday tasks such as walking and rising from a chair;

furthermore these problems are likely to be exacerbated by the trauma of undergoing major surgery.

In recognition of the relationship between the different joints and muscle groups throughout the body, and the impact that muscular dysfunction in one area of the body can have throughout other areas of the body (e.g. reduced thoracic mobility resulting in neck or shoulder pain, or lower back pain, the joint-by-joint approach to resistance training was developed by Michael Boyle and Grey Cook, among others (Boyle, 2010; Cook & Burton, 2010). The theory behind this approach identifies that in order for efficient movement to occur, a kinetic chain whereby energy and power is effectively transferred through the body must exist (Cook & Burton, 2010). The presence of deficiencies at any point in this kinetic chain therefore limits the effectiveness of the movement (Cook & Burton, 2010). As a result, Boyle and Cook argue resistance training should be functional, focusing on the movement and function of the joint rather than targeting specific muscles (Boyle, 2010; Cook & Burton, 2010). This view was based on the idea that each joint in the human body has specific functions relating to either mobility (e.g. ankle and hip joints) or stability (e.g. lumber spine/core and scapulae) (Table 2.10). To date these principles have not been applied to resistance training in PREHAB for surgical patients.

Table 2.10: Joint-by-joint primary training needs as proposed by Boyle (2010).

Joint	Primary training need
Ankle	Mobility (Sagittal)
Knee	Stability
Hip	Mobility (Multi-planar)
Lumber spine	Stability
Thoracic spine	Mobility
Scapula	Stability
Gleno-humeral	Mobility

Within the PREHAB studies reviewed in the preceding sections (section 2.5.1. to 2.5.3) it appears that resistance/flexibility training, if even included, is mainly supplementary to aerobic exercise. Whilst improving aerobic fitness is an attractive proposition, participation in such a programme is dependent on the patients being physically capable of performing prolonged periods moderate intensity aerobic exercise

and/or short bouts of high intensity intermittent exercise. As a consequence, this may result in the exclusion of patients who may not be physically capable of adhering to the PREHAB interventions discussed, however who may still benefit from a period of PREHAB. There is therefore an argument for placing greater emphasis on functional resistance/flexibility exercises aiming to enhance activities of daily living, especially when the period of time to surgery is likely to be limited.

To date, rather than focus on functional training, the resistance elements of PREHAB programmes have typically focused on isolated major muscle groups (e.g. quadriceps/hamstrings; biceps/triceps; abdominals) through the use of single joint exercises. For example, the studies by the Canadian research group led by Franco Carli (Carli et al., 2010; Gillis et al., 2014; Li et al., 2013; Table 2.9) included resistance band and free weight exercises such as bicep curls, triceps extension, deltoid lateral lifts and hamstring curls performed with a resistance to cause volitional fatigue within 10-12 repetitions. A criticism of these sorts of exercises however is that the movements involved often fail to replicate those of everyday activities, requiring less stabilisation and balance, and limiting the range of motion needed (Beckham & Harper, 2010). This criticism is even greater in machine based resistance training such as that used in Timmerman et al. (2011)(3 sets of 12-20 reps at 60-80% 1RM targeting arms, shoulders, chest, abdomen, back, hips, and legs). Even the multi-joint exercises such as squats which were included by Carli et al. (2010), Gillis et al. (2014) and Li et al. (2013) potentially fail to address the impact reduced ankle or hip mobility likely to have on performing the exercise correctly given the ageing populations studied (mean age: all > 60 years). Incorrect technique therefore will increase the risk of injury and potential impact of adherence and drop rates. Given none of the PREHAB studies to date appear to have addressed the importance of the relationships that exists between the joints and muscles within the kinetic chain as a whole, the potential role of more a focused and therefore inclusive functional resistance training in PREHAB represents a gap within the current literature.

2.5.5. Feasibility and adherence of PREHAB

When implementing an exercise intervention in a clinical population an interest in participation is essential in order to make it time efficient and cost effective. Table 2.11 displays information relating to recruitment and adherence in the PREHAB studies

reported above. In the eight studies to report information regarding recruitment uptake of eligible participants, recruitment figures ranged from an uptake of 29% (Sawatzky et al., 2014) up to 89% (Li et al., 2013). Common obstacles to recruitment involved lack of time/work commitments, distance to be travelled/lack of transportation and in some cases a general lack of interest. Whilst the recruitment uptake of 29% reported in Sawatzky et al., (2014) represents an uptake of less than one third of eligible participants, to put this figure into context in 2011-2012 the enrolment onto established CR programmes in the UK averaged only 43% of eligible patients (British Heart Foundation, 2013). The highest reported recruitment uptake was seen in the home based PREHAB studies by Carli et al. (2010) and Li et al. (2013) who reported recruitment of levels of 84% and 89% respectively. Utilising home based interventions therefore may represent the most effective means of recruiting participants as the highest uptake for hospital based programme was the 72% seen in CABG patients by Arthur et al. (2000).

Table 2.11: Patient recruitment uptake and retention to PREHAB studies.

Author	Surgery type	Study design	Home or hospital based	Study recruitment uptake	Reasons for non-participation (*more than one reason per participant)	Failures to complete programme (e.g. Intervention dropouts, failed to complete pre-surgery reassessment or became ineligible during study)	Number of sessions	Adherence	Exercise related adverse events
Arthur et al. (2000)	CABG	RCT	Hospital	249/347 (72%)	N/R	10/123 (8%)	14 [1 -57]	N/R	N/R
Rosenfeldt et al. (2011)	CABG	RCT	Both	117/123 (95%)	• None given	0/117 (0%)	N/R	N/R	N/R
Sawatzky et al. (2014)	CABG	RCT	Hospital	17/58 (29%)	• No time (n = 25) • Distance/no transport (n = 5) • No reason (n = 5) • Other (n = 5)	0/8 (0%)	9 ± 7	N/R	0
Benzo et al. (2011) Study 1	Lung	RCT	Hospital	9 patients in 18 months	• Patients and care providers unwilling to delay surgery (n = not stated)	0/5 (0%)	N/R	N/R	0
Benzo et al. (2011) Study 2	Lung	RCT	Hospital	19 patients in 12 months	N/R	1/10 (10%)	10 sessions in one week	100%	0

CABG: Coronary artery bypass graft; RCT: Randomised controlled trial; Obs: Observational ; N/R: Not reported;

Table 2.11 (continued): Patient recruitment uptake and retention to PREHAB studies.

Author	Surgery type	Study design	Home or hospital based	Study recruitment uptake	Reasons for non-participation (*more than one reason per participant)	Failures to complete programme (e.g. Intervention dropouts, failed to complete pre-surgery reassessment or became ineligible during study)	Number of sessions	Adherence	Exercise related adverse events
Bobbio et al. (2008)	Lung	Obs	Hospital	12 patients in 24 months	• Excluded if unable to adhere to all sessions (n = not stated)	0/12	N/R	16% fully adherent	N/R
Cesario et al. (2008)	Lung	Obs	Hospital	8/12 (66%)	N/R	0/8 (0%)	5.1 ± 1.9	97%	0
Coats et al (2013)	Lung	Obs	Home	16/40 (40%)	• No time (n = 6) • Lack of interest (n = 5) • Anxiety (n = 3) • Not specified (n = 6)	3/16 (19%)	N/R	78%	0
Jones et al (2009)	Lung	Obs	Hospital	25/35 (71%)	• Work commitments (n = 1) • Lack of interest (n = 6) • Distance/no transport (n = 1) • Already exercising (n = 2)	7/25 (28%)	27 ± 9	74 ± 16%	0

RCT: Randomised controlled trial; Obs: Observational ; N/R: Not reported;

Table 2.11 (continued): Patient recruitment uptake and retention to PREHAB studies.

Author	Surgery type	Study design	Home or hospital based	Study recruitment uptake	Reasons for non-participation (*more than one reason per participant)	Failures to complete programme (e.g. Intervention dropouts, failed to complete pre-surgery reassessment or became ineligible during study)	Number of sessions	Adherence	Exercise related adverse events
Carli et al (2010)	CRC	RIT	Home	133/159 (84%)	N/R	BS: 17/66 (26%) WB: 23/67 (34%) Total: 40/133 (30%)	N/R	16% fully adherent	N/R
Dronkers et al (2010)	GIC	RCT	Both	N/R	N/R	3/22 (14%)	5.1 ± 1.9	97%	0
Gillis et al (2014)	CRC	RCT	Home	89/106 (84%)	• Distance/no transport (n = 6) • Declined to participate (n = 8) • Non English or French speaking (n = 3)	7/45 (16%)	N/R	78%	0
Kim et al (2009)	CRC	RCT	Home	N/R	N/R	2/14 (14%)	27 ± 9	74 ± 16%	0
Li et al (2013)	CRC	CT	Home	46/52 (89%)	N/R	4/46 (9%)	N/R	45% fully adherent 70% two sessions a week	N/R

CRC: Colorectal cancer; GIC: Gastrointestinal cancer; RC: Rectal cancer; RIT: Randomised interventional trial; RCT: Randomised controlled trial; CT: Control trial; Obs: Observational ; N/R: Not reported; BS: Bike & strengthening exercises; WB: Walking & breathing exercises

Table 2.11 (continued): Patient recruitment uptake and retention to PREHAB studies.

Author	Surgery type	Study design	Home or hospital based	Study recruitment uptake	Reasons for non-participation (*more than one reason per participant)	Failures to complete programme (e.g. Intervention dropouts, failed to complete pre-surgery reassessment or became ineligible during study)	Number of sessions	Adherence	Exercise related adverse events
Timmerman et al (2011)	GIC	Obs	Hospital	15/36 (42%)	• Distance/no transport* (n = 21) • Insufficient time (n = 4)* • Lack of interest (n = 1)* • Too weak (n = 2)*	0/15 (0%)	9 (median) 8-11 IQR 5-15 range	84%	0
West et al (2015)	RC	CT	Hospital	N/R	N/R	0/22 (0%)	18	96%	0

CRC: Colorectal cancer; GIC: Gastrointestinal cancer; RC: Rectal cancer; RIT: Randomised interventional trial; RCT: Randomised controlled trial; CT: Control trial; Obs: Observational ; N/R: Not reported.

Once enrolled in an intervention, both patient retention and adherence can potentially influence the success of the programme. All six studies to report adherence based on mean percentage of sessions attended achieved levels in excess of 70% with the greatest adherence seen in hospital based studies by West et al. (2015) and Dronkers et al. (2010) (96% and 97% respectively). Alternatively, the studies by Carli et al. (2010) and Li et al. (2013) both reported adherence as a measure of participants achieving all scheduled sessions with only 16% fully adherent in Carli et al. (2010) and 45% in Li et al. (2013). This potentially highlights a limitation of home based programmes as in the absence of supervision, adherence may not be maintained. Furthermore, monitoring adherence to home based interventions is complicated by the reliance on the patient to provide an accurate account of self-reported engagement.

The use of PREHAB therefore seems to be feasible in terms of both high levels of patient recruitment and subsequent adherence. Key considerations when designing PREHAB programmes in terms of recruitment and adherence appear to be:

- Whether to utilise a home or hospital based programme: home-based may encourage recruitment whilst hospital based may promote improved adherence?
- Number of sessions per week: the more sessions per week may result in lower adherence however is attending five out of seven sessions per week better than having just three sessions per week that are fully adhered to? What are the cost implications of missed sessions?
- Training load and/or intensity: interventions with a high training intensity and/or load may increase the physical and physiological changes observed however whilst placing the individual at increased risk of injury.

2.5.6. A role for PREHAB in the ERAS pathway?

As is evident from the majority of studies reviewed above, a short period of PREHAB appears effective at improving pre-operative functional mobility in patients scheduled for a CABG or resection surgery for lung and colorectal cancer. Post-intervention improvements in 6MWT distance have been reported in patients scheduled for CABG (Sawatzky et al., 2014), lung resection (Cesario et al., 2007; Coats et al., 2013; Jones et al., 2007) and colorectal resection surgery (Kim do, Mayo, Carli, Montgomery, & Zavorsky, 2009; Li et al., 2013). Furthermore, participating in pre-surgical exercise appears to assist in regaining post-surgical functional mobility (Gillis

et al., 2014; Li et al., 2013; Sawatzky et al., 2014) with the effectiveness potentially greater than in those patients receiving post-surgery rehabilitation alone (Gillis et al., 2014). Ageing populations in particular can experience reduced muscular endurance and increased fatigue (Bautmans et al., 2010); therefore PREHAB potentially could have an important role in improving post-surgical recovery.

Any intervention with the ability to elicit improvements in cardiorespiratory fitness prior to surgery could potentially have clinically significant benefits. Associations have been made between post-surgical mortality and morbidity and various CPET derived variables. In gastrointestinal surgery, a cut-off point of <11 ml.kg.min for AT has been identified as a predictor of patients at a high risk of post-operative cardiorespiratory complications and mortality (Older et al., 1999; Wilson et al ., 2010) whilst a deficiency in ventilatory efficiency ($VE/VCO^2 > 34$) may also be predictive of poor post-operative mortality (Wilson et al., 2010). Of the studies to look at changes in CPET variables following PREHAB, Bobbio et al (2008) and West et al (2015) reported increases in VO_2 at AT following their respective interventions however, the results regarding VO_{2peak} remain equivocal with both post-intervention increases in VO_{2peak} (Bobbio et al., 2008; Carli et al., 2010; Jones et al., 2007; Timmerman et al., 2011; West et al., 2015) and no changes (Coats et al., 2013; Dronkers et al., 2010; Kim do et al., 2009) having been reported.

It is apparent from the variety in exercise interventions used throughout previous research that a general consensus regarding the most appropriate duration, intensity, frequency and modality is yet to be determined. Whilst interventions of high frequency and intensity may represent the best chance of improving cardiorespiratory fitness and strength, in previously sedentary and elderly populations these may increase the risk of injury whilst also proving demotivating in the absence of suitable social support. Moreover, in these populations where increased cases of impaired mobility may exist, such interventions can prove exclusive, with individuals unable to perform cycle/treadmill based exercise typically excluded. This is despite the fact improvements in functional mobility prior to surgery may translate into earlier mobilisation post-operatively, a component that strong emphasis is placed on in most ERAS guidelines (Fearon et al., 2005; Gustafsson et al., 2012).

In most instances, the intervention duration is determined by pre-surgical waiting time alone as delaying surgery to remove malignancies is likely to be counterproductive and goes against NHS target times for initiation of treatment within 31 days of diagnosis (Baker & Nakatudde, 2015). The exception to this is potentially when NACRT is part of the treatment course prior to surgery. Undergoing chemoradiotherapy has been reported to result in a clinically significant reduction in cardiorespiratory fitness in both oesophagus and rectal cancer patients awaiting surgery (Jack et al., 2014; West et al., 2014a). The typical 6-12 week recovery period that exists between the conclusion of treatment and undergoing surgery therefore represented an opportunity for longer periods of exercise training to be delivered (Levett & Grocott, 2015).

Whether the improvements in physical fitness achieved during a period of PREHAB is translated into improved clinical outcomes remains unclear. A reoccurring issue within these studies has either been that clinical outcomes such as LOS have not been the primary outcome measure or the studies were ran as pilot or feasibility studies, meaning they were underpowered to detect any potential changes. To date, only the CABG study of Arthur et al., (2000), for which hospital LOS was the primary outcome measure, has had a sufficient sample size to report a significant reduction (n = 246). All other studies (Carli et al., 2010; Li et al., 2013; Sawatzky et al., 2014) have failed to report a reduction in hospital LOS despite improvements in either 6MWT distance or VO_{2peak} being achieved. Similarly, as of yet, a significant reduction in either post-operative complications or HRQOL have yet to be identified as a result of a PREHAB intervention. The occurrence of complications post-surgery can often be multi factorial, influenced by factors including: the surgeons' experience (Kirchhoff et al., 2010), the individual's physical fitness (Older et al., 1999; Wilson et al., 2010); and for cancer patients, whether they received NACRT prior to surgery (Fujita et al., 2014) all contributing to the risk of complications developing. In respect to HRQOL, participation in physical activity and exercise has previously been reported to have the potential to improve mental well-being and HRQOL (Kvam, Kleppe, Nordhus, & Hovland, 2016; Rebar et al., 2015). Inadequate sample sizes mean meaningful changes in HRQOL may not be detected within the current PREHAB literature. Moreover, it may be argued that the use of generic HRQOL questionnaires may actually fail to capture the true multidimensional changes in QOL that may occur within patients undertaking PREHAB (Burke et al., 2013).

The addition of PREHAB to the ERAS pathway does appear to have the potential to elicit beneficial changes in patients awaiting surgery. The heterogeneity of the current literature means questions remain as to the most effective intervention to be used. Arguments can be made both for interventions targeting improved functional mobility both prior to and after surgery (as seen in Li et al. (2013)) as well as focusing on improving cardiorespiratory fitness (as in West et al. (2015)). In reality, one size may not fit all in terms of PREHAB, with individual patient needs and capabilities, equipment and facility availability and the time to surgery all factors likely to impact on design and success.

2.6. Review summary

The physical, physiological and psychological stresses associated with undergoing major resection surgery are well documented (Carli, 2015; Kehlet & Wilmore, 2002), with the introduction of ERAS having played an important role in modernising patient care and aiding post-operative recovery (Lv et al., 2012; Walter et al., 2009). As discussed in this review, clear interest appears to exist into incorporating PREHAB as a potential pre-operative component of the ERAS for colorectal resection surgery however, an absence of any pragmatic RCTs conducted within the 31 day target set in the UK for the start of cancer treatment means the practicalities of implementing such an intervention are unknown. Although improvements in different measures of physical functioning have been observed in the literature to date (Gillis et al., 2014; Li et al., 2013; West et al., 2015), there are questions over how inclusive many of the programmes are, especially in individuals with pre-existing mobility issues. Therefore the application of an inclusive PREHAB intervention with a focus on functional resistance training represents a gap in the literature. If possible to implement, along with any physical and HRQOL related benefits that may gained, there appears to be a potential for an upregulation in the Hsp and glutathione defence systems which may provide additional protection against the cellular stress surgery brings.

Chapter 3: Attitudes to exercise in the preparation for surgery: A questionnaire based study.

3.1. Introduction

When an individual undergoes a major surgical procedure or suffers a severe medical event it can often have a debilitating effect on the individual both physically and mentally. For example, following major abdominal surgery declines in muscular endurance and increased sensations of fatigue have been reported (Bautmans et al., 2010). As discussed in chapter 2, sections 2.4.1 to 2.4.6, in patients awaiting major elective surgery, it has been proposed that rather than waiting until after the patient has undergone surgery to start rehabilitation, a short term period of PREHAB may actually aid in preparing the individual for the stresses surgery brings (Carli & Zavorsky, 2005). As a relatively new area of research, limited evidence however exists regarding perceived attitudes to PREHAB and the potential barriers that may exist to participation. A larger evidence base however does exist for cardiac rehabilitation (CR) programmes.

Following a cardiac event, patients can experience a reduction in physical capacity (Dodson et al., 2012; Kamper, Stott, Hyland, Murray, & Ford, 2005), an impaired HRQOL (Gravely-Witte, De Gucht, Heiser, Grace, & Van Elderen, 2007; Westin et al., 1997) and the potential development of symptoms of anxiety and depression, both in the short and long term post-myocardial infarction (Lane, Carroll, Ring, Beevers, & Lip, 2002). As a means of aiding subsequent recovery, patients are often invited to undertake a period of rehabilitation. Cardiac rehabilitation is one of the most established programmes available in the UK, providing educational advice and support alongside exercise programmes to over 77,000 patients alone in 2013 (British Heart Foundation [BHF], 2014). Along with a reduction in total and cardiovascular mortality (Heran et al., 2011), participation in CR is reported to result in beneficial improvements in physical capacity (Rejeski et al., 2002), psychosocial well-being and HRQOL (Yu, Li, Ho, & Lau, 2003). Despite this, overall patient uptake is still only 45%; with less than half of those who are eligible actually enrolling (BHF, 2014). This illustrates the issues that remain in implementing exercise interventions as part of an overall medical treatment.

Although PREHAB has been proposed for use within a variety of different clinical populations, many of the barriers that exist to participation in CR such as a lack of time or interest may apply to PREHAB. Therefore, the aim of this preliminary experimental chapter was to examine the attitudes and perspectives of both the residents

and GPs of Hull and the East Riding of Yorkshire to the potential use of PREHAB as a means of preparing patients for surgery.

3.2. Methods

3.2.1. Ethical approval

The study was approved by the Sport, Health and Exercise Science Departmental Human Ethics Committee at the University of Hull and conducted in accordance with the declaration of Helsinki.

3.2.2. Development of the attitudes to exercise in the preparation for surgery questionnaires

A 20-item self-report questionnaire was developed to assess the attitudes of the general public to the potential use of PREHAB prior to major surgery (Appendix A). The questionnaire aimed to identify information relating to three main areas; 1.) the attitudes of the general public to the potential use of PREHAB prior to surgery; 2.) the most appropriate format for PREHAB and 3.) the possible perceived benefits and obstacles to PREHAB. To the author's knowledge, no psychometrically validated questionnaire assessing the attitudes and potential barriers to pre-operative exercise currently existed; a new questionnaire was designed according to the principles of questionnaire methodology (Boynton & Greenhalgh, 2004; Wall, DeHaven, & Oeffinger, 2002). All questions were investigator-generated following a review of pre-existing literature relating to PREHAB prior to surgery (Chapter 2 section 2.6) and, studies investigating barriers to participation and adherence to CR (Daly et al., 2002; De Vos et al., 2013; Grace et al., 2009). The first eight questions focused on identifying participant demographics, including gender, age and current self-reported activity levels, in order to determine the respondent characteristics. The next 11 questions then covered areas including attitudes to PREHAB (n = 6), the most appropriate format for PREHAB (n = 3), and the possible benefits (n = 1) and obstacles (n = 1) to participation. The questionnaire concluded with an open ended free text space inviting any additional comments, thoughts or concerns respondents had regarding PREHAB.

An amended 18-item self-report version of the questionnaire was developed for GPs (Appendix B). Fifteen of the 20 questions on the general public questionnaire were included on the GP questionnaire whilst two were removed as they were not deemed relevant and three were reworded to better focus on whether the GP would recommend participation in PREHAB and what populations they felt PREHAB was most applicable to. These were adapted from the 'Yoga for Cancer? An Oncologist's Perspectives questionnaire' (McCall, Ward, & Heneghan, 2015).

3.2.3. Administering the questionnaire

The questionnaire was available online via the Bristol Online Survey service (https://hull.onlinesurveys.ac.uk/attitudes-to-exercise-in-the-preparation-for-surgery-quest-2) for an eight week period between May and July 2015. Individuals were eligible to complete the questionnaire provided they were 18 years or older and resident in the Hull or the East Riding of Yorkshire area. The study was advertised via social media (e.g. Twitter, Facebook), emails (e.g. staff newsletter) and posters in the local community (e.g. shops, post offices). In order to target older populations who were less likely to access the online version, hard copies of the questionnaire were also distributed at two East Riding based Age UK over 50's social groups; two Hull based independent over 50's social groups and three community senior citizens groups.

Following an internet search of GP surgeries located in the Hull and East Riding of Yorkshire area, letters were sent to a total of 100 GPs (50 from Hull and 50 from the East Riding of Yorkshire) selected at random inviting them to participate in the GP version of the questionnaire. The questionnaire was available between May and July 2015 via the Bristol Online Survey service, (https://hull.onlinesurveys.ac.uk/gp-attitudes-to-exercise-in-surgery). To be eligible to participate, the GP needed to currently be practicing in the Hull and East Riding of Yorkshire area.

3.2.4. Data analysis

All nominal, ordinal and ratio data were presented as frequencies and absolute values. Analysis of free text responses was performed using a hierarchal content analysis as this approach allows the researcher to identify lower and higher order themes from the data available (Sparkes & Smith, 2013). This was performed in the

following stages; first free text responses were transcribed verbatim from the questionnaires before being read and re-read in order for a clear understanding of the replies to be developed. Next, any responses that were identified as reflecting a specific attitude or thought on PREHAB were highlighted and coded accordingly, forming a list of similar lower order raw data themes representative of the questionnaire responses. These raw data themes were then clustered together to form more inclusive higher order themes which are reported in the results section below (Sparkes & Smith, 2013).

3.3. Results

3.3.1. Participant characteristics

A total of 41 males (31.1%) and 91 females (68.9%) completed the general public attitudes to exercise in preparation for surgery questionnaire. Of the respondents, a total of 63 (47.7%) currently lived in Hull whilst 69 (52.3%) were from the East Riding of Yorkshire. Respondent age ranged from 21 to 81 years with 54.5% (n = 72) responses from individuals aged 21 to 39 years, 29.6% (n = 39) from those aged 40 to 59 years and 15.9% (n = 21) aged 60 years or older.

3.3.2 Attitudes to PREHAB

Eighty one percent (n = 107) of all respondents either agreed or strongly agreed that the concept of exercise PREHAB was a good idea in principle with 18.2% (n = 24) unsure (Table 3.1). Only one respondent disagreed (0.8%). Furthermore, 75.0% (n = 99) of respondents either agreed (45.5%; n = 60) or strongly agreed (29.5%; n = 39) that participating in PREHAB would help prepare patients for the surgery ahead whilst 4.5% (n = 6) disagreed. The remaining 20.5% (n = 27) were unsure how beneficial PREHAB would be. Unsurprisingly, 98.5% (n = 130) of respondents suggested they would be more likely to participate in PREHAB if the potential benefits were clearly outlined whilst 94.7% (n = 125) either agreed (56.1%; n = 74) or strongly agreed (38.6%; n = 51) that they would be more likely to participate if their doctor recommended it. Three respondents (2.3%) disagreed that a doctor's recommendation to PREHAB would increase the likelihood of participating.

Table 3.1. Respondent attitudes to the potential use of PREHAB prior to surgery.

		Males (n = 41)	Females (n = 91)	Combined (n = 132)
I believe the concept of exercise prehabilitation prior to surgery is a good idea in principle...	Strongly agree	16 (39%)	26 (28.6%)	42 (31.8%)
	Agree	15 (36.6%)	50 (54.9%)	65 (49.2%)
	Unsure	10 (24.4%)	14 (15.4%)	24 (18.2%)
	Disagree	0 (0%)	1 (1.1%)	1 (0.8%)
	Strongly disagree	0 (0%)	0 (0%)	0 (0%)
I believe that participating in exercise in the weeks before surgery would help prepare the individual for surgery...	Strongly agree	15 (36.6%)	24 (26.4%)	39 (29.5%)
	Agree	15 (36.6%)	45 (49.5%)	60 (45.5%)
	Unsure	10 (24.4%)	17 (18.7%)	27 (20.5%)
	Disagree	1 (2.4%)	5 (5.5%)	6 (4.5%)
	Strongly disagree	0 (0%)	0 (0%)	0 (0%)
If my doctor recommended exercise prehabilitation I would be more likely to participate...	Strongly agree	17 (41.5%)	34 (37.4%)	51 (38.6%)
	Agree	20 (48.8%)	54 (59.3%)	74 (56.1%)
	Unsure	3 (7.3%)	1 (1.1%)	4 (3%)
	Disagree	1 (2.4%)	2 (2.2%)	3 (2.3%)
	Strongly disagree	0 (0%)	0 (0%)	0 (0%)
I would be more likely to participate if the potential benefits were clearly explained…	Strongly agree	20 (48.8%)	44 (48.4%)	64 (48.4%)
	Agree	20 (48.8%)	46 (50.5%)	66 (50%)
	Unsure	0 (0%)	1 (1.1%)	1 (0.8%)
	Disagree	1 (2.4%)	0 (0%)	1 (0.8%)
	Strongly disagree	0 (0%)	0 (0%)	0 (0%)
If I was awaiting major surgery I would be interested in participating in an exercise prehabilitation programme...	Strongly agree	12 (29.3%)	31 (34.1%)	43 (32.6%)
	Agree	17 (41.5%)	40 (44%)	57 (43.2%)
	Unsure	7 (17.1%)	13 (14.3%)	20 (15.2%)
	Disagree	1 (2.4%)	1 (1.1%)	2 (1.5%)
	Strongly disagree	4 (9.8%)	6 (6.6%)	10 (7.6%)

Over half (57.6%, n = 76) of the respondents indicated that they would be more likely to participate in a PREHAB programme if it was fitness facility based rather than home based (42.4%; n = 56) (Table 3.2). When offered four more specific formats for PREHAB (group based classes, one to one with an instructor, fitness facility based intervention with guidance available if required or a DVD/leaflet guided home-based programme), 30.3% (n = 40) indicated a preference for the home based intervention. Out of the 40 respondents to select the DVD/leaflet guided home-based programme, 39 of them had indicated a preference for a home based programme rather than a fitness facility based intervention in the previous question. Of the formats more appropriate for fitness facility based activities, 29.5% (n = 39) expressed a preference for PREHAB sessions to be performed on a one to one basis under the supervision of an instructor,

22.7% (n = 30) a group class based approach and 17.4% (n = 23) a programme that could be performed individually but with guidance available if required. A total of 48.5% of respondents (n = 64) felt 3-4 sessions per week would be the most appropriate amount of sessions, closely followed by 45.5% (n = 60) preferring 1-2 sessions.

Table 3.2. Responses regarding preferred format for PREHAB.

		Males (n = 41)	Females (n = 91)	Combined (n = 132)
I would be more likely to participate in exercise prehabilitation if the activity was…	Home based	17 (41.5%)	39 (42.9%)	56 (42.4%)
	Fitness facility based	24 (58.5%)	52 (57.1%)	76 (57.6%)
I would be more likely to participate in exercise prehabilitation if the activity was…	Group based classes (e.g. exercise class style	7 (17.1%)	23 (25.3%)	30 (22.7%)
	One to one with an instructor	13 (31.7%)	26 (28.6%)	39 (29.5%)
	Performed on my own in a fitness facility but with guidance available if required	10 (24.4%)	13 (14.3%)	23 (17.4%)
	Detailed on a leaflet and/or DVD so it could be performed at home in my own time	11 (26.8 %)	29 (31.9%)	40 (30.3%)
I believe the most appropriate number of prehabilitation sessions per week would be…	1-2 session per week	15 (36.6%)	45 (49.5%)	60 (45.5%)
	3-4 sessions per week	23 (56.1%)	41 (45.1%)	64 (48.5%)
	5-6 sessions per week	3 (7.3%)	3 (3.3%)	6 (4.5%)
	7 + sessions per week	0 (0%)	2 (2.2%)	2 (1.5%)

A total of 84.1% (n = 111) of all respondents felt a faster recovery time after surgery may be a benefit of participating in PREHAB (Figure 3.1). Other popular perceived benefits of participation in PREHAB included improved physical fitness prior to surgery (79.5%, n = 105). Within the ‘other’ responses, two respondents indicated participation in PREHAB potentially would provide an opportunity to socialise with individuals with similar medical problems. A greater percentage of female than male

respondents identified PREHAB may have psychological benefits. A total of 12.7% (39.6%, n = 36 out of 91) and 12.4% (75.8%, n = 69 out of 91) more female respondents identified reduced anxiety and improved mental well-being respectively, as potential benefits of PREHAB compared to males (anxiety: 26.8%, n = 11 out of 41; mental well-being: 63.4%, n = 26 out of 41).

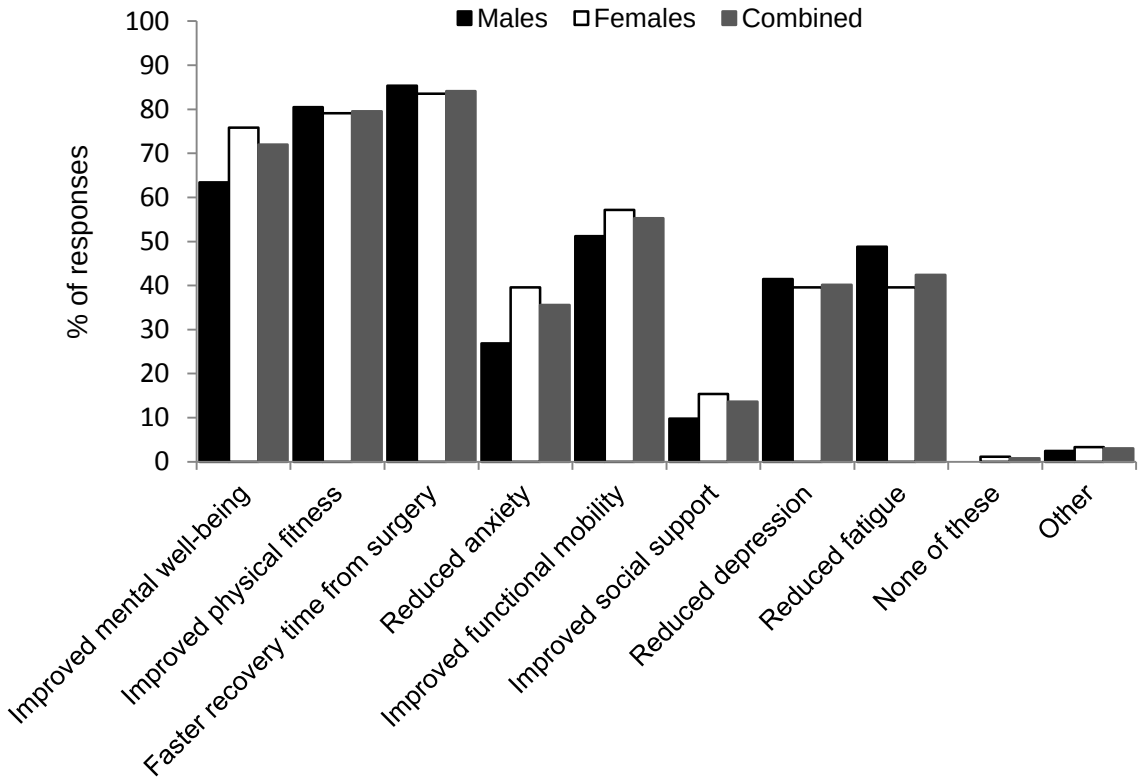


Figure 3.1 Respondents perceived benefits of participation in PREHAB

Respondents indicated that the three biggest perceived obstacles to participation in PREHAB were a lack of time (62.1%, n = 82), the potential financial cost (46.2%, n = 61) and work responsibilities (43.2%, n = 57) (Figure 3.2). Only 7.6% (n = 10) believed a lack of interest was an obstacle whilst 6.8% (n = 9) felt it not being perceived as beneficial would impact on participation. A larger percentage of males than females identified not knowing what was involved (+16.5% [Males: 34.1%, n = 14 out of 41; Females: 17.6%, n = 16 out of 91]), work responsibilities (+15.2% [Males: 53.7%, n = 22 out of 41; Females: 38.5%, n = 35 out of 91]) and family commitments (+10.9% [Males: 43.9%, n = 18 out of 41; Females: 33.0%, 30 out of 91]) as obstacles to participation. In contrast, a higher percentage of females identified not feeling physically capable of participating (+12.9% [Males: 14.6%, n = 6 out of 41; Females: 27.5%, n = 25 out of 91]), finding exercise tiring or painful (+9.8% [Males: 12.2%, n =

5 out of 41; Females: 22.0%, n = 20 out of 91]) and self-confidence (+7.6% [Males: 12.2%, n = 5 out of 41; Females: 19.8%, n = 18 out of 91]) as obstacles.

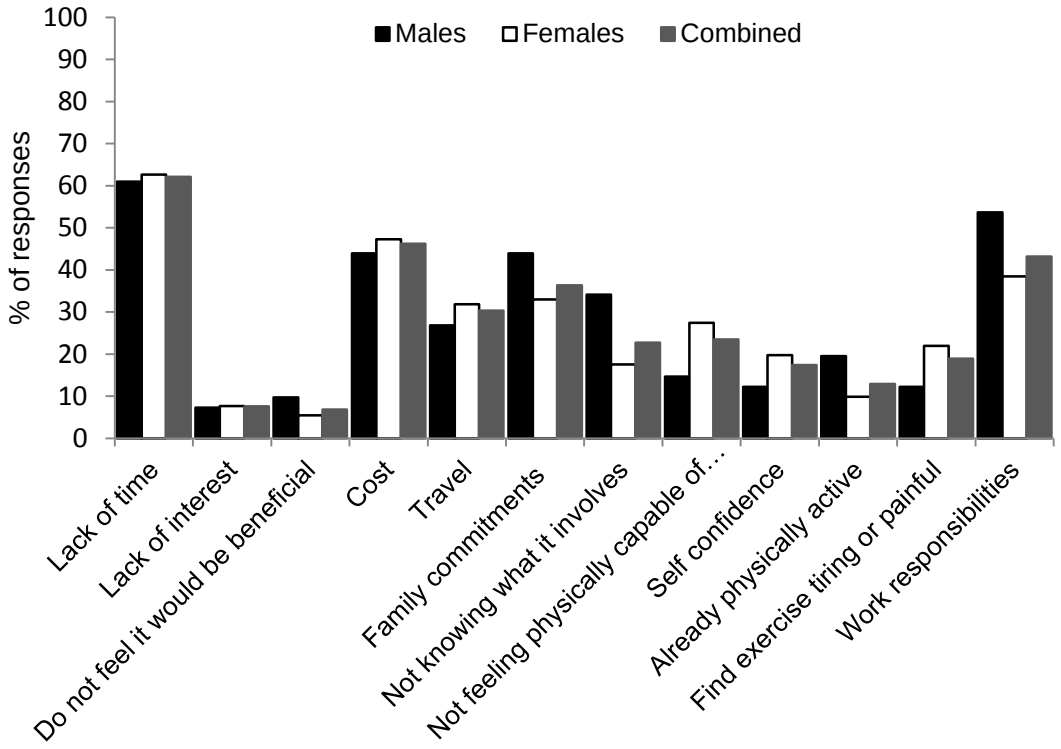


Figure 3.2 Respondents perceived obstacles to participation in PREHAB

Over 75% of all respondents either agreed (43.2%, n = 57) or strongly agreed (32.6%, n = 43) that if they were awaiting major surgery they would be interested in participating in PREHAB. Only 9.1% of all respondents either disagreed (1.5%, n = 2) or strongly disagreed (7.6%, n = 10) that they would be interested in PREHAB, with 15.2% (n = 20) unsure. A higher percentage of female respondents (+7.3% [Males: 70.8%, n = 29 out of 41; Females: 78.1%, n = 71 out of 91]) either agreed or strongly agreed they would be interested in PREHAB than male respondents (Table 4.1). Similarly, a higher percentage of respondents over 50 years of age (+8.4%) either agreed or strongly agreed they would be interested in PREHAB than those aged 18-49 years (18-49 years: 79.8%, n = 67 out of 84; Over 50 years: 88.2%, 30 out of 34; Figure 3.3).

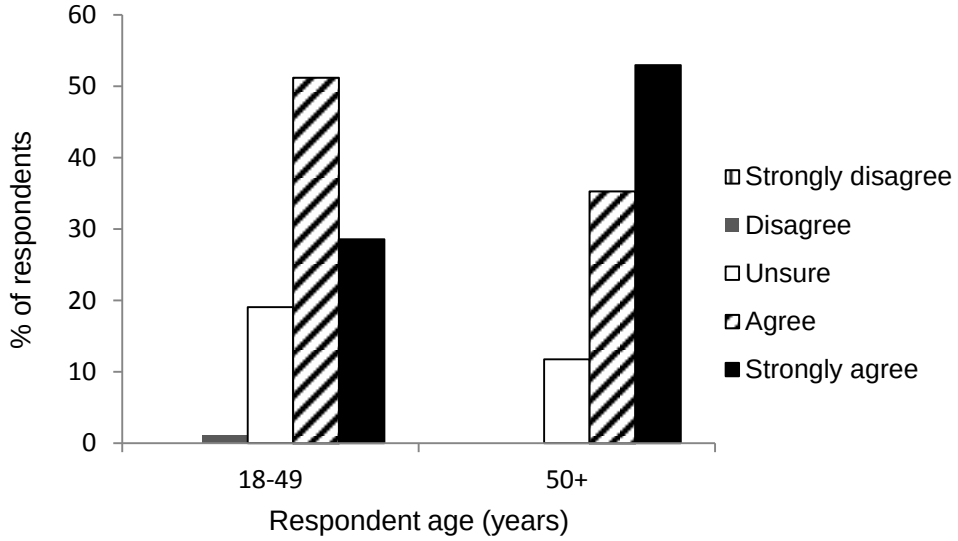


Figure 3.3 Respondent interest in PREHAB according to age

When asked whether they would be more likely to participate in PREHAB, rehabilitation, both or neither if all options were available, 80.3% (n = 106) of respondents indicated a preference to participate in both. Rehabilitation only was the second most popular option with 15.2% (n = 20) whilst only 3.8% (n = 5) suggested they would participate in PREHAB only. Only one respondent (0.8%) indicated they would be unlikely to participate in either option.

3.3.3. Free text responses

Free text responses which varied from short statements clarifying points from previous questions to statements expanding on overall thoughts on PREHAB were given by 38 (28.8%) of the 132 respondents. The raw data themes identified during analysis were then encompassed within two higher order themes: 1) benefits of PREHAB and 2) the challenges and concerns of PREHAB. These themes will be expanded on using specific free text responses from the respondent. Each response will be identified by participant ID, gender and age.

Benefits of PREHAB

Some respondents expressed a clear belief that PREHAB could provide benefits to physical fitness and subsequent recovery:

"I believe that surgical outcomes are improved by preparing the body in terms of fitness both before and after the surgery." (Respondent 42, Male aged 53 years)

"Physical fitness assists in promoting good mental health (but not always) and preparing for the rigours of a general anaesthetic and the trauma of invasive surgery which would undoubtedly involve incisions in muscles. The more physically able you are to cope with the recovery the quicker it may be." (Respondent 72, Male aged 67 years)

This was further emphasised within the patients who had identified themselves as previously having undergone a major surgical procedure:

"I think this is a good idea and would have welcomed this intervention before going under major surgery. I have since recommended to other people the importance of being fit/strong before undergoing any surgical procedure." (Respondent 59, Female age 40 years)

"I have had knee surgery and muscle loss was very significant and took >6 months to recover. Better muscle tone/strength beforehand can only aid recovery of muscle tissue and help protect the joint." (Respondent 98, Female aged 58 years)

Beyond any physical benefits that may be gained, a belief that some important psychological benefits could be gained was also expressed:

"I haven't ever had major surgery but I would imagine that if I was waiting for an operation I would feel quite powerless. Being able to do something like prehabilitation beforehand, that had been proven to be effective, would make me feel like I was doing something constructive towards my wellbeing." (Respondent 55, Female aged 26 years)

"I believe that regular enjoyable exercise is good for the mind as well as the body, so would help guard against the psychological challenges of surgery as well as the physical ones." (Respondent 19, Female aged 38 years)

"I believe exercise would be good for mental health and reduction in anxiety particularly prior to a stressful event e.g. surgery." (Respondent 114, Female aged 25 years)

However, despite these potential benefits, concern was expressed that the individuals who would be most likely to benefit from PREHAB are the ones least likely to participate:

"I really believe exercise prehabilitation is a great idea, although I am very conscious that it would be limited to those who are physically fit enough or capable to participate, especially depending on the type of surgery the patient is waiting for. I believe exercise is great for general wellbeing and reducing anxiety, but I would be doubtful the people who would really benefit from this before an operation, such as the elderly, are the least likely to participate. But generally, I think it would be a very pro-active move if exercise prehabilitation was more strongly encouraged to patients, and more information was given to them." (Respondent 66, Female aged 27 years)

Challenges and concerns of PREHAB

One of the greatest concerns expressed appeared to be the appropriateness of the PREHAB intervention for the surgical population involved:

"I assume that surgery is recommended as a result of physical discomfort which could restrict a person's ability to perform PREHAB before surgery. I.e. If you are waiting for knee replacement exercise may cause excess pain" (Respondent 12, Female aged 40 years)

"I can't imagine a very weak/sick patient being interested in a physical activity. This could elevate stress levels and lead to a deteriorating state of their health in the worst case." (Respondent 9, Female aged 31 years)

"Given that I had something wrong with me sufficiently serious to warrant surgery, then it is likely I would be unable to exercise either before or after." (Respondent 11, Male aged 54 years)

"I can understand why people would not want to participate as if they are injured they might worry about causing further damage and therefore not want to participate despite having the benefits explained to them." (Respondent 51, Female aged 22 years)

"Is it appropriate for all surgery? If awaiting foot surgery, how can exercise be performed." (Respondent 60, Female aged 33 years)

"Having had such surgery, I'm not sure I would have been physically able to do such exercises, but would have been willing to try." (Respondent 87, Female aged 40 years)

It was therefore acknowledged the suitability of PREHAB may be dependent on the specific individual or the surgical treatment required:

"I believe in some cases pre-surgery can be impossible as the patient could be in a lot of pain and unable to move. However in other cases it may help with surgery and recovery. It should be looked at on a case by case basis." (Respondent 13, Female aged 22 years)

"It is difficult to answer. I suppose it depends on the nature of the surgery needed. Exercise is a good thing in itself; are there any medical reasons for pre-surgery exercise?" (Respondent 49, Male aged 54 years)

The feasibility of actually implementing a PREHAB service into the current NHS structure was highlighted as another major issue:

"Without a dedicated service, or subsuming PREHAB into rehab services, it may be quite difficult to enable patients to perform PREHAB. Changing one's routine before major surgery (which in itself is a cause for concern) and picking up a new pastime, anecdotally, seem difficult to achieve. It could add an

additional, unnecessary stressor to the patient's life." (Respondent 47, Male aged 28 years)

"PREHAB would be great, but I had surgery twice in the last year and wasn't offered any rehab at all on either occasion, so it seems unlikely that PREHAB would be embraced by the Trust." (Respondent 57, Female aged 49 years)

Overcoming the stigma that some people have that exercise is not enjoyable remains an obstacle in promoting PREHAB:

"I would be more interested in spending time with my friends and family, and enjoying myself than doing exercise." (Respondent 41, Female aged 28 years)

"Exercise is not popular on the whole with residents in sheltered accommodation. Some have repeated surgery and attend weekly physio before and after. If exercises were practiced at a younger age e.g. as in China we might be a fitter race!" (Respondent 110, Female aged 81 years)

3.3.4. GP questionnaire

Out of the 100 invitations sent to general practitioners, no responses were received giving a respondent rate of 0%.

3.4. Discussion

Based on the responses to the general public questionnaire, residents of Hull and East Yorkshire would be interested in participating in PREHAB prior to major surgery providing the intervention was able to be accommodated into their lifestyle. However, a number of barriers to participation would need overcoming, some of which appear to be gender specific, in order to convert interest into actual enrolment onto a programme. Whether the same support would exist from the general practitioners in the region is unknown due to a lack of responses to the survey.

In the current study, a total of 94% of respondents either felt the most appropriate number of PREHAB sessions a week would be either 1-2 (45.5%) or 3-4

(48.5%) sessions per week. This is in agreement with De Vos et al. (2013) who reported that one of the barriers to participation in CR is a lack of time; a point reinforced in this study as the most likely barrier to participation (Figure 3.2). As the use of PREHAB has the potential to be used in variety of clinical populations (cancer, cardiac, orthopaedic patients), it was decided the questionnaire would not focus on a specific surgical procedure (hip/knee replacement surgery, colorectal or lung resection surgery). As a result, the respondents' preconceived ideas of exercise are likely to have influenced their responses. Given the non-procedure specific nature of the questionnaire, although respondents were asked what format they felt PREHAB should take (group or individual, home or facility based), they were not asked to identify what were viewed as the most appropriate forms of exercise for PREHAB given the variety that would exist across populations. This was also likely to have been evident in not being physically capable of participating (23.5%) and concerns that exercise was tiring and painful (18.9%) also being identified as obstacles to participation. Whilst it is acknowledged a more intensive programme of exercise training potentially represents the greatest opportunity for beneficial effects to occur prior to surgery, interventions which are too intensive have been reported as potentially intimidating to elderly and less able participants, limiting participation and thus proving counterproductive (Carli et al., 2010).

Two populations commonly reported as being difficult to recruit to CR programmes are participants of an older age (Grace et al., 2009) and those of female gender (Dunlay et al., 2009). A recent audit of CR in the UK (BHF, 2014) showed only 28-36% of eligible females actually accessed CR programmes across the different UK health regions. The responses in the current study however did not reflect this. A higher percentage of respondents aged 50 years or older (88.2%) either agreed or strongly agreed they would be interested in participating in PREHAB, than those aged 21-49 years (79.8%; Figure 3.3). Similarly, a slightly higher percentage of females (78.1%) agreed or strongly agreed that they would be interested in participating in PREHAB than males (70.8%) (Table 3.1). However, the current study has been based on responses to a hypothetical need for surgery whereas the existing studies in CR (De Vos et al., 2013; Dunlay et al., 2009; Grace et al., 2009) assessed the responses of individuals who had actually had a cardiac event and were therefore eligible for CR. This indicates that although an interest in participation within this population may exist, actually translating interest into enrolment remains a considerable challenge. In

hindsight, the inclusion of a question identifying the number of respondents who had previous experience of either having had major surgery themselves or knowing someone who had, may have been of interest. This would have helped identify whether opinions differed between individuals with prior experience of undergoing major surgery and those without.

The questionnaire revealed that some concerns existed regarding the appropriateness of PREHAB prior to certain surgical procedures, with knee replacement surgery identified by some respondents as a situation where PREHAB may not be suitable. Despite this, a number of previous studies have demonstrated that PREHAB interventions are safe and feasible in patients awaiting hip and knee arthroplasty or replacement surgery (Desmeules, Hall, & Woodhouse, 2013; Rooks et al., 2006; Swank et al., 2011) as well as other surgeries such as colorectal and lung resections (Carli et al., 2010; Dronkers et al., 2010; Jones et al., 2007), aortic abdominal aneurism repairs (Barakat et al., 2014; Kothmann et al., 2009) and CABG (Arthur et al., 2000; Sawatzky et al., 2014). Effective communication of the suitability of a PREHAB intervention and what it entailed would therefore be essential in both its recommendation and implementation, to overcome any concerns caused by misconceptions relating to its appropriateness for the individual. This is reflected in the current study where not knowing what was involved (34.1%) was the fifth most identified barrier to participation in male respondents behind lack of time (61.0%), work responsibilities (53.7%), family commitments (43.9%) and cost (43.9%; Figure 3.2). Interestingly, not knowing what was involved was identified as only the ninth biggest obstacle (17.6%) to participation in females, with not feeling physically capable of participating (27.5%), finding exercise tiring or painful (22%) and self-confidence (19.8%) viewed as greater challenges. This would suggest certain obstacles to participation are potentially more prevalent in specific genders.

Whilst a similar percentage of male and female respondents agreed that participating in PREHAB could improve physical fitness (Males: 80.5%; Females: 79.1%) and aid in faster recovery (Males: 85.4%; Females: 83.5%; Figure 3.1); over 12% more of female respondents identified either improved mental well-being or reduced anxiety prior to surgery as potential benefits of PREHAB compared to males. This may reflect that the females in this sample population are more receptive to the potential psychological benefits exercise may have on mental well-being, a point

reinforced within the free text comments. Alternatively, it may be argued that females could be more conscious or concerned with the psychological stresses that are associated with major surgery. This would be in keeping with the idea that the symptoms of depression and anxiety are reported to be more prevalent in females both in the general population (Piccinelli & Wilkinson, 2000) as well as following adverse medical diagnosis (Linden et al, 2012).

The absence of any responses from the GPs contacted was disappointing, especially as a failure of healthcare professionals to promote attendance in CR has been reported as a barrier to participation (Grace et al., 2008; O'Connell, 2014). Whether the lack of response is an indication of the current interest or support for PREHAB in GP's from the Hull and East Yorkshire region is impossible to establish. It is plausible that current workload demands rather than a lack of support for PREHAB contributed to the absence of replies, although this is obviously speculation. Initially, it was intended that consultant surgeons would also be contacted regarding the study however gaining access to the required numbers needed for analysis proved unfeasible in the timescale available. A Delphi study by Boereboom, Williams, Leighton, and Lund (2015), aimed to establish a consensus opinion on the use of PREHAB in elderly colorectal cancer patients. Although the sample size of 19 consultant colorectal surgeons was relatively small, within the 21 statements for which a consensus opinion was formed, support was evident for the use of PREHAB as a safe method of improving aerobic fitness prior to surgery. Moreover, beliefs that positive post-operative benefits (e.g. earlier mobilisation, reduced LOS) may be achieved as a result of PREHAB were also present. Interestingly, following three rounds of questioning, no consensus was reached by the consultants as to whether PREHAB would be deliverable in their respective hospitals. In order to facilitate patient participation in interventions such as PREHAB and rehabilitation programmes, healthcare professionals ranging from the surgical consultants and doctors to the nursing and physiotherapy teams, to name a few, need to support referrals to the intervention and promote the potential benefits it may bring. Therefore, future studies need to be performed to establish the level of support that exists for PREHAB in other members of the multidisciplinary team (e.g. physiotherapists, nurses) that are involved in the treatment of surgical patients.

A particular strength of this study was the specificity of the population targeted. The target population for any PREHAB intervention in this thesis would be residents

from the Hull and East Riding of Yorkshire region who had been referred for elective surgery. As regional variations exist throughout the UK in terms of physical activity levels (Townsend, Wickramasinghe, Williams, Bhatnagar, & Rayner, 2015), attitudes to exercise may subsequently vary across the UK. Focusing the questionnaire on residents of Hull and the East Riding of Yorkshire region only ensured the results were applicable to the recruitment area of the study in chapter 5.

Limitations do however exist within this study. Firstly, the use of a newly designed questionnaire rather than adapting a previously validated questionnaire may affect its validity. The decision to use this approach was taken as there is currently no psychometrically validated questionnaire assessing the attitudes and potential barriers to pre-operative exercise. Ideally during the preparation of the questionnaire, interviews or focus groups would have been held with individuals who had previously experienced major surgery in order to guide question development. Unfortunately limited access to such patients and insufficient available time meant this ultimately was not possible. This meant the questions were investigator-generated; however this was only after an extensive review of the current literature relating to PREHAB and barriers to CR.

Another limitation of the study was the heterogeneity of the participant population who responded. Although the questionnaire was open to all individuals aged 18 years of age or older with no upper age limit, over 50% of the responses came from individuals aged 21-39 years whilst only 15.9% of responses came from individuals aged 60 years or older. This was despite targeting older individuals through the attendance of local community groups such as those for over 50's. Ideally the response rate would have been higher in this population given that: 1) in some cases, such as hip or knee replacement surgery, those of an older age are the ones who are most likely to be undergoing surgery and 2) along with individuals of lower physical fitness, it may be argued that ageing populations may benefit the most from participating in PREHAB. However, psychosocial factors such as family support, or a lack of it, have also been reported to affect attendance at CR (Clark et al., 2012). This is potentially reflected in the current study by the preference for a facility based intervention, which is likely to promote an environment with increased social interaction. As a result, the attitudes of family members and friends who may not require PREHAB themselves are also important, as they could influence the enrolment of older participants who are eligible for PREHAB.

In summary, within the Hull and East Riding of Yorkshire region, residents would appear to be willing to embrace the application of PREHAB prior to surgery, particularly if the potential benefits were identifiable. The design of any such programme would require consideration of potential obstacles, such as cost implications, both to the participant and the organisation providing the service, as well as the required time commitment to the individual and accessibility to the programme.

Chapter 4: The test–retest reliability of five tests of physical functioning in apparently healthy adults

4.1. Introduction

Undergoing a major surgical procedure can have a debilitating effect on the individual with prolonged periods of immobilisation promoting acute insulin resistance, reduced body mass and muscle wasting (Carli, 2015); all of which accentuate the decay in physical functioning. As a typically ageing population, colorectal cancer patients are likely to be at increased risk of these detrimental effects. Ageing is associated with declines in cardiorespiratory fitness, muscular strength and endurance, and/or a loss of balance (Hakola et al., 2011; Samson et al., 2000), all of which can contribute to impaired physical functioning and health related quality of life in an ageing population (Yümin, Şimşek, Sertel, Öztürk, & Yümin, 2011).

The uses of simple field tests remain a popular metric by which to assess changes in physical functioning in both clinical and ageing populations. Various tests have been developed to assess the different components that can impact on the functional status of an individual. Poor performance of the TUG, which is considered a measure of both balance (Shumway-Cook et al., 2000) and functional mobility (Podsiadlo & Richardson, 1991), has been associated with increased incidences of falls in elderly populations (Shumway-Cook et al., 2000) whilst the 6MWT distance has been associated with all-cause mortality in chronic heart failure patients (Ingle, Cleland, & Clark, 2014b). Furthermore, low scores on HGD, which is a strength deficit tool, is associated with mortality, complications and increase LOS (Bohannon, 2008). An important aspect to these tests is that they often need only a short administration time and do not require specialist equipment making them assessable in a host of clinical settings, easy to administer and simple for the patient/client to understand and perform. They do, however, have certain limitations as their sensitivity to change over longer periods is potentially compromised by the presence of measurement error and variation in individual performance.

An understanding of the test-retest reliability is therefore imperative in interpreting the results of each specific test. Interclass correlation coefficients (ICC) remain one of the most frequently used statistical methods for assessing test-retest reliability (Atkinson & Nevill, 1998), however these only provide a measure of relative reliability and therefore provide no indication of measurement error. As a measure of absolute reliability, the standard error of measurement (SEM) allows measurement error

to be displayed in the same units as the original measurement (Stratford & Goldsmith, 1997). Additionally, the minimum detectable change (MDC) can be calculated as the smallest difference between repeated trials that is not due to chance variation (Haley & Fragala-Pinkham, 2006). The aim of this chapter was therefore to establish the test-retest reliability and absolute reliability of the five tests of physical functioning that were to be used in the main experimental study of this thesis. By establishing reference values of measurement error for the five tests, this would aid in interpreting the effectiveness of the PREHAB intervention used in chapter 5.

4.2. Methods

4.2.1. Ethical Approvals

Ethical approval was obtained from the Sport, Health and Exercise Science Departmental Human Ethics Committee at the University of Hull. All participants were provided with the participant information sheet informing them of the nature of the research and provided with opportunity to ask any questions. Participants were screened for eligibility using a departmentally approved medical questionnaire. All participants were required to provide written informed consent prior to commencing participation.

4.2.2. Participants

Apparently healthy males and females aged 30-75 years were recruited from the local community via advertisement. Individuals were excluded if any of the following criteria were present: a previous history of heart conditions, family history of sudden death, any current musculoskeletal and/or orthopaedic conditions, history of fracture within the last year, uncorrected visual impairment, recent history of dizziness or fainting, any vestibular disorders, a systolic blood pressure of more than 180 mmHg or diastolic blood pressure of more than 120 mmHg, pregnancy, a preexisting severe physical disability, resting heart rate of more than 120 bpm or shortness of breath with minimum exertion.

4.2.3. Experimental design

As the purpose of this chapter was to test the test-retest reliability of the five assessment measures rather than inter-rater reliability, all trials were conducted by a single tester; this ensured maximum consistency for data collection of each variable. Participants were required to attend two identical testing sessions separated by approximately four weeks. Both sessions were conducted at the same time of day in order to control for circadian variation (Atkinson & Reilly, 1996) and participants were asked to refrain from strenuous exercise in the 24 hours preceding each visit. The order of testing was the TUG, followed by the FTSTS, SCT, HGD and finally the 6MWT.

4.2.4. Outcome measures

i. Timed up and go (TUG)

Introduced by Podsiadlo and Richardson (1991) as an adaptation of the Mathias, Nayak, and Isaacs (1986) 'get up and go test', the TUG has been used as a measure of a person's functional mobility and balance (Bischoff et al., 2003; Podsiadlo & Richardson, 1991). In community dwelling older adults (65-95 years), poor performance (time taken > 13.5 seconds) has been shown to be related to a history of fall incidences (Shumway-Cook, Brauer, & Woollacott, 2000) whilst similar findings have also been found for individuals with Parkinson's disease (Nocera et al., 2013).

Same day test-retest reliability has been reported to be good to high in various populations including: community dwelling older adults (Intraclass correlation coefficients [ICC]: $ICC_{2,1}$: 0.95-0.97) (Steffen, Hacker, & Mollinger, 2002), individuals with Alzheimer's disease ($ICC_{2,2}$: 0.985-0.988) (Ries, Echternach, Nof, & Gagnon Blodgett, 2009) and patients with advanced chronic organ failure ($ICC_{2,1}$: 0.85-0.98) (Mesquita et al., 2013). Over longer periods of time, the reliability has been reported to be lower with Jette, Jette, Ng, Plotkin, and Bach (1999) reporting an ICC of 0.74 (model not stated) in elderly frail individuals when retested with a median gap of 14 days (range: 0 to 132 days).

A plastic chair 40 cm from the floor and 39 cm deep but with no armrests was used for the TUG. Participants were provided the following instructions prior to having

the test demonstrated to them:

> *"The aim of this test is to stand up from an upright seated position with your back against the chair backrest, walk 3 metres before turning 180° around the cone and returning to the seated position on the chair. This test is to be performed as fast as you can but in a controlled safe manner. The test will start with the count of THREE, TWO, ONE, GO, with you beginning the first standing movement on GO and the test finishing once you have returned to the seated position."*

Participants completed an untimed familiarisation trial following a demonstration by the researcher. Following the familiarisation trial, the mean time measured in seconds from three trials was taken.

ii. Five times sit to stand (FTSTS)

Assessing an individual's ability to perform five consecutive sit to stand manoeuvres as fast as possible in a controlled manner, the FTSTS was used as a measure of lower extremity strength and power. When performed on the same day, test-retest reliability has previously been reported to be high. Goldberg, Chavis, Watkins, and Wilson (2012) reported an ICC of 0.95 in community dwelling females aged 60 years or over. Test-retest reliability measured using ICC over longer periods than 24 hours have been reported to be more varied, ranging from 0.67 to 0.975 (Bohannon, Bubela, Magasi, & Gershon, 2011; Jette et al., 1999). Jette et al. (1999) reported an ICC of 0.67 in elderly frail individuals (n = 89), however the duration between testing sessions ranged between 0-132 days suggesting results may have been effected by a time interaction effect. Two separate studies by Schaubert and Bohannon (2005a, 2005b) reported ICCs of 0.81 and 0.82 respectively for three repeated trials, each separated by a 6 week period. All three studies calculated ICCs based on a single performance of the FTSTS during each testing session, however the mean of multiple tests is often recommended (Portney & Watkins, 2008).

A plastic chair 40 cm from the floor and 39 cm deep but with no armrests was used for the FTSTS. Participants were provided the following instructions prior to having the test demonstrated to them:

"The aim of this test is to perform five sit to stand movements as fast as you can but in a controlled safe manner. From an upright seated position with your back against the chair backrest and your arms crossed over your chest, you are required to stand up and sit down a total of five consecutive times as fast as you can. The test will start with the count of THREE, TWO, ONE, GO, with you beginning the first standing movement on GO and the test finishing once you have returned to the seated position for the fifth time"

Participants completed one untimed familiarisation trial before completing three experimental trials each with one minute rest in between as required. Mean time in seconds from the three trials was calculated and used in the analysis.

iii. Stair climb test (SCT)

The SCT assesses the functional ability of an individual to ascend a flight of stairs, a task requiring lower limb power, strength and balance, and has predominantly been utilised in orthopaedic populations (Fransen, Nairn, Winstanley, Lam, & Edmonds, 2007; Lin, Davey, & Cochrane, 2001; Zeni & Snyder-Mackler, 2010). Variations in the SCT have included differing numbers of steps to climb and requirements to either ascent and decent the stairs or ascent only (Bennell, Dobson, & Hinman, 2011). For this thesis, a 5 step ascent only SCT was used due to the availability of facilities at the hospital. Test-retest reliability data for the SCT is limited. Lin et al. (2001) reported ICCs of 0.94 (ascent only) and 0.96 (decent only) for a 4 step SCT when performed in 106 sedentary knee and hip osteoarthritis patients. For a 5 or 9 step SCT (ascent and decent combined) performed in 25 knee osteoarthritis patients, (Rejeski et al., 1995) observed correlations of r = 0.93 when the tests were performed 2 weeks apart. This decreased to r = 0.75 when the tests were separated by 3 months.

A purpose built flight of 5 stairs with a step height of 20 cm and depth of 27 cm was used. All participants received a demonstration and the following standardised instructions:

"The aim of this test is to ascend five steps as fast as you can but in a controlled safe manner. You will begin from a standing position with both feet together and you are permitted to use the handrail if required. The test will start with the

count of THREE, TWO, ONE, GO, with you beginning the ascent on GO and the test will finish once both feet are flat on the top step."

Following a demonstration, participants completed one untimed familiarisation trial before completing three experimental trials each with one minute rest in between as required. Time taken to complete the task was recorded in seconds and the mean of all three trials calculated and used in the analysis.

iv. Handgrip dynamometry (HGD)

Handgrip dynamometry was used as a measure of the maximum isometric strength of the hand and arm muscles (Lemmink, Han, de Greef, Rispens, & Stevens, 2001). An association has been observed between impaired muscle function and malnutrition (Wang et al., 2005) with low grip strength associated with increased risk of complications and prolonged hospitalisation following surgery (Bohannon, 2008). In 28 apparently healthy participants (mean age: 45.7 ± 23.5 years [range: 14 to 85 years]), ICCs of 0.965 (95% CI: 0.926 to 0.983) and 0.948 (95% CI: 0.890 to 0.975) were reported for the left and right hand respectively when performing the test 4 to 10 days apart (Bohannon et al., 2011). Similar values were observed for the dominant (ICC: 0.96 [95% CI: 0.88 to 0.99) and non-dominant (ICC: 0.95 [95% CI: 0.83 to 0.98) hands of haemodialysis patients (n = 38; mean age: 60.3 ± 15.8 years) when the test was repeated 1 to 2 weeks apart (Segura-Orti & Martinez-Olmos, 2011).

To perform the test the participants received the following instructions:

"The aim of this test is to measure your maximal grip strength. Standing in an upright position with your hands positioned by your sides and holding the handgrip dynamometer (TKK 5001 Grip A, Takei Scientific Instruments, Shiba, Japan) in your dominant hand, on GO, I would like you to squeeze as hard as possible for three seconds before relaxing. I would then like you to swap the dynamometer to your non-dominant hand were we will the repeat the test."

The grip length on the dynamometer was adjusted as required to suit each participant's hand size and the setting was documented for the repeat testing. Performance of the task was alternated between the dominant and non-dominant hand

with a total of three repetitions performed per hand. The mean of all three trials calculated and used in the analysis.

v. Six minute walk test

The 6MWT is a practical sub-maximal walking test which requires a minimal amount of equipment and that has been reported to be both valid (Moriello, Mayo, Feldman, & Carli, 2008) and reliable (Kervio, Ville, Leclercq, Daubert, & Carre, 2004), making it a popular test within a number of clinical settings. Used as a measure of aerobic fitness and endurance capacity, the 6MWT has been utilised within both cardiac (Bellet, Adams, & Morris, 2012) and pulmonary rehabilitation as a prognostic tool (Jenkins, 2007) and as an outcome measure in interventional studies (Rejbi et al., 2010). The distance covered in the 6MWT has been reported to be a strong predictor of morbidity and mortality in patients with heart failure (Ingle, Cleland, & Clark, 2014a), whilst a decline in 6MWT distance has been associated with mortality in individuals with COPD (Polkey et al., 2013). Whilst the relative reliability of the 6MWT has been reported to be good to high (ICC: > 0.90) in both healthy (Steffen et al., 2002) and clinical populations (Hamilton & Haennel, 2000; Hanson, McBurney, & Taylor, 2012), it has been reported that without sufficient familiarisation a learning effect can be evident. Within a population of healthy 60-70 year olds, Kervio et al. (2003) reported a minimum of two familiarisation trials were required to limit the learning effect when completing five tests over the space of three days.

A single trial of the 6MWT was performed in accordance to the guidelines outlined by the American Thoracic Association (ATS; 2002) ,using a 30 metre walking course with 3 metre intervals marked. Following a 10 minute period of seated rest, during which measures of resting heart rate, blood pressure and oxygen saturation were taken, participants were provided with the following instructions adapted from the guidelines issued by the ATS (2002):

> *"The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary however the timing will continue. You may lean against the wall while resting, but resume*

walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Each time you pass the starting point, it will be marked down as one completed lap. As the time progresses, I will let you know as each minute passes as well as once you enter the final 15 seconds. Once the six minutes are up, I will say stop at which point I would like you to stop where you are and I will come across to you.

Are you clear on all the instructions and ready to start? Ok, just to reiterate please remember the object of the test is to walk AS FAR AS POSSIBLE for 6 minutes but however don't run or jog. If you are ready, we shall start the test on GO."

Once ready, the participant was positioned at the start of the course and the test started. Standardised words of encouragement delivered in an even tone were provided each minute as instructed by the ATS (2002). These were as follows:

After the first minute, participants were told: *"That's 1 minute gone, you are doing well."*
Following the second minute: *"Two minutes gone, keep up the good work."*
After three minutes the participant was informed: *"You are halfway there, well done. Do you feel ok?"*
Following the fourth minute, participants were told: *"Keep up the good work. Only 2 minutes to go."*
As the test reached the final minute participants were instructed *"Final minute, almost finished."*
When the test was 15 seconds from completion participants were told: *"In a moment I'm going to tell you to stop. When I do, just stop right where you are and I will come to you."*

At the end of the 6 minutes, participants were instructed to stop and a chair was taken across allowing the participant to be seated. The total distance walked was recorded as the primary outcome measure along with immediate post-test measures of heart rate and oxygen saturation (SaO_2) as secondary outcome measures. Finally

participants were then monitored post-test until heart rate returned to near pre-test values to ensure their well-being.

4.2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 20 (SPSS Inc., Chicago, IL) with the exception of the Bland-Altman plots which were performed using SigmaPlot Version 12 (Systat Software, San Jose, CA, USA). To assess whether the assumption of normality of distribution was plausible, all data was assessed using the Shapiro-Wilks test and verified through visual inspection of histograms, quantile-quantile plots and box plots in conjunction with statistics for skewness and kurtosis. As all data conformed to the assumption of normal distribution, parametric statistical procedures were used. Central tendency was reported as mean ± standard deviation [range]. Differences between the two testing sessions for each assessment measure were assessed using Paired Sample T-tests.

Relative reliability was assessed using the ICC model 3 (Shrout & Fleiss, 1979). As the mean of three trials was used for the TUG, FTSTS, SCT and HGD, test-retest reliability was measured using $ICC_{3,2}$ model. The 6MWT, which involved a single trial in each session, used the $ICC_{3,1}$ model. Absolute reliability was expressed using 95% limits of agreement (95% LOA) (Bland & Altman, 1986), SEM and minimum detectable change at a 95% confidence interval (MDC_{95}). The 95% LOA represents the expected range of difference scores for each test. The SEM allowed measurement error to be displayed in the same units as the original measurement and was calculated using the formula:

$$SEM = SD \times \sqrt{(1\text{-}ICC)}$$

where SD was the standard deviation for all observations from test sessions 1 and 2 and ICC was the reliability coefficient. Measurement error was also expressed as a percentage of the mean ($SEM_{\%}$) using the formula:

$$SEM_{\%} = (SEM/mean) \times 100$$

This represents the smallest change required to indicate real change in a group of participants. MDC_{95} was calculated to represent the magnitude of change required to exceed the anticipated measurement variation, measurement error and variability of participants with 95% confidence (Haley & Fragala-Pinkham, 2006). The formula used for calculating MDC_{95} was:

$$MDC_{95} = SEM \times 1.96 \times \sqrt{2}$$

where the value of 1.96 represents the 95% CI and $\sqrt{2}$ accounted for the added uncertainty in measurement associated with repeated trials. Statistical significance was set at $p \leq 0.05$ for all tests.

4.3. Results

4.3.1. Participant characteristics

In total, 35 participants (18 males and 17 females; age 54.6 ± 12.1 years [range: 30 to 74 years], height 170.9 ± 11.0 cm [range: 145.6 to 195.6 cm], body mass 78.4 ± 17.8 kg [range: 43.0 to 119.3 kg]) were recruited. The mean number of days between trials was 27.9 ± 1.5 days [range: 24 to 33 days]. Thirty-one (17 males and 14 females) of the 35 participants reported their self-reported physical activity level as either moderately active or active. Three participants (1 male and 2 females) were sedentary whilst one female reported their physical activity level as highly active.

4.3.2. TUG, FTSTS and SCT

A mean percentage improvement in the performance time of the TUG (3.4%; range: -10.4 to +16.0%), FTSTS (3.9%; range: +20.5 to -23.7%) and SCT (1.7%; range: +12.4 to -0.3%) was seen between the first and second visit. The improvement however was only significant ($p < 0.05$) for the TUG and FTSTS (Table 4.1). The results relating to both the relative (ICC) and absolute reliability (LOA, SEM & MDC) of the TUG, FTSTS and SCT are displayed in Table 4.2. All three tests demonstrated good test-retest reliability with high ICCs ranging from 0.96 to 0.98. Out of the three tests, the SCT displayed the greatest absolute reliability with the SEM represented as a percentage of the mean being 2.8% whilst the FTSTS had the greatest measurement error at 5.8% of

the mean. When analysed based on gender, although mean performance time for all three tests was faster in males (Table 4.1), the difference was not significant (all $p > 0.05$). The magnitude of the ICCs for all three tests remained similar in males (ICCs: 0.97 to 0.98) and females (ICCs: 0.94 to 0.97) compared to when all participants were combined (ICCs: 0.96 to 0.98). In respects of absolute reliability, the greatest variability between genders was observed in the FTSTS.

Table 4.1: Between session performance differences for the TUG, FTSTS and SCT.

		Session 1	Session 2	Mean difference [95% CI]	*p* value
TUG (s)	Males (n = 18)	5.98 ± 1.41 [4.20; 8.89]	5.70 ± 1.20 [4.01; 8.60]	-0.28 ± 0.38 [-0.46; -0.09]	0.007
	Females (n = 17)	6.46 ± 1.44 [4.21; 9.21]	6.31 ± 1.78 [4.12; 8.41]	-0.15 ± 0.40 [-0.36; 0.06]	0.159
	Combined (n = 35)	6.21 ± 1.42 [4.20; 9.21]	6.00 ± 1.21 [4.01; 8.60]	-0.21 ± 0.40 [-0.35; -0.08]	0.003
FTSTS (s)	Males (n = 18)	10.96 ± 2.86 [6.20; 17.50]	10.61 ± 2.94 [5.76; 17.87]	-0.36 ± 0.38 [-0.75; 0.04]	0.073
	Females (n = 17)	11.87 ± 2.94 [6.45; 19.64]	11.33 ± 2.67 [7.07; 17.74]	-0.54 ± 1.33 [-1.22; 0.14]	0.113
	Combined (n = 35)	11.40 ± 2.89 [6.20; 19.64]	10.96 ± 2.79 [5.76; 17.87]	-0.44 ± 1.06 [-0.81; -0.08]	0.019
SCT (s)	Males (n = 18)	2.79 ± 0.45 [2.13; 3.68]	2.73 ± 0.46 [2.03; 3.61]	-0.05 ± 0.11 [-0.11; -0.00]	0.048
	Females (n = 17)	2.85 ± 0.51 [1.93; 3.69]	2.80 ± 0.58 [1.71; 3.83]	-0.04 ± 0.19 [-0.14; 0.05]	0.348
	Combined (n = 35)	2.82 ± 0.48 [1.93; 3.69]	2.77 ± 0.51 [1.71; 3.83]	-0.05 ± 0.15 [-0.10; +0.01]	0.061

Mean ±standard deviation [Range]; 95% CI: 95% confidence intervals; s: seconds

Table 4.2: Reliability data for the TUG, FTSTS and SCT.

		$ICC_{3,2}$ [95% CI]	95% LOA	SEM	SEM%	MDC_{95}	$MDC_{95\%}$
TUG	Males (n = 18)	0.97 [0.86; 0.99]	-1.02; +0.47	0.23	3.89	0.63	10.79
	Females (n = 17)	0.97 [0.92; 0.99]	-0.95; +0.63	0.22	3.37	0.60	9.33
	Combined (n = 35)	0.97 [0.93; 0.99]	-0.99; +0.56	0.22	3.67	0.62	10.18
FTSTS	Males (n = 18)	0.98 [0.94; 0.99]	-1.90; +1.19	0.43	3.94	1.18	10.92
	Females (n = 17)	0.94 [0.82; 0.98]	-3.14; +2.06	0.71	6.12	1.96	16.92
	Combined (n = 35)	0.96 [0.91; 0.98]	-2.55; +1.66	0.58	5.19	1.60	16.09
SCT	Males (n = 18)	0.98 [0.95; 0.99]	-0.27; +0.16	0.06	2.13	0.16	5.91
	Females (n = 17)	0.97 [0.92; 0.99]	-0.41; +0.33	0.09	3.32	0.26	9.21
	Combined (n = 35)	0.98 [0.95; 0.99]	-0.34; +0.25	0.08	2.80	0.22	7.77

ICC: Intraclass correlation coefficient; 95% CI: 95% confidence interval; 95% LOA: 95% limits of agreement; SEM: Standard error of measurement; MDC_{95}: Minimum detectable change at the 95% confidence interval

4.3.2. HGD

Out of the 35 participants, 31 were right hand dominant. No significant difference was seen in grip strength for either the dominant or non-dominant hand between trials ($p > 0.05$) (Table 4.3). Regardless of which hand was used, relative test-retest reliability was good. Absolute reliability was similar for both dominant and non-dominant hand, with the dominant hand displaying less variability than the non-dominant hand (Table 5.4). As would be expected, male grip strength was greater in both the dominant (mean difference: +15.2 kg [95% CI 11.2 to 19.1]; $p < 0.001$) and non-dominant (mean difference: +13.7 kg [95% CI: 9.0 to 18.4]; $p < 0.001$) hand compared to females. Despite there being a significant difference in grip strength for the non-dominant hand in females between sessions (1.1 ± 2.0 kg [95% CI: 0.0 to 2.1]; $p < 0.05$), test-retest reliability based on gender remained similar for both hands when compared to all participants combined (males ICC: 0.94 to 0.95; females ICC: 0.93 to 0.95; combined ICC: 0.98).

Table 4.3: Between session performance differences for the HGD.

		Session 1	Session 2	Mean difference [95% CI]	*p* value
HGD Dom (kg)	Males (n = 18)	40.8 ± 6.4 [27.5; 51.8]	40.5 ± 7.0 [25.7; 52.3]	-0.4 ± 3.2 [-2.0; -1.2]	0.616
	Females (n = 17)	25.4 ± 5.0 [18.0; 33.3]	25.6 ± 5.1) [16.7; 33.2]	-0.2 ± 2.6 [-0.4; 0.1]	0.816
	Combined (n = 35)	33.4 ± 9.7 [18.0; 51.8]	33.2 ± 9.7 [16.7; 52.3]	-0.1 ± 2.9 [-1.1; 0.9]	0.794
HGD Non-dom (kg)	Males (n = 18)	37.4 ± 7.9 [19.7; 52.7]	37.7 ± 9.0 [15.7; 50.0]	+0.3 ± 3.5 [-1.5; 2.0]	0.743
	Females (n = 17)	23.3 ± 5.1 [16.2; 34.0]	24.4 ± 4.8 [17.3; 33.5]	+1.1 ± 2.0 [+0.0; 2.1]	0.043
	Combined (n = 35)	30.5 ± 9.7 [16.2; 52.7	31.2 ± 9.8 [15.7; 50.0]	+0.7 ± 2.9 [-0.3; 1.6]	0.185

Mean ±standard deviation [Range]; Dom: dominant hand; Non-dom: non-dominant hand; 95% CI: 95% confidence intervals; kg: kilogram

Table 4.4: Reliability data for the HGD.

		$ICC_{3,2}$ [95% CI]	95% LOA	SEM	SEM%	MDC_{95}	$MDC_{95\%}$
HGD Dom	Males (n = 18)	0.94 [0.84; 0.98]	-6.72; +5.94	1.60	3.94	4.45	10.94
	Females (n = 17)	0.93 [0.82; 0.98]	-4.88; +5.17	1.27	4.99	3.53	13.84
	Combined (n = 35)	0.98 [0.96; 0.99]	-5.80; +5.54	1.42	4.28	3.95	12.79
HGD Non-dom	Males (n = 18)	0.95 [0.84; 0.98]	-6.66; +7.12	1.75	4.66	4.84	12.91
	Females (n = 17)	0.95 [0.84; 0.98]	-2.83; +4.95	1.11	4.65	3.07	12.88
	Combined (n = 35)	0.98 [0.96; 0.99]	-4.98; +6.29	1.47	4.77	4.08	13.22

ICC: Intraclass correlation coefficient; 95% CI: 95% confidence interval; 95% LOA: 95% limits of agreement; SEM: Standard error of measurement; MDC_{95}: Minimum detectable change at the 95% confidence interval; Dom: dominant hand; Non-dom: non-dominant hand

4.3.3. 6MWT

A mean improvement of approximately 5.6 metres (+0.9%) was seen between the first and second visit although this was not significant ($p > 0.05$) (Table 4.5). No significant difference was seen between sessions for SaO_{2pre}, SaO_{2post}, HR_{pre}, HR_{post} or HR_{ave} (all $p > 0.05$). The high ICC and narrow accompanying 95% CI demonstrated good test-retest reliability for the 6MWT (Table 4.6). Furthermore, the values reported

for both 95% LOA and MDC_{95} were similar whilst the SEM of 13.7 metres ($SEM_{\%}$ - 2.3%) represented a low value of measurement error. Although mean distance walked was further in males (619.2 ± 80.0 metres) compared to females (581.8 ± 79.1 metres), the difference was not significant ($p > 0.05$). In the 2nd session for males, the mean distance walked was significantly further (+12.1 metres; +2.0%; $p < 0.05$) however no difference between sessions was evident for females (-1.2 metres; 0.2%; $p > 0.05$). Despite the difference in males between sessions neither the relative nor absolute reliability of the 6MWT was greatly affected.

Table 4.5: Between session performance and physiological differences for the 6MWT.

		Session 1 Mean ± SD [Range]	Session 2 Mean ± SD [Range]	Mean difference [95% CI]	*p* value
Distance walked (m)	Males (n = 18)	613.2 ± 73.9 [486; 726]	625.3 ± 86.9) [483; 759]	+12.1 ± 20.7 [1.8; 22.4]	0.024
	Females (n = 17)	576.7 ± 78.3 [437; 699]	575.5 ± 75.1) [451; 705]	-1.2 ± 14.5 [-8.7; 6.2]	0.729
	Combined (n = 35)	595.5 ± 77.2 [437; 726]	601.1 ± 84.1) [451; 759]	+5.6 ± 18.9 [-0.9; +12.1]	0.087
HR_{pre} (bpm)	Males (n = 18)	68.1 ± 12.2 [52; 94]	69.7 ± 9.5 [54; 84]	1.6 ± 9.6 [-3.2; 6.4]	0.503
	Females (n = 17)	72.3 ± 13.4 [52; 98]	68.6 ± 10 [52; 88]	-3.7 ± 9.3 [-8.5; 1.1]	0.119
	Combined (n = 35)	70.1 ± 12.8 [52; 98]	69.1 ± 9.6 [52; 88]	-1.0 ± 9.7 [-4.3; +2.3]	0.546
HR_{post} (bpm)	Males (n = 18)	107.4 ± 22.0 [78; 165]	110.6 ± 23.4 [71; 161]	13.2 ± 11.2 [-2.4; 8.6]	0.248
	Females (n = 17)	112.4 ± 24.5 [76; 166]	110.7 ± 23.7 [80; 159]	-1.7 ± 6.2 [-4.9; 1.5]	0.275
	Combined (n = 35)	109.8 ± 23.1 [76.0; 166]	110.6 ± 23.2 [71; 161]	+0.8 ± 9.3 [-2.4; +4.0]	0.616
HR_{ave} (bpm)	Males (n = 18)	109.1 ± 20.1 [83.5; 157.0]	110.3 ± 21.2 [75; 151]	1.2 ± 7.9 [-2.7; 5.2]	0.522
	Females (n = 17)	112.6 ± 16.8 [84; 140]	111.4 ± 17.2 [84; 142]	-1.3 (6.4) [-4.6; 2.0]	0.420
	Combined (n = 35)	110.8 ± 18.4 [84; 157]	110.8 ± 19.1 [75; 151]	+0.0 ±7.2 [-2.5; +2.5]	0.998
$SaO2_{pre}$ (%)	Males (n = 18)	97.9 ± 1.0 [96; 99]	96.9 (1.6) [94; 99]	-1.0 ± 2.0 [-2.0; -0.0]	0.046
	Females (n = 17)	98.0 ± 1.1 [95; 100]	97.5 (1.6) [94; 100]	-0.5 ± 1.3 [-1.2; 0.3]	0.187
	Combined (n = 35)	97.9 ± 1.0 [95; 100]	97.2 ± 1.7 [94; 100]	-0.7 ± 1.7 [-1.0; +0.5]	0.073
$SaO2_{post}$ (%)	Males (n = 18)	97.7 ± 1.5 [93; 100]	96.7 ± 1.8 [91; 98]	-0.9 ± 2.3 [-2.1; 0.2]	0.094
	Females (n = 17)	97.0 ± 2.6 [89; 99]	97.5 ± 2.2 [91; 100]	0.5 ± 1.3 [-0.2; 1.3]	0.135
	Combined (n = 35)	97.4 ± 2.1 [89; 100]	97.1 ± 2.0 [91; 100]	-0.3 ± 2.0 [-0.9; +0.5]	0.440

HR_{pre}: Heart rate prior to 6MWT; HR_{post}: Heart rate post 6MWT; HR_{ave}: Average heart rate; $SaO2_{pre}$: Oxygen saturation prior to 6MWT; $SaO2_{post}$: Oxygen saturation post 6MWT

Table 4.6: Reliability data for the 6MWT.

		$ICC_{3,2}$ [95% CI]	95% LOA	SEM	SEM%	MDC_{95}	$MDC_{95\%}$
6MWT	Males (n = 18)	0.96 [0.86; 0.99]	-28.4; +52.6	16.3	2.6	45.3	7.3
	Females (n = 17)	0.98 [0.95; 0.99]	-29.6; +27.1	9.9	1.7	27.3	4.7
	Combined (n = 35)	0.97 [0.94; 0.99]	-31.4; +42.7	13.7	2.3	37.8	6.3

ICC: Intraclass correlation coefficient; 95% CI: 95% confidence interval; 95% LOA: 95% limits of agreement; SEM: Standard error of measurement; MDC_{95}: Minimum detectable change at the 95% confidence interval

4.4. Discussion

The aims of this chapter were 1). To establish the test-retest reliability of five tests of physical functioning often used within clinical studies when performed approximately four weeks apart and 2). To calculate LOA, SEM and MDC, giving an indication of absolute reliability between repeated tests. All five tests used in this study displayed good test-retest reliability, exceeding the ICC threshold of 0.90 previously reported to be required for a clinical test (Portney & Watkins, 2008). Whilst the use of ICCs provide an indication of the relative reliability of a test, the inclusion of a measure of absolute reliability is important in order to gain an understanding of whether real change has actually occurred. In this study, despite good test-retest reliability being seen for all the tests used, considerable individual performance variability was present for some tests (in particular the FTSTS), highlighting the need to incorporate both measures of relative and absolute agreement when assessing the reliability of a test (Costa-Santos, Bernardes, Ayres-de-Campos, Costa, & Amorim-Costa, 2011).

Of the five tests included in this study, the 6MWT is perhaps the most frequently used acting as a means of assessing the effectiveness of different intervention programmes (Carli et al., 2010) as well as a predictor of both cardiorespiratory fitness (Ross, Murthy, Wollak, & Jackson, 2010) and clinical outcomes (Ingle et al., 2014b). As in this study, good test-retest reliability has been observed in a number of other populations including cardiac patients (ICCs: 0.88 to 0.97) (Demers, McKelvie, Negassa, & Yusuf, 2001; Hamilton & Haennel, 2000; Hanson et al., 2012), type 2 diabetics (ICC: 0.99) (Alfonso-Rosa, Del Pozo-Cruz, Del Pozo-Cruz, Sanudo, & Rogers, 2014) and the elderly (ICCs: 0.87 to 0.93) (Harada, Chiu, & Stewart, 1999). It

is however often reported that at least one, if not more, familiarisation trials are required in order to alleviate any potential learning effect and thus achieve a consistent baseline measurement for the 6MWT (Hamilton & Haennel, 2000; Hanson et al., 2012; Kervio et al., 2003).

In healthy individuals aged 60-70; it was only from the third trial that the measurement became reliable when performing five 6MWTs over a 1 week period (Kervio et al., 2003). Between both the 1st and 2nd, and 2nd and 3rd trials a mean increase of ~20 metres was reported; representing a 3.7 and 3.8% increase between trials. An average improvement of 8 ± 5% (+47 metres) in the second of two trials performed on the same day was observed in healthy individuals aged 50-85 years (Troosters, Gosselink, & Decramer, 1999). Both Hanson et al. (2012) and Hamilton and Haennel (2000) et al. reported a learning effect occurred between trials within a CR setting despite reporting good relative reliability (ICC: 0.91 and 0.97 respectively). An 11.8% (+52 metres) increase in distance walked was observed in Hanson et al. (2012) between the 1st and 2nd trial and this increased to 19.1% (+85 metres) between the 1st and 3rd trial. Furthermore, whether the three tests were performed on the same day or spread over a week did not alter the presence of the learning effect (Hanson et al., 2012). Although the improvement was smaller, Hamilton and Haennel (2000) observed a 3.5% (+18 metres) increase between the 1st and 2nd trial and 5.6% (+29 metres) between the 1st and 3rd trial. These improvements therefore may be reflective of a more conservative approach being taken to the 1st trial by the participant when the physical demand of the 6MWT is unknown.

Whilst performing repeated trials of the 6MWT on the same day has been shown to be physically tolerable in clinical populations (Kervio et al., 2003; Kristjánsdóttir, Ragnarsdóttir, Einarsson, & Torfason, 2004), it may not always be feasible. In the current study only a 0.9% (+5.6 metres) increase was witnessed between trials when all participants were combined. Even in males alone, where a 2.0% (+12.1 metres) increase in distance walked was observed during the 2nd trial compared to the 1st, the magnitude of the change was lower than some of the values previously reported (Hamilton & Haennel, 2000; Kervio et al., 2003; Troosters et al., 1999). The difference between these previous studies and the current study may partly be explained by a couple of factors.

To a certain extent that any learning effect gained through previously performing the test may be attenuated by the longer period (4 weeks) between trials compared to those repeated over a shorter period of time (1 to 14 days) (Hamilton & Haennel, 2000; Kervio et al., 2003; Troosters et al., 1999). The absence of a significant difference in HR_{post}, HR_{ave} or SaO_{2post} between the sessions (Table 4.5) would suggest there was no increased or decreased physical effort exerted by participants during the 2nd trial, potentially supporting the presence of an attenuated learning effect. Alternatively, the higher basal physical activity levels of the participants in this study compared to these previous studies (Hamilton & Haennel, 2000; Kervio et al., 2003; Troosters et al., 1999) may have also contributed to the smaller magnitude of change as the scope for improvement may have been less.

It is acknowledged that direct comparisons between this study and those using clinical populations are difficult, as considerable variation does exist between population groups. The SEM (13.7 metres) and MDC_{95} (37.8 metres) seen in the current study were comparable to those reported in older type 2 diabetics (SEM = 9.88 metres; MDC_{95} = 27.37 metres) by Alfonso-Rosa et al. (2014). These values however do differ from those seen in both elderly (SEM: 32-34 metres; MDC_{95}: 88.7-95 metres; Harada et al., 1999) and cardiac (SEM: 18.4 - 32.6 metres; MDC_{95}: 50.9 - 90.3 metres; Demers et al., 2001; Hamilton & Haennel, 2000; Montgomery & Gardner, 1998) populations. Again this may be a reflection of the physical activity status of the participants used within this study reaffirming the need to consider patient characteristics and conditions in determining changes in performance.

Unlike with the 6MWT, the presence of a significant statistical decrease in time taken to perform the TUG and FTSTS between the first and second sessions suggested a learning effect was present. Similar improved FTSTS performance times have previously been reported in trials separated by 4-10 days (Bohannon et al., 2011) up to six weeks (Schaubert & Bohannon, 2005a; Schaubert & Bohannon, 2005b). Despite this, the ICC for all three studies was in excess of 0.80 indicating good correlation and agreement between trials. The ICC of 0.97 for the TUG in the current study (Table 4.2) exceeded that of Jette et al. (1999), who reported an ICC of 0.74 in elderly frail individuals. However, the difference in study populations is likely to have influenced the reduced ICC in Jette et al. (1999) compared to the current study. It is also worth noting that whilst the median number of days between trials was 14 days in Jette et al.

(1999), the overall range between trials varied from 0 days to 132 days. It is therefore plausible that the decrease in test-retest reliability, as indicated by ICC, was related to a true change in the study populations' ability to perform the FTSTS; especially in the individuals with the largest number of days between trials.

The results relating to the relative reliability of the FTSTS when performed with an extended period between trials have previously been varied (Bohannon, 2011). In trials separated by 4-10 days, Bohannon et al. (2011) reported good test-retest reliability (ICC: 0.96; 95% CI: 0.92 to 0.98) in community-dwelling men and women aged 15-85 years. In contrast, when the interval between trials has been longer, lower ICC's have tended to be reported. In two studies by Schaubert & Bohannon (2005a; 2005b) in which testing sessions were separated by 6 weeks, ICCs of 0.82 (95% CI: 0.68 to 0.92) and 0.81 (95% CI: not stated) respectively were reported. In the current study, despite the 4 week period between tests, test-retest reliability remained good with the ICC of 0.96 far exceeding those seen in the two aforementioned studies.

This difference could potentially be explained by a number of factors, including the presence of a shorter four week period between testing sessions in the current study as opposed to six weeks (Bohannon, 2011; Schaubert & Bohannon, 2005a; 2005b). Furthermore, the sample sizes used in both these studies (n = 21; Schaubert & Bohannon, 2005a; and n = 11; Schaubert & Bohannon, 2005b) were smaller than those of the current study (n = 35). More pertinent factors however are probably the differences in participant ages between the studies as well as the moderately active to active physical activity levels of the participants in this study. It is therefore acknowledged that the mean ages in both Schaubert and Bohannon studies (2005a; 75.0 ± 5.9 years [range: 65 to 85 years] and 2005b; 75.5 ± 5.8 years [range: 65 to 85 years]) make their findings more generalisable, especially to older populations where the FTSTS is more traditionally used, than the current study (54.6 ± 12.1 years [range: 30 to74 years]). Despite this, the current study adds to the existing literature by providing reference values for the potential measurement error of the five tests investigated in sample more reflective of the clinical population studied in chapter 5.

Whilst the TUG and FTSTS displayed good relative test-retest reliability in the current study, the absolute reliability for the tests did reflect the presence of considerable individual variation in the performance of each. Inconsistencies in the

agreement of relative and absolute reliability measures have previously been observed making the use of a combined approach important (Costa-Santos et al., 2011). The FTSTS was the most variable with a $SEM_{\%}$ of 5.8% and $MDC_{95\%}$ of 16.09%. These values were less than the $SEM_{\%}$ of 6.3% and $MDC_{95\%}$ of 17.5% reported by Goldberg et al. (2012) when performing repeated trials on the same day in apparently healthy older female participants. Furthermore, Goldberg et al. (2012) indicated a $MDC_{95\%}$ of 17.5% may be considered a low minimum change percentage. Further variation existed in the level of absolute reliability depending on the measure by which it was assessed.

The use of 95% LOA as a measure of absolute reliability in the current study reflected the most conservative method. The 95% LOA for the FTSTS suggested a change of over 2.55 seconds was required to detect real change compared to the 1.60 seconds according to the MDC_{95} (Table 4.2). Understanding the variation present in both the performance of the test and the different methods of calculating absolute reliability could be important when assessing any change present in repeated performances.

Although in the current study the SCT displayed good relative test-retest reliability (ICC: 0.98; 95% CI: 0.95 to 0.99) and absolute reliability (SEM: 0.08 seconds; MDC_{95}: 0.22 seconds), the results remain difficult to interpret. Variations of the SCT have been used in a variety of different populations including those with orthopaedic limitations and the elderly. The intra-session reliability in elderly individuals (mean age 69.4 years) with hip and/or knee osteoarthritis was reported to be good with an ICC of 0.94 (95% CI: 0.75 to 0.98) and SEM of 0.28 seconds seen for a four step ascent only SCT (Lin et al, 2001). When performing a five step SCT including both the ascent and descent of the stairs two weeks apart, Rejeski et al. (1995) reported good test- retest reliability (ICC: 0.93; 95% CI: not reported) in patients with knee osteoarthritis. Despite similar ICCs being reported in Lin et al. (2001), Rejeski et al. (1995) and the current study, making comparisons between the studies is difficult. The physical activity status along with the absence of any limiting condition such as osteoarthritis that may have impaired the ability of the participants in this study to climb stairs, means the performance time of 2.77 seconds is faster than those reported in either Lin et al. (2001) (4.17 ± 2.80 seconds) or Rejeski et al. (1995) (10.21 ± 4.45 seconds). It is therefore acknowledged the SCT results are difficult to generalise beyond the present study.

This study is not without limitations. The use of an apparently healthy population with a relatively wide age range (30 to 74 years) in this study means the results cannot be directly generalised to those of a specific clinical population although they are probably more representative of the general population. Ideally, this study would have been conducted in colorectal cancer patients however such a population was not accessible. Furthermore, given the sample size, stratification based on factors such as age, gender and self-reported physical activity was not possible. The sub-analysis based on gender alone (Tables 4.2, 4.4 and 4.6) did not differ greatly between the genders for any of the tests in the current study, however whether a more pronounced difference would be observed with a larger sample size cannot be dismissed.

Whilst reference values for the tests examined in this study exist in many clinical and ageing populations where their use is potentially more suited, circumstances occur where these tests may be used outside of such populations meaning values such as those found in the current study remain important. Although colorectal cancer is predominantly considered a disease of an ageing population, the number of cases diagnosed per year begins to rise from 40 years of age onwards (CRUK, 2016a) representing a relatively wide age range. Furthermore, unlike cardiac patients and those of some other cancers (e.g. lung cancer), the diagnosis of colorectal cancer is not always accompanied by the presence of other co-morbidities or physiological limitations. The results from this study therefore are relevant in informing the next chapter as well as providing important reference values to support the pre-existing literature and future studies relating to these age ranges.

In conclusion, this study demonstrated test-retest reliability for the TUG, FTSTS, SCT, HGD and 6MWT when performed with a 4 week period between sessions in apparently healthy adults aged 30-74 years exceeding the ICC threshold of above 0.90 that is required for a clinical test (Portney & Watkins, 2008), whilst also providing reference values for absolute reliability. Although not directly related to colorectal cancer patients, the reference values obtained from this study aid in interpreting the effectiveness of the PREHAB intervention used in chapter 5. For example, based on the MDC_{95} observed in this study, an improvement in 6MWT distance of 37.8 metres (or 6.3% of the participant's baseline score) can be assumed with greater confidence to be reflective of a true meaningful change rather than as a consequence of either

measurement error and individual variability in chapter 5. Beyond this thesis, these reference thresholds for LOA, SEM and MDC_{95} provided clinicians and researchers alike information to identify meaningful changes in test performance where more sophisticated facilities and techniques may not be available.

Chapter 5: The feasibility of PREHAB in elective colorectal surgery patients and its effects on physical functioning, HRQOL and clinical outcomes.

5.1. Introduction

Since first being pioneered by Kehlet and Wilmore (2002, 2005), the introduction of the multimodal ERAS approach to colorectal surgery has been reported to reduce post-operative LOS and complications without increasing the risk to the patient (Varadhan et al., 2010; Walter et al., 2009; Zhuang et al., 2013). Despite low levels of pre-operative physical fitness being associated with poor post-operative outcomes (Snowden et al., 2013; Wilson et al., 2010), PREHAB programmes are not currently part of the ERAS approach. Whilst examples of improved functional capacity (Jones et al., 2007; Li et al., 2013) and aerobic fitness (Kim do et al., 2009; West et al., 2015) as well as reductions in anxiety and depression (Carli et al., 2010; Coats et al., 2013; Li et al., 2013) have been reported in colorectal and lung cancer resection patients, evidence remains equivocal with other instances where no measurable beneficial effects have also been found (Dronkers et al., 2010). Even in studies where improvements in functional capacity and/or aerobic fitness have been observed following PREHAB in colorectal and lung cancer patients, there has been no evidence in a reduction in LOS (Li et al., 2013). In CABG surgery patients, Arthur et al. (2000) did report a one day reduction in LOS following a twice weekly PREHAB intervention (~mean duration 8.2 weeks) however no sufficiently powered studies in any cancer patients have been conducted to date. The presence of considerable variation in the intensity, volume, duration and modalities of the interventions used as well as the differing population characteristics of each study means making comparisons between existing studies is difficult.

Many of the previous studies have incorporated PREHAB interventions lasting four weeks or more (chapter 2, Tables 2.5, 2.7 and 2.9). However, in the UK the NHS guidelines dictate that in patients with a diagnosis of cancer, treatment starts within 31 days of the decision to treat (Baker & Nakatudde, 2015). The time available to implement a PREHAB programme in a UK hospital setting can therefore often be limited to just a 2-4 week period unless NACRT is required first. In studies using a similar time period, the results of PREHAB interventions are more equivocal. In a randomised controlled pilot study by Dronkers et al. (2010) no change in aerobic fitness or physical functioning (measured using the TUG) was observed following a 2-4 week, twice weekly 60 minute hospital based PREHAB programme in the Netherlands. This

raises the question whether in line with the current NHS timescales; does sufficient time exist for PREHAB to be applied prior to major colorectal resection surgery?

In chapter 3, an interest in the potential application of PREHAB prior to major surgery appeared evident in the Hull and East Riding of Yorkshire region with 75% of respondents indicating that they would consider participating in PREHAB if awaiting major elective surgery. The aim of this chapter was therefore to determine the feasibility of implementing a PREHAB programme into a NHS secondary care hospital, with the length of the intervention dictated by wait time to surgery (~2-6 weeks) alone. This included aspects of recruitment and subsequent patient follow-up, the logistics of implementing the programme and suitability of the intervention to the patients. Whether participation in PREHAB was sufficient to induce beneficial changes in functional capacity, clinical outcomes or HRQOL in patients awaiting major elective colorectal surgery was also investigated.

5.2. Methods

5.2.1. Ethical Approvals

Ethical approval was granted by the NHS Yorkshire & The Humber – Humber Bridge NRES committee (13/YH/0322) on 23rd October 2013 and Research and Development approval (R1573) from the Hull and East Yorkshire NHS trust on 16th January 2014. Three substantial amendments were subsequently submitted and approved. Approval for substantial amendment 1, which was a change from the generic HRQOL questionnaire, the SF-32 to the cancer specific EORTC-C30 questionnaire was issued on 30th January 2014. Substantial amendment 2 was for an extension of the exercise period from 2-4 weeks to 2-6 weeks and was approved on the 24th July 2014. Finally, substantial amendment 3 extended the inclusion criteria to include patients with benign disease in response to poor initial recruitment and was approved on the 23rd December 2014. Written informed consent was obtained from all participants prior to commencing and all testing followed the principles outlined by the Declaration of Helsinki.

5.2.2. Participants

Between March 2014 and July 2015, 23 participants who were listed for elective colorectal resection surgery at Castle Hill Hospital (HEY Hospitals NHS Trust) were recruited to a randomised controlled pilot study. The initial inclusion criteria was for all adult patients (aged 18 or older) identified at the weekly MDT meeting as being listed for elective colorectal cancer resection surgery at Castle Hill Hospital (HEY Hospitals NHS Trust) to be screened for eligibility. Following substantial amendment 3, this was extended to include patients with benign disease who were scheduled for any elective colorectal resection surgery. Potential participants were screened for initial eligibility by the study doctor for any health conditions that may prohibit their participation in the study before being approached. Exclusion criteria for the study included any of the following; a history of any heart related disease, current severe infection or fever, any uncontrolled metabolic diseases, uncontrolled asthma, a resting heart rate of more than 120 bpm, systolic blood pressure of more than 180 mmHg or diastolic blood pressure of more than 110 mmHg, any recent cerebrovascular accident, pregnancy, any pre-existing physical disability that would affect the participation in physical activity or an unwillingness to allow their GP to be informed of their participation in the study. Additionally, participants were excluded if the known expected time to surgery was less than two weeks. Receiving preoperative chemotherapy or radiotherapy was not a reason for exclusion from the study. If the participant appeared eligible according to the information available, the participant was approached, provided with the participant information sheet and given a minimum of 24 hours to consider participation. If the patient expressed an interest in participating, eligibility was confirmed and informed consent obtained.

5.2.3. Experimental design

A two group pilot randomized controlled parallel design with repeated measures was used in this trial. Following the consenting process, the initial baseline assessment was completed. Body mass (kg) and height (cm) were measured using digital scales accurate to ± 100 g (Seca Robusta 813, Hamburg, Germany) and Seca Stadiometer (Seca Leicester Height Measure, Birmingham, UK) and a venous blood sample was taken (described in detail in chapter 6, section 6.2.x). Two HRQOL questionnaires, the Hospital Anxiety and Depression Scale (HADS) and EORTC QLQ C30 were then

completed by the participant before the TUG, FTSTS, SCT, HGD and 6MWT were performed. Participants were then randomly assigned to receive either standard care (control group) or participate in a 2-6 week exercise PREHAB intervention using a random number sequence in blocks of 20 generated prior to the start of the study. The number sequence was generated using an online random number generator (https://www.randomizer.org/) and the assignments were sealed in opaque envelopes and opened in sequence following consent and once baseline assessments had been completed.

Participants randomised to the PREHAB group were required to perform a VO_{2peak} test from which the aerobic component of the PREHAB intervention was prescribed. Participants then attended three weekly PREHAB sessions up until the date of surgery. Changes in physical activity levels were monitored between baseline assessment and pre-operative reassessment in both groups through the completion of self-reported physical activity dairies. The HRQOL questionnaires and physical functioning tests were repeated at readmission to hospital on the day before surgery.

5.2.4. VO_{2peak} test

Participants randomised to the PREHAB group were asked to perform a graded cycling exercise test (VO_{2peak} test) to volitional exhaustion on an electronically braked cycle ergometer (Ergo Bike Premium, Daum electronic Gmbh, Furth, Germany). Participants completed a 3 minute seated rest period on the cycle ergometer prior to beginning the exercise test. A step protocol was used, starting with an initial workload of 20 watts (W) for 2 minutes and increasing by 5-25 W per minute until volitional fatigue was reached. Participants were instructed to provide maximal effort with the test continuing until the participant either reached volitional exhaustion, was unable to maintain a cadence of 60 rpm for more than 30 seconds despite encouragement, or the participant developed a sign or symptom that indicated early termination of an exercise test according to the ATS/American College of Chest Physicians (ACCP) guidelines (ATS/ACCP, 2003). Breath by breath analysis of gas exchange was collected in order to establish objective measures of cardiorespiratory fitness (Quark B2, Cosmed Srl, Rome, Italy). Prior to each exercise test, the system was calibrated according to the manufacturer's specifications, initially with ambient air and then followed by standard

gas (17.05 % O_2 and 4.98% CO_2). VO_{2peak} was calculated as the mean of the three highest consecutive 15 second oxygen consumption readings during the test.

5.2.5. PREHAB exercise programme

Participants were asked to attend three supervised exercise sessions per week at the University of Hull with the duration of the programme determined by wait time to surgery alone. Each exercise session was individualised to the participant and lasted approximately 60 minutes. Each session aimed to include:

- Five minute warm-up on a cycle ergometer at 40-50% heart rate reserve (HRR)
- Three or four circuits of exercises targeting ankle ROM (Section 5.2.5. *i*), medial gluteal activation (Section 5.2.5.*vii*), thoracic-spinal (T-spine) mobility (Section 5.2.5. *iv*), and shoulder function (Section 5.2.5. *v).*
- Up to 25 minutes of moderate intensity aerobic exercise on a cycle ergometer at 40-60% HRR and/or a perceived exertion of between 11-13 on the Borg scale.
- Two or three circuits of exercises targeting hip flexor ROM (Section 5.2.5.*ii*), gluteal activation (Section 5.2.5.*iii*), whole kinetic chain movement (Section 5.2.5.*viii*) and core control (Section 5.2.5.*vi*).
- Cool down (walking and stretching).

Although the intervention consisted of both aerobic and resistance training, the primary focus of the programme was to address common movement deficits observed in the general population, and in particular those individuals with a sedentary lifestyle, through the use of a functional resistance training programme. As discussed in section 2.4.4, previous PREHAB studies that have included resistance training within their intervention have predominantly focused on single joint exercises targeting the major muscle groups individually (Carli et al., 2010; Gillis et al., 2014; Li et al., 2013). The emphasis of these exercise has often been based on either performing specified numbers of repetitions and sets at a percentage of 1 RM (Dronkers et al., 2010; Timmerman et al., 2011) or achieving volitional fatigue (Carli et al., 2010; Gillis et al., 2014; Li et al., 2013) (Table 2.9). There is however an argument for applying a more functional approach to resistance exercises in PREHAB aimed at improving the performance of activities of daily living as well as reducing the risk of developing injuries when performing unaccustomed exercise. In response to this gap in the literature, the

resistance and flexibility exercises delivered in this thesis were based on the joint by joint approach to functional training developed by Michael Boyle and Gray Cook (Boyle, 2010) and based on the principles originally suggested by Shirley Sahrmann (Sahrmann, 2002). This approach are based on the view that each joint in the human body has specific functions (e.g. ankle and hip joints require mobility; knee and lumbar spine require stability; Section 2.5.4, Table 2.10) and dysfunction in one muscle group and/or joint can impact on the function of joints further up or down the body (Boyle, 2010). For example, a reduction in hip mobility as a result of the hip flexor tightness commonly associated with prolonged sitting (and sedentary behaviour) can affect gluteal activation, which especially in elderly populations may impact on their ability to perform simple tasks such as standing from a chair. The exercises included in the resistance/flexibility element of the intervention therefore targeted increasing ankle, hip and T-spine mobility whilst improving knee, lumbar spine/core and scapula stability by using a series of functional exercises based on the joint-by-joint approach.

As a guide to prescription, exercises were selected from Table 5.1 with the difficulty of the task increasing from left to right in the table. The exercises targeting hip flexor ROM, gluteal activation and core control were included in the second resistance circuit in order to address the potential tightening of hip flexors and related reduction in gluteal activity as well as the reduced core control, experienced as a result of prolonged cycling included as the aerobic component of the programme used here. Each specific starting exercise was determined on a participant by participant basis with the programme tailored to the individual. When possible, progressions were applied every two to three sessions however this was dependent on the participant's ability to demonstrate correct technique and participant-reported difficulty. Depending on the exercise being performed, progressions involved either increased repetitions/duration followed by added resistance or progression on to the next exercise. Whenever resistance was added, the number of repetitions was reduced in order to control for training volume. Given the population involved, there were occasions when regression was required for individual sessions (e.g. participant-reported fatigue or muscle soreness at the start of session), in these instances, repetitions/resistance/duration was reduced accordingly.

Table 5.1: Exercises and progressions used during PREHAB programme

Focus Area	Exercises (Increasing difficulty →)			
Ankle ROM	Seated heel / toe mobilization	Ankle mobilization	Heel Walking	Sit to stand (with variations in foot placement)
Progressions	*Reps*	*Reps*	*Dis*	*Reps*
Hip flexor ROM	Standing hip flexor stretch	Lying hip flexor stretch	Split squat	Rear foot elevated split squat
Progressions	*Dur*	*Dur*	*Reps*	*Reps; Res*
Gluteal activation	Bilateral lying gluteal bridge	Cook hip lift	Foot elevated single leg gluteal bridge	Shoulders elevated bilateral and unilateral gluteal bridge
Progressions	*Reps*	*Reps*	*Reps*	*Reps*
T-Spine mobility	Seated postural exercise variations	Standing postural exercise variations	Lying T-spine mobilization with roller (sagittal and transverse)	Foot raised thoracic extension
Progressions	*Reps*	*Reps*	*Reps*	*Reps; Res*
Shoulder function	Band pull apart variations	Band resisted external rotation	Seated row with bands	Lying scapular setting
Progressions	*Reps; Res*	*Reps; Res*	*Reps; Res*	*Reps; Res*
Core control	High kneeling band anti-rotation	Band resisted side shuffles	Suitcase carry	Ball passes
Progressions	*Reps; Res*	*Reps; Res*	*Reps; Res*	*Reps; Res*
Medial gluteal activation	Band resisted sit to stand	Side lying bent leg hip abduction	X-band walks	
Progressions	*Reps; Res*	*Reps; Res*	*Reps; Res*	
Whole kinetic chain	Kettlebell swings	Dumbbell push press		
Progressions	*Reps; Res*	*Reps; Res*		

ROM: Range of movement; Reps: Added repetitions; Added resistance; Dis: Added distance; Dur: Duration held

Consistent with previous PREHAB studies, an aerobic element was included. Initial prescription of aerobic exercise was based on the performance of the VO_{2peak} test with the corresponding HRR calculated from heart rate at peak exercise and the resting heart rate used to set the initial level of exercise. In the first session, 10 minutes of cycling was performed by participants, many of whom were unaccustomed to bike based exercise. As tolerated, duration of cycling was increased by 2-5 minutes per session up to a maximum duration of 25 minutes. Subsequent progressions and regressions in exercise intensity were based on participant heart rate response and/or participant self-reported perceived exertion on the Borg 6-20 scale (Borg, 1982).

Any inability to perform any component of the programme (e.g. any of the whole kinetic chain exercises or being unable to achieve the target duration of cycling) was not a reason for exclusion and the intervention was adapted to ensure continued participation.

i. Ankle range of motion exercises

Seated heel/toe mobilisation

Starting from an upright seated position with both feet positioned flat on the floor (Figure 5.1 a), participants were asked to alternate between dorsiflexion (Figure 5.1 b) and plantar flexion (Figure 5.1 c) of their ankle in a slow controlled manner. A total of 15-20 repetitions were completed per set.

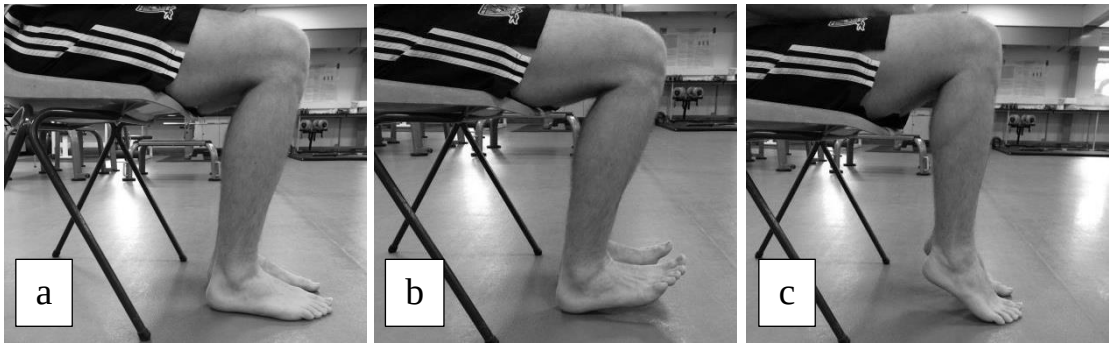


Figure 5.1: Seated heel/toe mobilisation (a) Starting with feet flat on the floor, (b) the participant alternated between dorsiflexion and (c) plantar flexion of ankle.

Ankle mobilisation

Resting both hands against a wall, participants were instructed to place one foot flat on the floor so the tip of the toes was approximately 5 cm away from the wall and the knee and hip slightly flexed. The other foot was then positioned behind the body with the leg extended (Figure 5.2 a). Participants then extended the hip, moving the front knee directly forward towards the wall travelling over the front foot resulting in the dorsiflexion of the ankle (Figure 5.2 b). Participants were instructed to position the front foot at a distance from the wall which ensured that the heel of their front foot did not rise from the floor, as the knee travelled over the toes. If they were unable to

achieve this, they were instructed to move the front foot closer to the wall and try again. Each set involved performing a total of 15-20 repetitions per leg.

Figure 5.2: Ankle mobilisation (a) From the starting of forefoot flat on the floor with both hands positioned against the wall (b) the participant moved the knee forward over the foot, resulting in dorsiflexion of the ankle.

Heel walking

From a standing upright posture, participants were instructed to point their toes upwards so only their heels were in contact with the floor. They were then required to walk 10 metres like this before turning around and walking back. As a progression of this exercise the distance walked was increased.

Sit to stand (with variations in foot placement)

Sitting with an upright posture on a chair from the ground, participants were asked to position both feet flat on the floor approximately one shoulder width apart (Figure 5.3 a). Participants were asked to rise from the seated position to an upright standing position before returning to the seat. Key instructions to the participants were to ensure the standing phase finished with the activation of the gluteus maximus muscle. Initially 8 repetitions were completed per set with subsequent progressions including increased repetitions per set and changes in height from which the exercise was performed. Foot placement was altered to either a narrower (Figure 5.3 b) or wider stance (Figure 5.3 c) to change the range of movement required in the ankle.

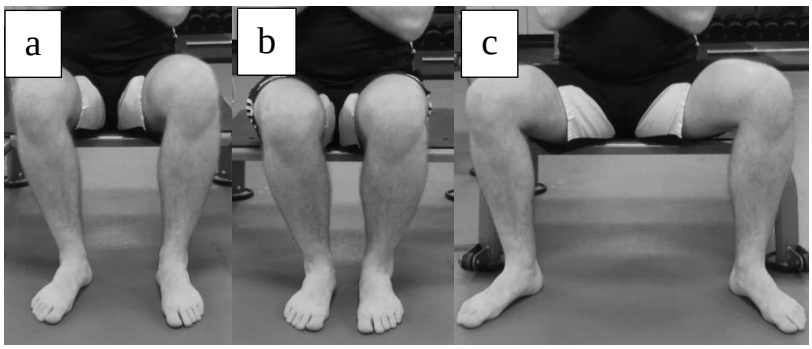


Figure 5.3: Sit to stand (with variations in foot placement) (a) Sitting with both feet flat on the floor approximately one shoulder width apart, the participant raised from the seated position to an upright standing position before returning to the seat. Foot placement was altered to either (b) narrow or (c) wider stance to change the range of movement required in the ankle.

ii. Hip flexor range of motion exercises

Standing hip flexor stretch

Participants were asked to assume the lunge position, with one foot out in front and the other positioned behind the torso, maintaining a straight upright posture of the chest and head (Figure 5.4 a). From this position, the participant gently lowered the rear knee towards the ground, maintaining the front knee's position over the ankle until they felt a slight stretch of the hip flexor of the rearmost leg, at which point they held the position for 15 seconds (Figure 5.4 b) before slowly returning to the start position. The exercise was then replicated on the opposite side with a total of three repetitions completed on either side. The exercise was progressed by increasing the time each stretch was held for to 30 seconds. For individuals who struggled with balance, platforms were positioned at either side of the participants to provide additional support.

Figure 5.4: Standing hip flexor stretch (a) Starting in the lunge position with a platform to the side for support (b) the participant lowered their rear knee to the ground where position was held for 15-30 seconds.

Lying hip flexor stretch

Participants were instructed to lie on a treatment table, with their legs overhanging the bottom of the bed. From this position, the instructor assisted with the flexion of one hip upwards whilst the other leg was allowed to relax into an extended position. The participant was asked to say when they felt a slight 'pull' on the hip flexor muscles, at which point the position was held for the count of 15 seconds before being returned to the starting position. The exercise was then replicated on the opposite side with a total of three repetitions completed on either side. The exercise was progressed by increasing the time each stretch was held for to 30 seconds.

Split squat

Starting from the same position as for the standing hip flexor stretch but with their hands positioned behind their head (Figure 5.5 a), participants lowered their back knee to the ground whilst keeping the front knee over the ankle (Figure 5.5 b). Once completing the downward phase of the movement, the participants were required to drive back upwards immediately pushing through the heel of the front foot finishing the movement with the activation of the gluteus maximus. Participants then completed the same movement on the opposite leg. Initial prescription consisted of sets of 8 repetitions with the exercise progressed firstly by increasing the number of repetitions performed per set (sets of 8, 10 or 12 repetitions) followed by the addition of 1-5 kg extra resistance (depending on the individual). When resistance was added, the number of

repetitions was reduced to the initial starting point of 8 repetitions before being progressed again. If required, participants could then be progressed to the rear foot elevated split squat.

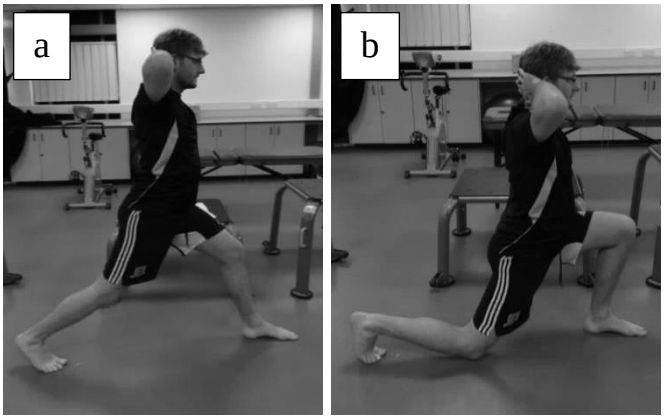


Figure 5.5: Split squat (a) From a lunge starting position, (b) the participant lowered their rear knee to the ground in a controlled manner before immediately pushing up through the front heel to return to the starting position.

Rear foot elevated split squat

The exercise was performed in a similar manner to the split squat but with the rear foot elevated on a platform meaning only one instead of two stable points of contact existed with the floor (Figure 56 a). The downward phase of the movement was performed until the knee was almost touching the ground and the front thigh was parallel to the ground (Figure 5.6 b). As with the split squat, participants were required to drive back upwards immediately pushing through the heel of the front foot finishing the movement with the activation of the gluteus maximus (Figure 5.6 c). The same movements were then completed on the opposite leg. Initial prescription consisted of sets of 6 repetitions with the exercise progressed firstly by increasing the number of repetitions performed per set (sets of 6, 8 or 10 repetitions) followed by the addition of 1-5 kg extra resistance (depending on the individual). When resistance was added, the number of repetitions was reduced to the initial starting point of 6 repetitions before being progressed again.

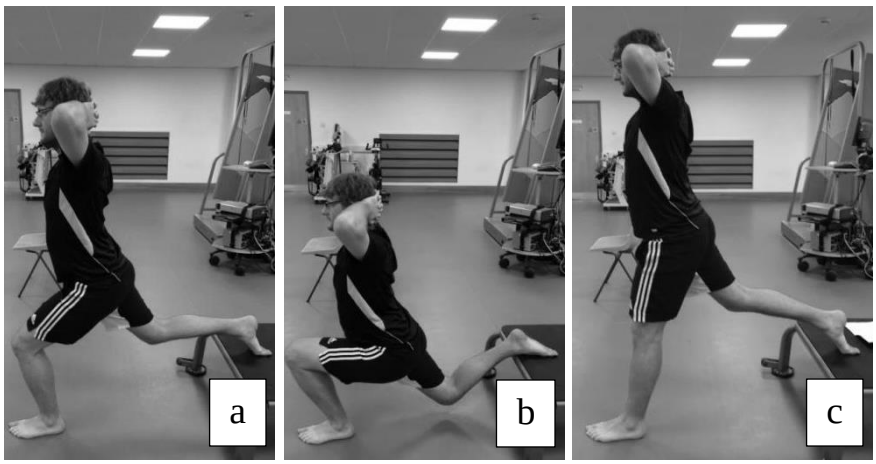


Figure 5.6: Rear foot elevated split squat (a) Starting with the front foot as the only stable point of contact with the floor (b) the participant slowly lowers their rear knee (c) before pushing back up through the heel of the front foot.

iii. Gluteal activation exercises

Bilateral lying gluteal bridge

Participants were asked to take up a supine position on the floor with their knees flexed at 90° angles so only their heels were in contact with the ground. Arms were positioned by their sides in order to aid stability (Figure 5.7 a). Pushing through their heels, participants were instructed to drive their hips upwards, activating their gluteus maximus and forming a straight line along the thighs and torso (Figure 5.7 b). Having held that position for 3 seconds, participants then slowly lowered themselves down to the resting supine position. Initial prescription consisted of sets of 8 repetitions with the exercise progressed firstly by increasing the number of repetitions performed per set (sets of 8, 10 or 12 repetitions).

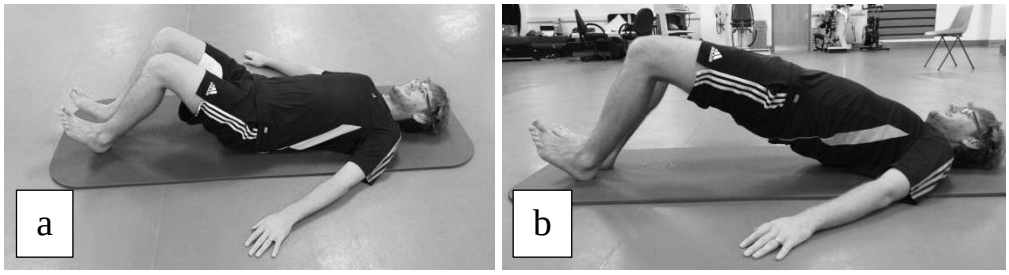


Figure 5.7: Bilateral lying gluteal bridge (a) Lying with knees flexed at 90°, (b) the participant drives the hips upwards forming a straight line along thighs and torso.

Cook hip lift

Initially starting in the supine position with both feet flat on the floor, the participant placed a tennis ball against their ribs before pulling one leg towards their chest, securing the ball in position (Figure 5.8 a). Pushing downwards on the floor with the heel of the other foot, participants extended their hip whilst maintaining the ball position against the ribs with the other leg (Figure 5.8 b). Having held that position for 3 seconds participants then lowered themselves to the floor before repeating the exercise. Initial prescription started with 6 repetitions per set with additional repetitions (sets of 6, 8 or 10 repetitions) added as the progression. The participants then completed the same movement on the opposite leg.

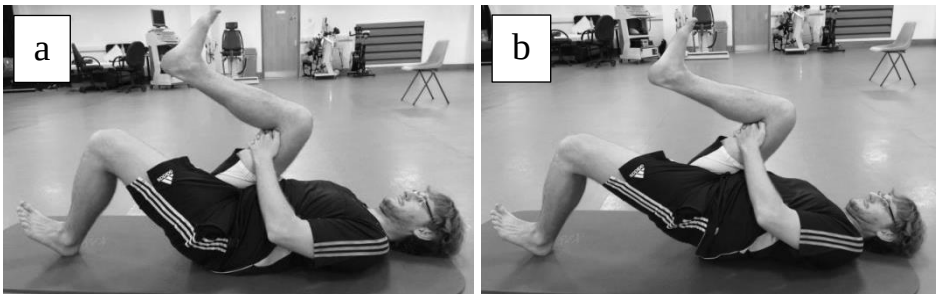


Figure 5.8: Cook hip lift (a) Securely holding a tennis ball between thigh and chest of one leg, (b) the participant pushes up with the opposite leg extending hip.

Foot elevated single leg gluteal bridge

Lying in the supine position, participants held one leg vertically in the air whilst the other leg was positioned on a step block (Reebok, Amsterdam, Netherlands) with the knee flexed at 90° and only the heel in contact (Figure 5.9 a). Arms were positioned by their sides so to aid support. As with the bilateral gluteal bridge, participants pushed through the heel of the foot which was positioned on the block, extending the hip until a straight line along hip and torso was formed (Figure 5.9 b). Having held the position for 3 seconds, participants slowly lowered themselves back to the resting supine position before repeating the movement. The same movement was then completed on the opposite leg. Initial prescription consisted of sets of 6 repetitions with the exercise progressed by increasing the number of repetitions performed per set (sets of 6, 8 or 10 repetitions).

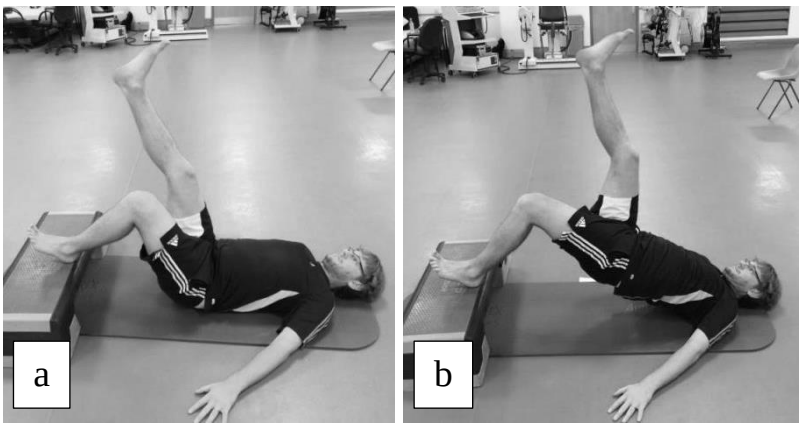


Figure 3.9: Foot elevated single leg gluteal bridge (a) Lying down with one leg straight in the air and the other foot positioned on a step block, (b) the participant pushes up through their heel, extending the hip forming a straight line along hip and torso.

Shoulders elevated bilateral gluteal bridge

Participants were instructed to rest the bottom of the shoulder blades on an exercise bench, so that the base of their scapular was level with the edge of the bench and their arms were extended to the sides for support. Both feet were placed flat on the floor with their hips flexed (Figure 5.10 a). Participants then extended their hips, pushing through their heels and contracting the gluteus maximus until the thighs and torso formed a straight line (Figure 5.10 b). This position was held for 3 seconds before the participants slowly lowered themselves back to the resting position and repeated the exercise. Starting with initial sets of 6 repetitions, the exercise progressed by increasing the number of repetitions performed per set (sets of 6, 8 or 10 repetitions).

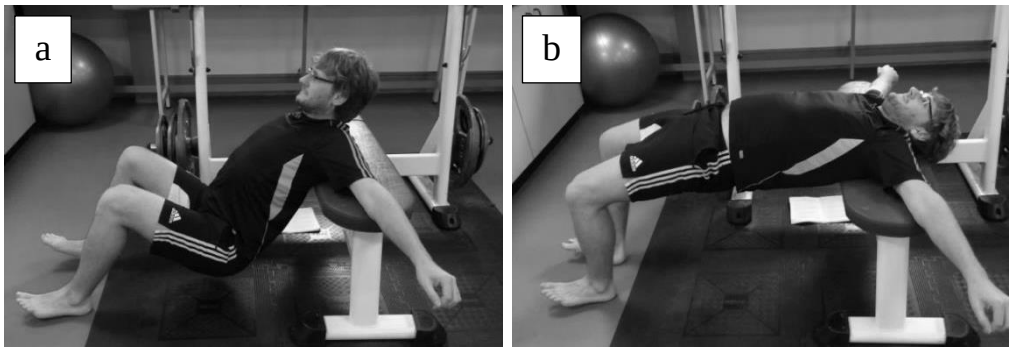


Figure 5.10: Shoulders elevated bilateral gluteal bridge (a) With shoulders positioned on the bench, knees and hips flexed and both feet flat on the floor, (b) the participant pushed through their heels and extended their hips until the thighs and torso formed a straight line.

Shoulders elevated unilateral gluteal bridge

The exercise was performed and progressed in the same manner as the shoulders elevated bilateral gluteal bridge with the exception of two key points; firstly, participants had only one foot in contact with the floor meaning only one stable point of support existed (Figure 5.11 a). Secondly, the alternate leg was flexed and held in position with a 90° angle at both the hip and knee (Figure 5.11 b). The exercise was performed using both legs.

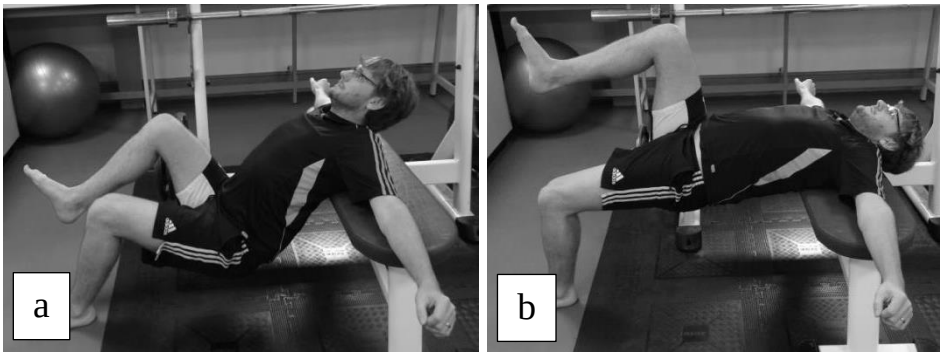


Figure 5.11: Shoulders elevated unilateral gluteal bridge (a) With shoulders positioned on the bench, one foot flat on the floor and the other leg positioned with 90° angle at the hip and knee, (b) the participant pushed through their heels and extended their hip until the thighs and torso formed a straight line.

iv. T-spine mobility exercises

Seated and standing postural exercise variations

This exercise was performed from either a seated or standing position. Participants were instructed to take up their normal neutral position, from which they slumped forward to a position of poor posture. This included shoulders forward, back arched and head tilted forward resulting in an enclosed chest cavity (Figure 5.12 a). Participants were then required to straighten their back, pushing their shoulders back and down so they were in a position of good posture (Figure 5.12 b). A total of 8-12 repetitions were performed per set.

Figure 3.12: Standing postural exercise (a) From a slumped position, (b) the participant straighten their back and pushed their shoulders back forming a straight upright posture.

Lying t-spine mobilisation with roller (Transverse)

Participants were instructed to lie on their side with their bottom leg, back and neck forming a straight line. Their top leg was flexed at a 45° angle at the hip with their knee resting on a foam roller and their arms extended in front of them (Figure 5.13 a). Maintaining the contact between their top leg and the foam roller to minimise hip rotation, participants were told to rotate their uppermost arm backwards as far as possible in a controlled manner, following the movement with their eyes, stopping when they could no longer see their arm (Figure 5.13 b). They then returned to the starting position. A total of 10-15 repetitions were performed on each side.

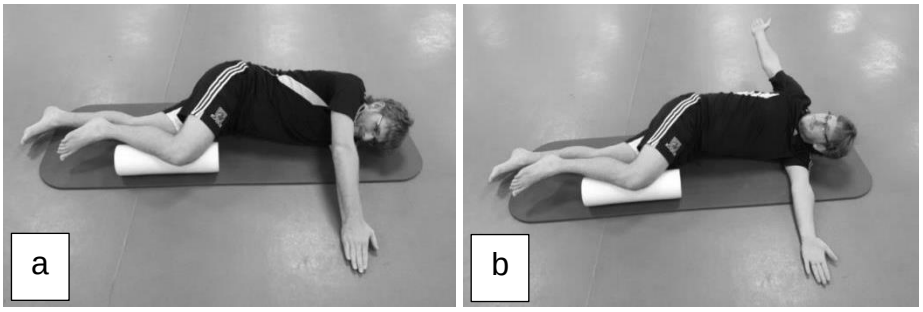


Figure 5.13: Lying t-spine mobilisation with roller (Transverse) (a) Lying on the their side with the bottom leg, back and neck straight and the top leg flexed at the hip and knee, and resting on a foam roller, (b) the participant rotated their arm backwards, following the movement with their eyes.

Lying t-spine mobilisation with roller (Sagittal)

In the supine position with knees flexed at 45°, participants were instructed to position a foam roller at the base of the scapular. With their core set and hands positioned behind their head, participants extended their spine, held the position for the count of 3 before relaxing (Figure 5.14 a). Raising their bottom into the air, the participant rolled forward on the foam roller for a couple of centimetres (Figure 5.14 b) before lowering themselves back down and repeating the stretch. This was repeated until the foam roller was positioned at the top of the shoulders.

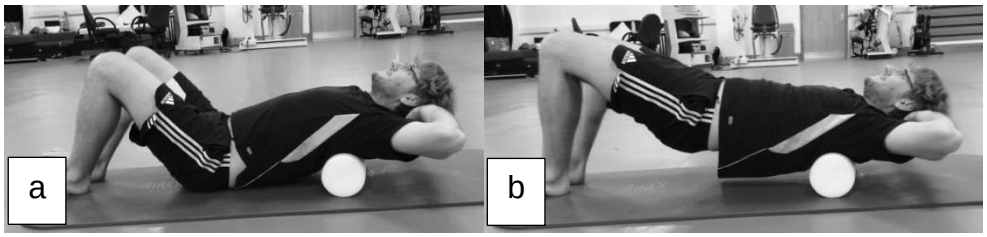


Figure 5.14: Lying t-spine mobilisation with roller (Sagittal) (a) With a foam roller positioned at the base of the scapular, the participant extends their spine for the count of 3. (b) Having relaxed, the participant rolls with gluteals in the air before repeating the stretch.

Foot raised thoracic extension

With one foot elevated on a platform and the other foot positioned behind on the floor, participants held a medicine ball in front of their torso using both hands (Figure 5.15 a). Moving forward in a controlled manner, with flexion of the front knee and the extension of the rear hip, participants raised the ball upwards over their head, following the trajectory of the medicine ball with their eyes, resulting in the extension of their back (Figure 5.15 b) before returning to the starting position. Participants then swapped legs and repeated. Initially 8 repetitions per set were performed using a 2 kg medicine ball with the exercise progressed by increasing the repetitions per set (sets of 8, 10 or 12 repetitions). If required, the exercise was then progressed by increasing medicine ball weight (5 kg) however the number of repetitions performed was reduced back to the initial starting point of 8 before being progressed again.

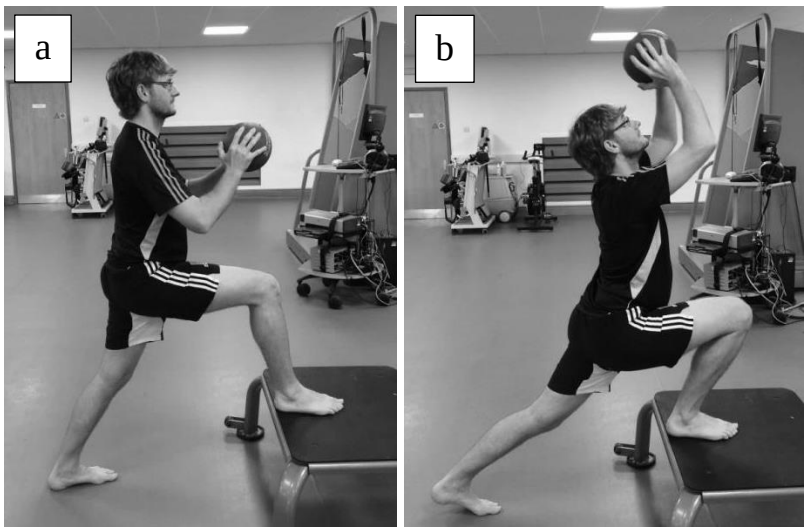


Figure 5.15: Foot raised thoracic extension (a) Holding a medicine ball in front of the torso with one foot elevated in front and the other positioned behind, (b) in one continuous movement, the participant moved forward flexing the front knee and hyperextending the rear hip whilst raising the ball upwards over their head, following the trajectory of the medicine ball with their eyes.

v. Shoulder function

Lying scapular setting

Lying in the supine position on an exercise bench with their feet up, participants were asked to push themselves forward ensuring their scapulae were back and down. With arms extended vertically in the sagittal plane and holding a weight (2.5 – 5.0kg) in each hand, participants moved the weights up and down repeatedly by extending (Figures 5.16 a) and retracting (Figures 5.16 b) their scapulae. The number of repetitions performed started at 15 before being progressed to 20 repetitions if tolerated. The exercise was further progressed by adding extra weight however this was always coincided with a reduction in the number of repetitions performed.

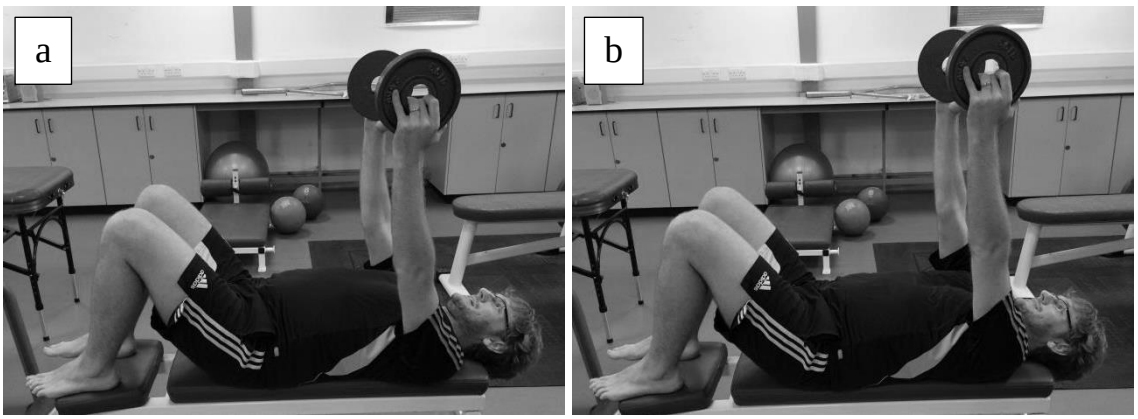


Figure 3.16: Lying scapular setting (a) Lying down with arms extended vertically and a weight in each hand, (b) the weights were slowly lifted up and down by extending and retracting the scapulae.

Band pull aparts

Three different variations of the band pull aparts were used. The starting posture for all three was a standing upright position with shoulders back and the core set. Variation 1 consisted of the participant holding a resistance tube (Pullum Sports, Luton, UK) with a pronated grip and arms extended forwards in the sagittal plane (Figure 5.17 a). Keeping the arms extended, with the band at chest level, participants slowly horizontally abducted and adducted their arms (Figure 5.17 b). Variation 2 was the same as variation 1 except the band was held with a supinated grip (Figure 5.17 c). For variation 3, holding a resistance band in a pronated grip with arms extended above their head (Figure 5.16 d), participants actively depressed their scapulae and flexed their elbows to pull their arms downwards, extending the resistance tube (Figure 5.16 e) before returning to the starting position. For all variations, the initial resistance used was dependent on the individual, with three levels of resistance tube available (beginner, professional, advanced). The number of repetitions performed began at 8 per set, with the exercise progressed by the addition of extra repetitions (sets of 8, 10 or 12). Participants were progressed by moving onto the next resistance tube however this always coincided with an initial reduction in repetitions performed.

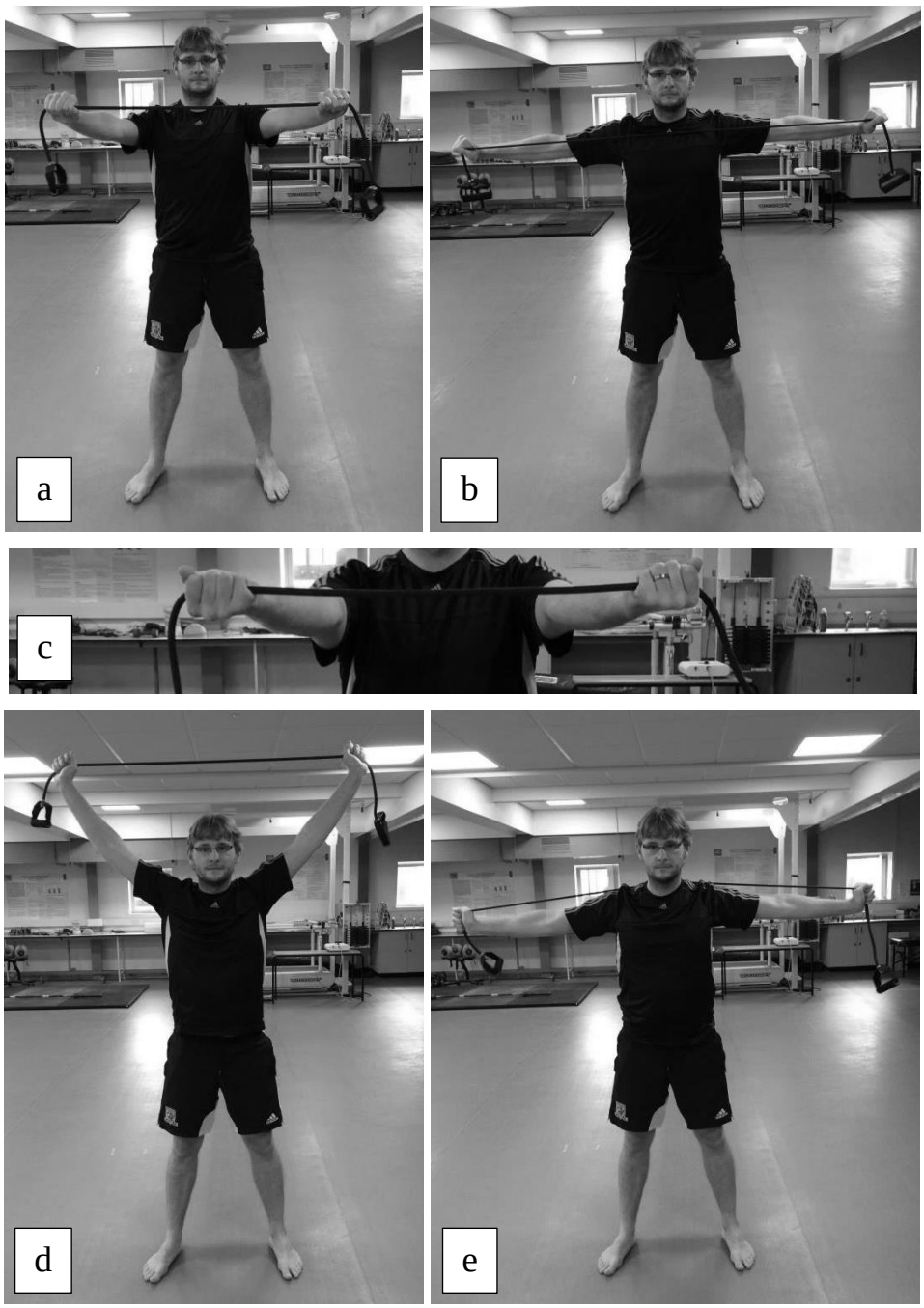


Figure 5.17: Band pull aparts (a) Variation 1: Holding a resistance band with a pronated grip and arms extended in front, (b) the arms were slowly abducted and adducted horizontally. (c) Variation 2: This was also performed with a supinated grip. (d) Variation 3: a resistance band was held in a pronated grip with arms extended above their head, (e) scapulae were depressed and elbows flexed to pull the arms downwards extending the resistance tube.

Band resisted external rotations

From a side on starting posture, participants were instructed to hold one end of a securely anchored resistance tube (Pullum Sports) with the hand furthest away from the

anchor point. With the elbow flexed to 90°, so the medial side of the arm was in contact with the ribs (to ensure contact was maintained, participants were required to hold a towel between their elbow and ribs) (Figure 5.18 a); participants externally rotated their shoulder, slowly stretching the resistance tube (Figure 5.18 b) before returning to the starting position. The exercise was then replicated on the opposite side. The number of repetitions performed began at 8 per set, with the exercise progressed by the addition of extra repetitions (sets of 8, 10 or 12). Participants were progressed by moving onto the next resistance tube however this always coincided with an initial reduction in repetitions performed.

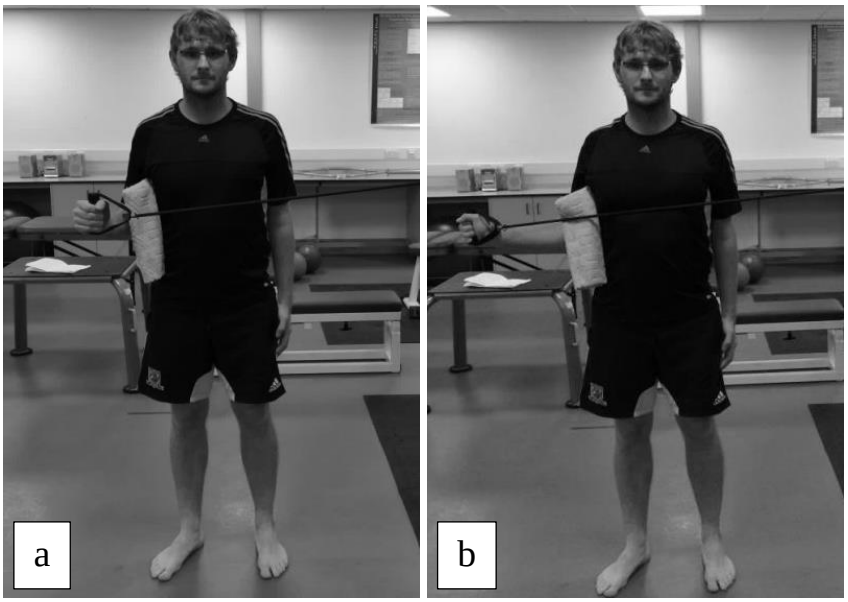


Figure 5.18: Band resisted external rotations (a) Holding a resistance band with elbow flexed and a towel trapped against their ribs, (b) the participant slowly externally rotated the shoulder outwards before returning to the starting position.

Seated row

Seated on the floor with legs extended in front so their feet were resting against a step box and maintaining an upright back posture with their core set and shoulders back; participants held the handles of a securely anchored resistance tube (Pullum Sports) in a neutral grip with arms extended in front horizontally (Figure 5.19 a). From this position, participants slowly brought their arms towards themselves, by first retracting their scapulae and then flexing their elbows and externally rotating their wrists 90° until their palms were pronated (Figure 5.19 b) before returning to the original starting position. The number of repetitions performed began at 8 per set, with

the exercise progressed by the addition of extra repetitions (sets of 8, 10 or 12). Participants were progressed by moving onto the next resistance tube however this always coincided with an initial reduction in repetitions performed.

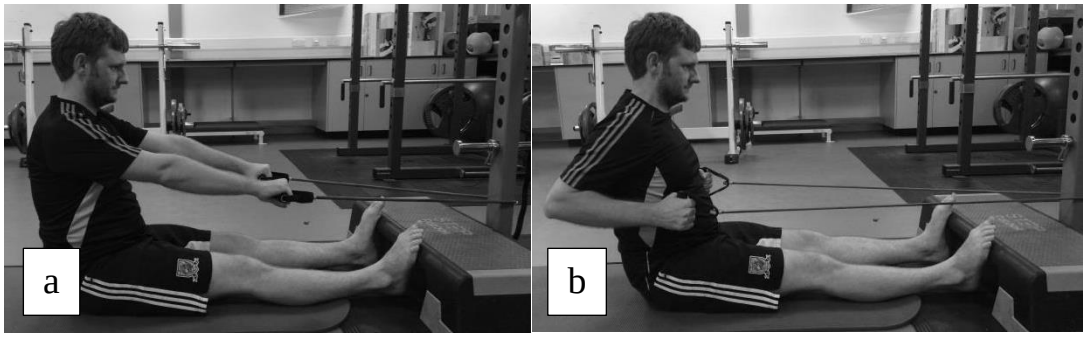


Figure 5.19: Seated Row: (a) Holding the handles of a securely anchored resistance tube in a neutral grip with arms extended in front horizontally, (b) the participant retracted their scapulae and slowly flexed their elbows whilst externally rotating their wrists as they brought their arms towards them.

vi. Core control

High kneeling band anti-rotation

In a high kneeling position, participants were instructed to set their core, whilst holding one end of a securely anchored resistance band (Pullum Sports) close to their chest with both hands using a pronated grip (Figure 5.20 a). From this position, participants extended their arms forward horizontally, increasing the tension in the band (Figure 5.20 b). Participants held this position for the count of three, resisting the urge to rotate inwards towards the anchor position. The number of repetitions performed began at 8 per set, with the exercise progressed by the addition of extra repetitions (sets of 8, 10 or 12). Resistance could also be added either by the participants moving further from the anchor point or by moving onto the next resistance tube. When resistance was increased, the number of repetitions performed was reduced.

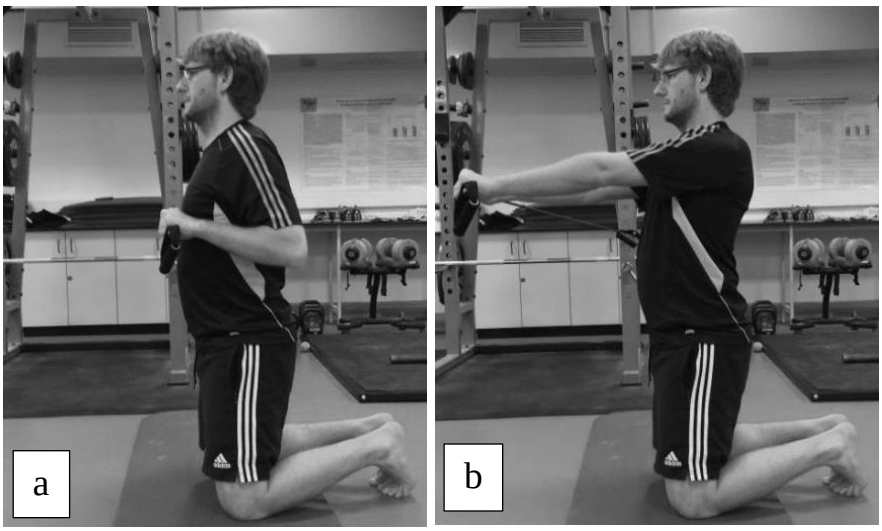


Figure 5.20: High kneeling band anti-rotation: (a) In a high kneeling position with their core set, the participant held one end of a securely anchored resistance band close to their chest with both hands using a pronated grip. (b) The participant then extended their arms forward horizontally, increasing the tension in the band.

Band resisted side shuffles

Standing in a side on position with their core set and arms extended horizontally in front of their torso, participants held one end of a securely anchored resistance tube (Pullum Sports) with both hands using a pronated grip (Figure 5.21). Taking three small steps sidewards, participants gradually increased the tension in the band, resisting the rotational force generated by the tension in the band. After the third step, the position was held for the count of two, before turning and returning to the starting position. Starting with 8 repetitions per set, the exercise was progressed firstly with the addition of extra repetitions (sets of 8, 10 or 12) and then with the additional of extra resistance by changing to the next resistance tube. When resistance was increased, the number of repetitions performed was reduced until the participant was ready to progress again.

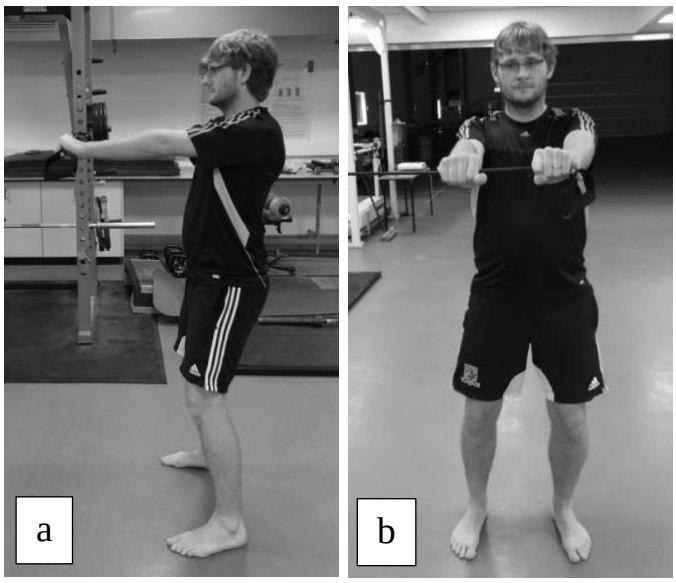


Figure 5.21: Band resisted side shuffles: (a) Standing in a side on stance with core set, arms extended in front (b) holding one end of a securely anchored resistance band with a pronated grip. The participant took three small sideward's steps, gradually increased the tension in the band.

Ball passes

Standing in an upright posture with their core set and arms extended in front of their torso, participants had a medicine ball passed to their right hand side from behind. Participants were required to rotate to the right, collect the ball in both hands before rotating to the left maintaining a set core and arms extended forwards. This was performed 8-12 times per set with the exercise then repeated starting from the left hand side. Initially, 8 repetitions per set were performed using a 2 kg medicine ball with the exercise progressed by increasing repetitions per set (sets of 8, 10 or 12 repetitions). If required, the exercise was then progressed by increasing medicine ball weight (5 kg) however the number of repetitions performed was reduced back to the initial starting point of 8 before being progressed again.

Suitcase carry

Standing in an upright posture with their core set and shoulders pulled back and down, participants held a dumbbell or kettlebell in one hand. Participants then walked 10 metres; turned 180° before walking back, maintaining the starting posture by resisting excessive side bending caused by the weight imbalance (Figure 5.22). The

exercise was progressed by increasing the distance walked by 10 metre increments with the option of increasing the weight carried as appropriate.

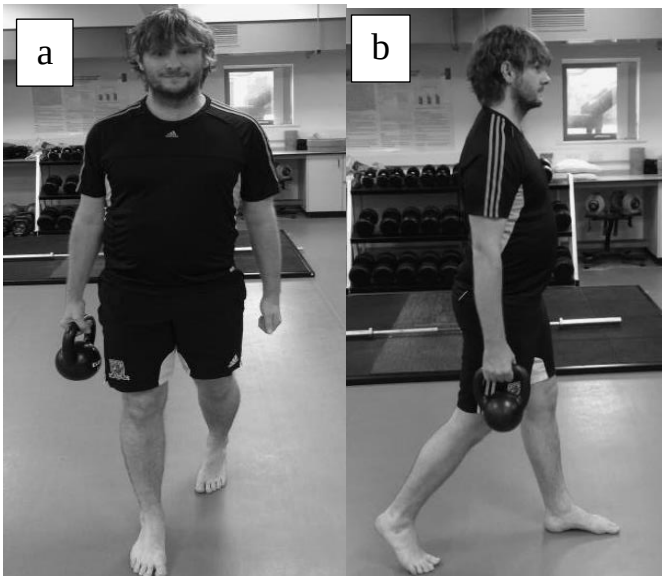


Figure 5.22: Suitcase carry: Standing with a kettlebell in one hand, in an upright posture with their core set and shoulders pulled back and down, the participant began walking, resisting excessive side bending caused by the weight imbalance

vii. Medial gluteal activation

Band resisted sit to stand

Sitting with an upright posture on a chair from the ground, participants were asked to position both feet flat on the floor approximately one shoulder width apart. With either a 13 inch resistance band (2.3-9.0 kg or 4.5-13.6 kg; Pullum Sports, Luton, UK) placed around the legs and positioned slightly below the knees (Figure 5.23), participants were asked to rise from the seated position to an upright standing position before returning to the seat. Key instructions to the participants included asking them to resist the band's attempt to draw the knees inwards in order to activate the gluteus medius and ensuring the standing phase finished with the activation of the gluteus maximus muscle. Initially 8 repetitions were completed per set with subsequent progressions including increased repetitions per set, increased band resistance and changes in height from which the exercise was performed.

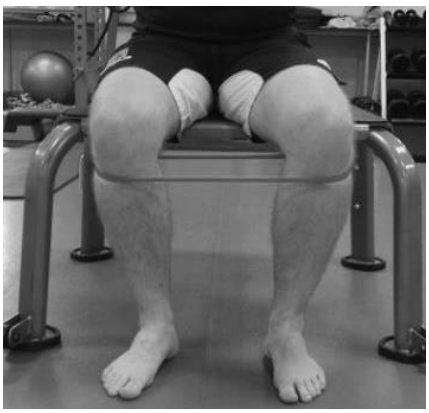


Figure 5.23: Band resisted sit to stand. With a resistance band positioned slightly below the knees, the participant raised to an upright standing position before returning to the seat.

Side lying bent leg hip abduction

With a 13 inch resistance band (Pullum Sports) placed around their thighs, participants were asked to lie on their sides with their knees flexed at 45° and hips flexed at 45° so that the soles of their feet were level with their spine. Hips and shoulders were positioned one over the other forming a vertical line (Figure 5.24 a). Participants then rotated their uppermost hip upwards, holding the position at the top (Figure 5.24 b) before returning to the resting position. Throughout the movement both feet were required to remain together. In order to ensure the movement came from the hip rather than the lumbar spine rotation, the instructor stood behind the participant with their leg resting against the participants back ensuring hips and shoulders remained in the starting vertical position. Generally sets began with 8 repetitions with either additional repetitions or greater resistance added as the individual progressed. The movement was then repeated on the opposite side. Starting with 8 repetitions per set, the exercise was progressed firstly with the addition of extra repetitions (sets of 8, 10 or 12) and then with the additional of extra resistance by changing to the next resistance band. When resistance was increased, the number of repetitions performed was reduced until the participant was ready to progress again.

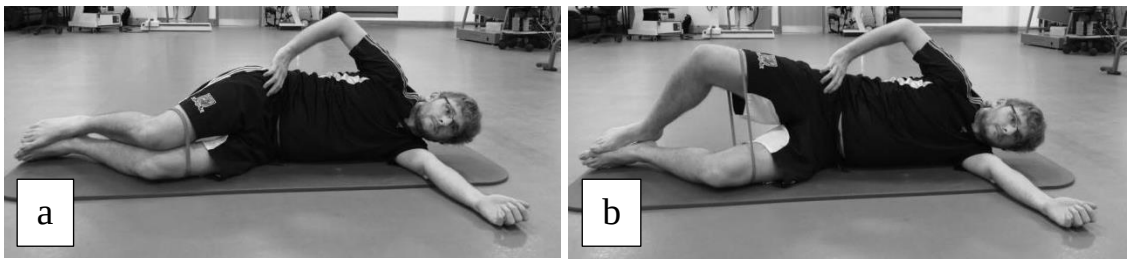


Figure 3.24: Side lying bent leg hip abduction (a) Lying on their side with a resistance band placed around their thighs and their knees and hips flexed (b) the participant then rotated their hip upwards.

X-band walks

Standing in a straight upright posture with their feet positioned one shoulder width apart; participants placed a 41 inch resistance band (2.2-15.9 kg; Pullum Sports) under the soles of their feet, crossed the band over forming an X and held the top of the band with both hands ensuring tension was present (Figure 3.25 a). Participants then took a side step with one leg against the resistance of the band to extend their stance to approximately two shoulder widths apart (Figure 3.25 b) before the other leg followed in a controlled manner to return to the one shoulder width apart stance (Figure 3.25 c). A total of 5 side steps were taken in one direction before they repeated the movement in the opposite direction. The exercise was progressed initially by the addition of extra side steps. The level of resistance could also be increased by either altering the hand positioning to generate greater band tension or by changing to the next resistance band.

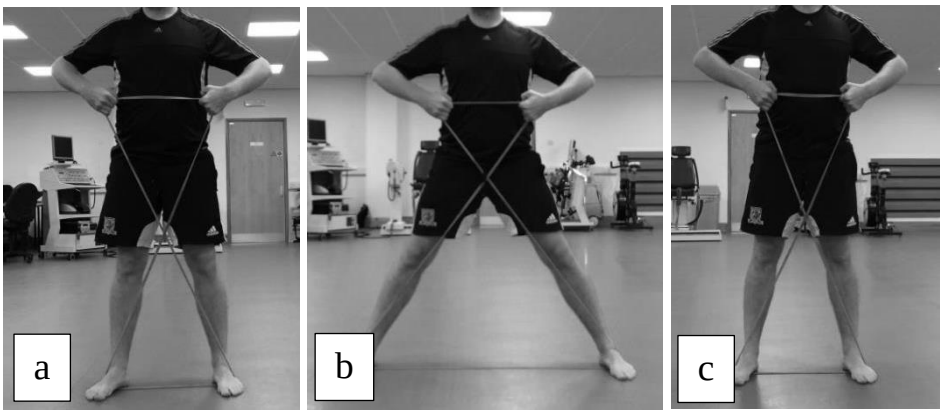


Figure 3.25: X-band walks (a) Positioned with feet shoulder width apart and a resistance band under the soles of their feet and held with both hands forming an X, (b) the participant takes a side step against the resistance of the band extending the stance to two shoulder widths (c) before following with the other leg, returning to the starting stance.

viii. Whole kinetic chain exercises

Kettlebell swing

The kettlebell swing consisted of three phases, the starting position, the upwards phase and the downwards phase. The starting position involved the participants standing with their feet approximately one shoulder width apart and with their hips and knees slightly flexed. Participants then held a single kettlebell (Eleiko Sports, Chicago, USA) with both hands so the weight hung between their legs (Figure 5.26 a). Once in this position, they began gently swinging the kettlebell backwards and forwards in order to gain momentum. This constituted the start of the upwards phase. Maintaining straight arms as the kettlebell travelled forward, participants extended their hips and knees, propelling it upwards in an arch until it has travelled to shoulder level or just above (Figure 5.26 b). Almost immediately the transition into the downward phase occurred as the kettlebell returned back downwards during which time the participants flexed their hips and knees again ensuring they returned to the original starting position before repeating. Starting with 8 repetitions per set, the exercise was progressed by increasing the number of repetitions performed (sets of 8, 10 or 12 repetitions) followed by increased kettlebell weight (with fewer repetitions).

Figure 5.26: Kettlebell swing (a) Starting with feet shoulder width apart, knees and hips flexed, (b) the kettlebell is swung forward maintaining extended arms forming an arch finishing with the kettlebell at shoulder level.

Push press

As with the kettlebell swing, the push press involved three phases, the counter movement phase and the upwards phase. The starting position consisted of the participants standing with their feet approximately one shoulder width apart with a straight upright posture. In this stance, participants held a single dumbbell in one hand positioned above their shoulder with their elbow flexed (Figure 5.27 a). From this position the participants began the countermovement phase, slightly flexing their hips and knees at a moderate speed moving the dumbbell downwards in a straight path (Figure 5.27 b). Having dipped approximately 5-10 cm, participants immediately began the upwards phase, extending their knees, hips and arm thus driving the dumbbell in a straight path upwards where it was held for one second (Figure 5.27 c) before being lowered back down slowly to the starting position. To begin with this was performed 8 times, before swapping the dumbbell to the opposite hand and repeating. The exercise was progressed by increasing the number of repetitions performed (sets of 8, 10 or 12 repetitions) followed by reducing repetitions but increasing dumbbell weight.

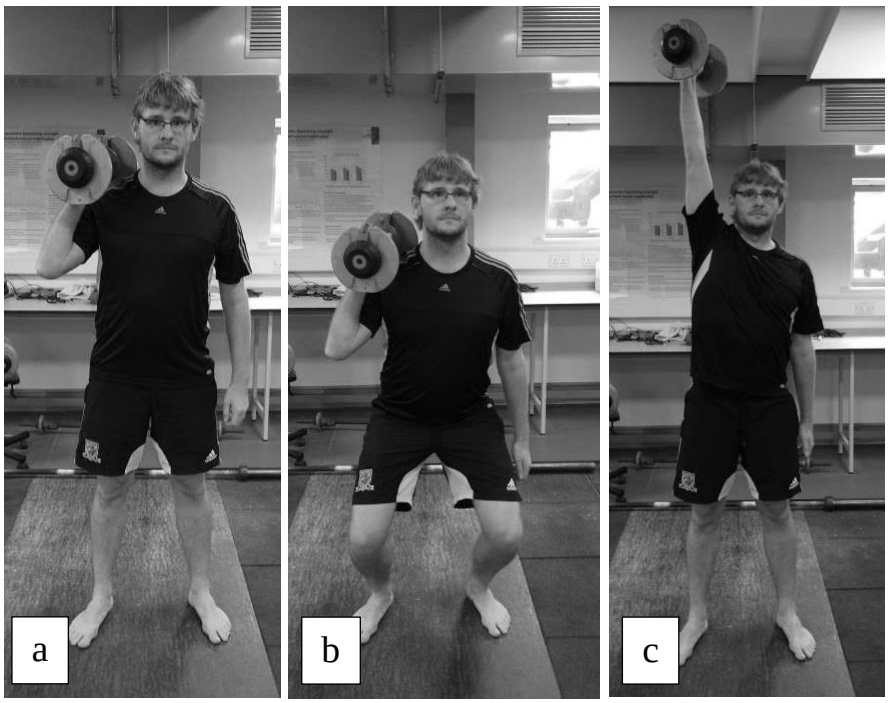


Figure 5.27: Push press (a) Starting position – feet shoulder width apart with arm flexed at the elbow, holding dumbbell above shoulder, (b) Counter movement phase – flexion of knees and hips as body moved downwards, (c) Upwards phase – from the dipped position participant drives upwards extending the knees, hips and elbow.

5.2.6. Outcome measures

i. Hospital Anxiety and Depression Scale

The HADS (Appendix C) was designed by Zigmond and Snaith (1983) as a method to assess patient anxiety and depression in clinical and medical settings over the previous seven days. Consisting of a 14-item scale, half of the HADS relates to the anxiety subscale and half to the depression subscale allowing both aspects to be assessed individually as well as providing an overall measure of patient distress. With each question scored on a four point scale ranging from 0 to 3, sub section scores range from 0 to 21 with the higher scores reflective of increased anxiety or depression. A score of ≥8 has been reported as the lower cut off point for the possible presence of anxious or depressive symptoms, with a score ≥11 indicative of the probable existence of a disorder (Terluin, Brouwers, van Marwijk, Verhaak, & van der Horst, 2009).

ii. EORTC QLQ-C30

The EORTC QLQ-C30 is a quality of life instrument developed for cancer patients (Aaronson et al., 1993) (Appendix D). Comprising of 30 core questions, the EORTC QLQ-C30 assesses five functional scales (physical functioning, role functioning, emotional functioning, cognitive functioning and social functioning), nine symptom scales/single items (fatigue, nausea and vomiting, pain, dyspnoea, insomnia, appetite loss, constipation, diarrhoea and financial difficulties) and a global health status/quality of life (GHS/QOL) scale. Scoring for each sub-scale can range between 0-100. A high score on either the functional or GHS/QOL scale represents a high level of functioning or QOL; whilst a high symptom scale score represents a high level of symptoms. The EORTC QLQ C30 (Version 3) is the most current version of the questionnaire and therefore was used within this thesis. In the EORTC QLQ C30 (Version 3), questions 1 to 28 are answered on a four point scale (“Not at all”, “A little”, “Quite a bit” and “Very much”) with questions 29 and 30 on a seven point scale (1 = Very poor, 7 = Excellent).

iii. Timed up and go test

Timed up and go was performed as described in chapter 4.

iv. Five times sit to stand test

Five times sit to stand was performed as described in chapter 4.

v. Stair climb test

Apart from a flight of stairs located within the hospital ward being used (same dimensions as purpose built stairs), the SCT was performed as described in chapter 4 (page 95).

vi. Handgrip dynamometry

Handgrip dynamometry was performed as described in chapter 4.

vii. Six minute walk test

Due to space restrictions at the hospital, the course was reduced to a distance of 10 metres marked out in two metre intervals. All other aspects of the 6MWT were performed as described in chapter 4.

viii. Other outcomes

Clinical outcomes such as length of hospital stay, incidences of post-operative complications and the occurrence of any adverse events were recorded. Adherence rates to the PREHAB intervention as well as reasons for non-attendance were documented.

5.2.7. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 20 (SPSS Inc., Chicago, IL). In line with the recommendations into the analysis of pilot studies (Lancaster, 2015; Lancaster et al., 2004), the primary analysis this chapter focused on descriptive statistics with hypothesis testing forming the secondary analysis. A common criticism of hypothesis testing in pilot and feasibility studies is that they are not designed or powered to detect the effectiveness of an intervention increasing the chance

of type I and type II errors occurring. Normal distribution of data was determined by the visual inspection of quantile-quantile plots, histograms and box plots. Normally distributed continuous variables were reported as mean ± standard deviation whilst non-normally distributed data was reported as median (interquartile range [IQR]). Range [range] was displayed for both normally and non-normally distributed data. Between groups was completed using either Independent Sample T-tests for normal distribution or Mann-Whitney U test for non-normally distributed data. Within group effects of the PREHAB intervention were performed using the Wilcoxon Rank-Sum test due to the non-normal distribution of the relevant data. Statistical significance for all tests was set at $p < 0.05$.

5.3. Results

5.3.1. Study recruitment

Figure 5.28 depicts the flow of participants through the study. During the 18 month recruitment period (February 2014 to July 2015), a total of 198 eligible patients were listed for major elective colorectal cancer resection surgery. Of these, 84 patients were excluded due to insufficient time until surgery (< 2 weeks) meaning 114 patients were approached about participating in the study. A total of 21 cancer patients consented to participate equating to a consent rate of 18.4%. From January 2015 onwards, inclusion criteria were expanded to include resection surgery for benign disease resulting in the further recruitment of two additional patients. One cancer patient withdrew from the study prior to baseline assessment meaning 22 were randomised into either the control (Cancer: n = 10; Benign: n = 1) or PREHAB group (Cancer: n = 10; Benign: n = 1). One further cancer patient was withdrawn from the PREHAB group two days prior to surgery having completed the entire PREHAB programme due to an unrelated adverse event. Clinical data was therefore available for 11 patients (Cancer: n = 10; Benign: n = 1) in the control group and 10 patients (Cancer: n = 9; Benign: n = 1) from the PREHAB group. Furthermore, two patients from the control group and one from the PREHAB group (all cancer) failed to complete the pre-operative reassessments meaning data from the functional capacity tests and the HRQOL questionnaires was available for 9 patients only in each group.

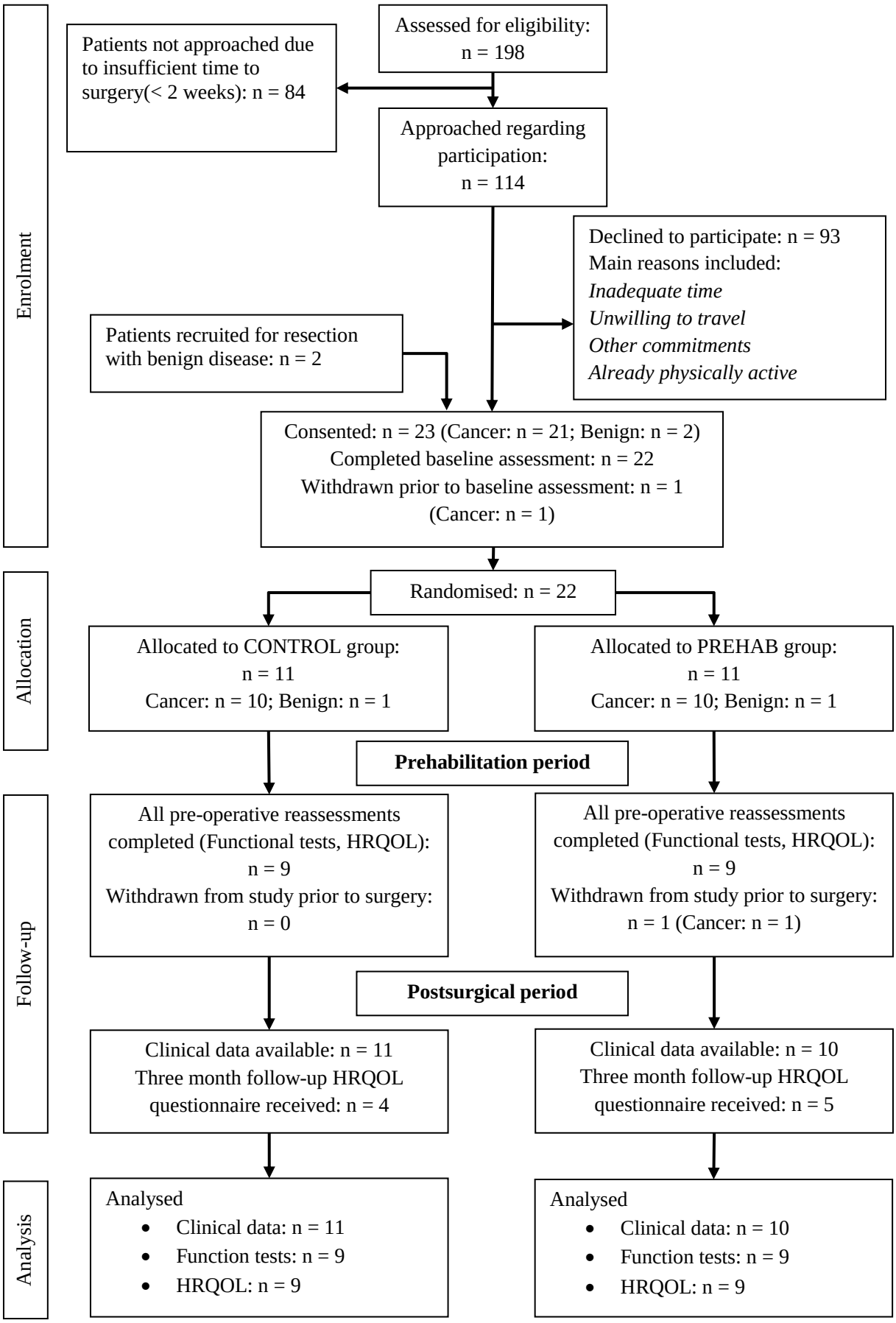


Figure 5.28: CONSORT diagram for the trial

5.3.2. Patient physical and surgical characteristics

Baseline physical and surgical characteristics are displayed in Table 5.2. Randomisation resulted in a 64% male dominance in the control group compared to a 60% female dominance in the PREHAB group. There was no significant difference between groups for age (mean difference: -0.65 years, 95% CI: -11.27 to 9.98; t =-0.127; p = 0.900), height (mean difference: 8.20 cm, 95% CI: -1.59 to 18.00; t = 1.75; p = 0.096), body mass (mean difference: 2.70 kg, 95% CI: -15.79 to 21.19; t = 0.306; p = 0.763) or BMI (mean difference: -2.47 kg.m^2, 95% CI: -7.13 to 2.19; t = -1.11; p = 0.281). There was no significant difference between groups for number of days between baseline assessment and surgery (Z = -0.918; p = 0.359). In the PREHAB group, baseline VO_{2peak} was 17.4 ± 5.7 ml.kg.min [range: 10.5 to 27.2 ml.kg.min].

Table 5.2: Baseline patient descriptives

	Control (n = 11)		PREHAB (n = 10)		Control vs. PREHAB *p* value
Age (Years)	63.5 ± 12.5 [37; 83]		64.1 ± 10.5 [46; 79]		0.900[a]
Gender ratio (M:F)	7:4		4:6		
Height (cm)	169.1 ± 11. 9 [147.5; 185.0]		160.9 ± 9.2 [145.5; 176.6]		0.096[a]
Body mass (kg)	81.1 ± 25.0 [44.7; 119.0]		78.4 ± 13.1 [57.4; 101.4]		0.763[a]
BMI (kg/m^2)	27.8 ± 5.7 [19.9; 38.1]		30.3 ± 4.3 [24.4; 38.6]		0.281[a]
Smoker	Yes	2	Yes	0	
	No	9	No	10	
Previous smoker	Yes	5	Yes	3	
	No	6	No	7	
Drinks alcohol	Yes	5	Yes	7	
	No	6	No	3	
Comorbidities and previous medical history	Hypertension	3	Hypertension	5	
	Type II Diabetes	2	Type II Diabetes	2	
	Osteoarthritis	2	Asthma/COPD	2	
	Hypothyroidism	1	Crohn's disease	1	
	Raynaud's disease	1	Ulcerative colitis	1	
	Gastro-oesophageal reflux disease	1	Liver cirrhosis	1	
			Breast cancer	1	
	Chronic kidney disease	1	Osteoarthritis	1	
Neoadjuvant Chemoradiotherapy	Yes	3	Yes	4	
	No	8	No	6	
Duration between baseline assessment and surgery (Days)[#]	16.0 (6) [14; 33]		23.0 (14) [13; 35]		0.359[b]

Mean ± Standard Deviation [Range]; Median (Interquartile range) [Range]; [a]Independent sample t-tests; [b]Mann Whitney U Test

5.3.3. Exercise prescription and adherence

A total of 69 out of a potential 77 sessions were attended by the 10 patients randomised to the PREHAB group who went on to receive surgery as scheduled, giving an overall patient adherence of 89.6%. Individual patient adherence ranged from 75% to 100%, with five out of 10 patients attending all sessions. Reasons for missing sessions included a pre-arranged holiday (1 session), attendance at family events such as

weddings and funerals (3 sessions), work commitments (2 sessions) and transport issues (2 sessions). The mean number of sessions attended was 6.9 ± 2.3 [range: 3 to 10 sessions] with a mean PREHAB period of 22.0 ± 7.5 [range: 13 to 35] days. Mean session duration was 61.0 ± 5.6 minutes, of which a mean of 22.5 ± 4.0 minutes was aerobic exercise (Figure 6.2). Mean percentage of HRR during aerobic exercise was 53.1 ± 6.7%. Patient 4 who was withdrawn from the study prior to surgery, completed all scheduled sessions (14/14 sessions; mean session duration: 59.6 ± 5.1 minutes; mean aerobic exercise duration: 23.9 ± 4.1 minutes; mean percentage of HRR during aerobic exercise: 59.6 ± 5.1%).

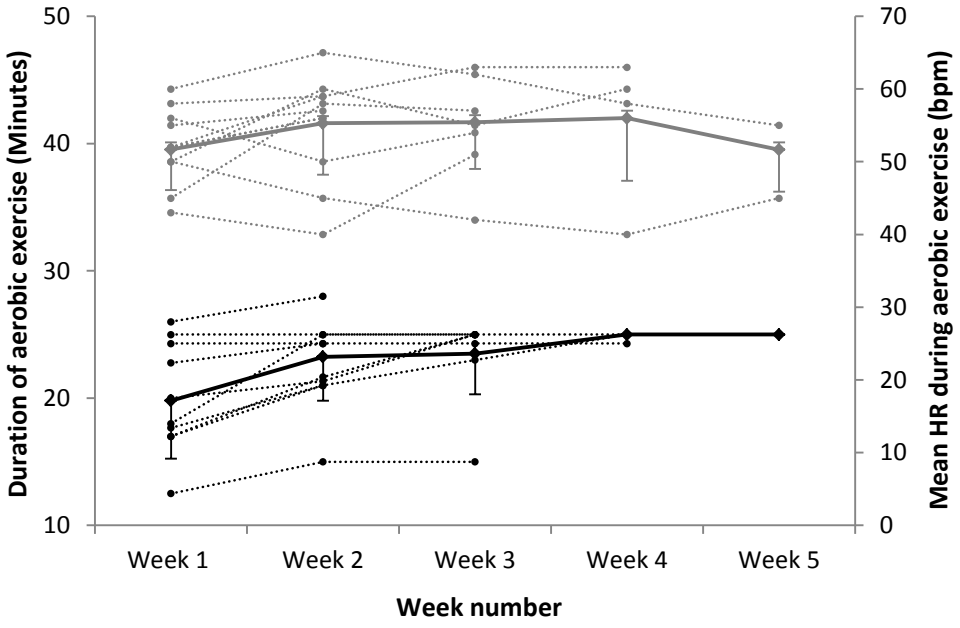


Figure 5.29: Progression in mean aerobic exercise duration (solid black line) and mean HRR per week (solid Grey lines) for the 10 PREHAB patients to complete pre-operative reassessment. (Dotted lines represent each individual patient)

Table 5.3 illustrates the total number of sets completed, the mean number of repetitions per set and the range in HRR achieved for each exercise used during resistance circuits one and two. Considerable individual variation existed in both the exercises that the patients were capable of performing and the percentage of HRR it elicited. All patients managed to complete resistance circuit one, however, two patients were unable to perform any of the exercises from resistance circuit two. Furthermore, two patients were unable/unwilling to perform continuous cycling for longer than 10 minutes due to self-reported physical discomfort.

Table 5.3: Combined total number of sets performed and number of reps [range] per set from resistance circuits one & two in the PREHAB group (n = 10).

	Ankle ROM	Reps	Total Sets	% HRR	Medial gluteal activation	Reps	Total Sets	% HRR	T spine mobility	Reps	Total Sets	% HRR	Shoulder function	Reps	Total Sets	% HRR
Resistance Circuit 1	Seated heel/toe mobilisation	15-20	9	N/R	Band resisted sit to stand	8-12	33	35-45	Seated/standing postural variations	12-15	18	20-25	Lying scapular setting	15-20	8	10-20
	Ankle mobilisation	15-20	156	30-60	Side lying bent leg hip abduction	8-15	84	30-50	Lying t-spine mobilisation	8-12	72	35-45	Band pull aparts	8-12	119	25-65
	Sit to stand	8-12	39	20-45	X-band walks	8-12 steps	90	40-70	Foot raised thoracic extension	8-12	60	50-65	Band resisted external rotations	8-12	51	25-65
													Seated row	8-12	43	30-60
	Hip flexor ROM	**Reps**	**Total Sets**	**% HRR**	**Gluteal activation**	**Reps**	**Total Sets**	**% HRR**	**Whole kinetic chain**	**Reps**	**Total Sets**	**% HRR**	**Core control**	**Reps**	**Total Sets**	**% HRR**
Resistance Circuit 2	Standing hip flexor stretch	3 x 15-30 s	62	35-55	Bilateral lying gluteal bridge	8-12	93	30-45	Kettlebell swing	8-15	24	50-90	High kneeling band anti-rotation	8-12	50	40-60
	Lying hip flexor stretch	3 x 15-20 s	7	N/R	Cook hip lift	8-12	34	N/R	Push press	8-12	83	55-80	Band resisted side shuffles	8-12	89	40-80
	Split squat	8-12	65	60-75	Shoulders elevated bilateral gluteal bridge	8-12	9	70					Suitcase carry	10-20 metres	9	N/R
													Ball passes	8-12	55	30-40

%HRR: minimum and maximum value recorded as % of HRR (values do not include all patients; N/R: Heart rate not recorded for this exercise in any patients.
N.B. Values for heel walking, rear foot elevated split squat, foot elevated single leg gluteal bridge & shoulders elevated unilateral gluteal bridge not included as they were not performed

5.3.4. Clinical outcomes

Table 5.4 displays the surgical data and clinical outcomes for both groups. Distribution of patients with colonic cancer (Control: n = 4; PREHAB: n = 3), rectal cancer (Control: n = 6; PREHAB: n = 6) and benign disease (Control: n = 1; PREHAB: n = 1) was similar between study groups as was number of surgeries performed open (Control: n = 6; PREHAB: n = 5) and laparoscopically (Control: n = 5; PREHAB: n = 5). When analysed based on study group alone, median LOS was two days longer for the PREHAB group than the control group although the difference was not significant ($Z = 0.459$; $p = 0.646$). Insufficient numbers were available to perform statistical sub-analysis of LOS based on prior treatment however median LOS was similar between groups regardless of whether the patients had received NACRT prior to surgery or not. All reported post-operative complications were of a surgical nature, with the number of patients (PREHAB: n = 3; Control: n = 4) experiencing complications and total number of complications (PREHAB: n = 3; Control: n = 5) were comparable between groups. Five of the seven patients to experience post-operative complications occurred in rectal cancer patients. There was no evidence of a link between pre-operative physical fitness and complications with three of the seven patients to experience a complication achieving a higher than age predicted 6MWT distance, three patients below the predicted 6MWT distance and one as expected.

Table 5.4: Surgical information and clinical outcomes

	Control (n = 11)		PREHAB (n = 10)		Control vs. PREHAB (*p* value)
Diagnosis	Rectal	6	Rectal	6	
	Caecal	2	Caecal	2	
	Sigmoid	1	Transverse colon	1	
	Splenic flexure	1	Diverticular Disease	1	
	Diverticular Disease	1			
Histology at surgery (TNM classification of malignant tumours)	T2N0M0	1	T3N0M0	6	
	T3N0M0	3	T3N1M1	2	
	T3N1M0	3	No residual malignancy	1	
	T3N2M0	1	Benign	1	
	T3N1M1	1			
	T4N0M0	1			
	Benign	1			
Length of stay[a]					
All patients	8 (5) [6; 27]		10 (7) [5; 12]		0.646[b]
Neoadjuvent therapy (n = 3 & n = 4)	12 (-) [8; 13]		10 (4.3) [7; 12]		
No neoadjuvent therapy (n = 8 & = 6)	8 (10.5) [6; 27]		8 (7) [5; 12]		
Patients with Complications	4 (36%)		3 (30%)		
Total Complications	5		3		
	Illeus	2	Illeus	2	
	Anastamotic leak	1	Wound infection	1	
	Wound Infection	2			

TNM: Tumour, Nodes, Metastasis; AR: Anterior resection; APR: Abdominoperineal resection
[a] Median (Interquartile range) [Range]; [b] Mann Whitney U Test

5.3.5. Physical functioning tests

As the data violated the assumptions of normal distribution, non-parametric statistical methods were used. At entry to the study, there was no significant difference between groups for any of the tests (all $p > 0.05$; Table 5.5). Time to perform the TUG decreased in all nine patients in the PREHAB group between baseline and pre-operative reassessment whereas performance declined in seven of the nine patients in the control group (Figure 5.30 a & b). This translated into an improved median performance time for PREHAB group ($Z = -2.668$; $p = 0.008$) whilst a non-significant increase was seen in the control group ($Z = -1.424$; $p = 0.154$). Median difference between baseline and pre-operative reassessment was significantly different in the PREHAB group compared to the control group ($Z = -2.782$; $p = 0.005$). Similar results were seen for the SCT

where all nine patients improved performance in the PREHAB group but only three improved in the control group (Figure 5.30 c & d). Median difference between baseline and pre-operative reassessment was again significantly different between the PREHAB and control group (Z = -2.696; $p = 0.007$). For the FTSTS, six patients from each group reduced performance time at pre-operative reassessment (Figure 5.30 e & f) however the difference was not significant for either (Control: Z = -1.007; $p = 0.314$; PREHAB: Z = -1.660; $p = 0.097$).

Table 5.5: Differences in TUG, FTSTS and SCT performance between the control and PREHAB groups.

		Baseline	**Pre-op**	**Base vs. Pre-op (*p* value)[a]**	**Median difference**	**Between group comparison (*p* value[b])**
TUG (s)	Control (n = 9)	6.73 (1.93) [5.42; 10.03]	7.12 (2.42) [5.12; 10.14]	0.154	0.11 (1.16) [-0.52; 1.36]	0.005
	PREHAB (n = 9)	6.35 (2.36) [5.43; 9.68]	5.80 (2.52) [4.76; 9.56]	0.008	-0.35 (0.60) [-1.04; -0.10]	
Baseline comparison (*p* value)[b]		1.000				
FTSTS (s)	Control (n = 9)	11.67 (2.68) [9.88; 14.76]	11.42 (5.68) [7.52; 16.30]	0.314	-0.37 (2.89) [-2.81; 1.88]	0.895
	PREHAB (n = 9)	10.27 (4.06) [9.03; 14.36]	11.08 (5.05) [7.40; 14.40]	0.097	-0.73 (1.59) [-1.79; 1.00]	
Baseline comparison (*p* value) [b]		0.436				
SCT (s)	Control (n = 9)	2.86 (0.69) [2.22; 3.83]	2.96 (0.93) [2.11; 4.54]	0.314	0.05 (0.56) [-0.33; 0.71]	0.007
	PREHAB (n = 9)	2.65 (0.48) [2.03; 4.21]	2.44 (0.53) [1.93; 3.47]	0.008	-0.27 (0.17) [-0.74; -0.10]	
Baseline comparison (*p* value) [b]		0.377				

Median (Interquartile range)[Range]; [a]Wilcoxon Signed Ranks Test; [b]Mann Whitney U Test

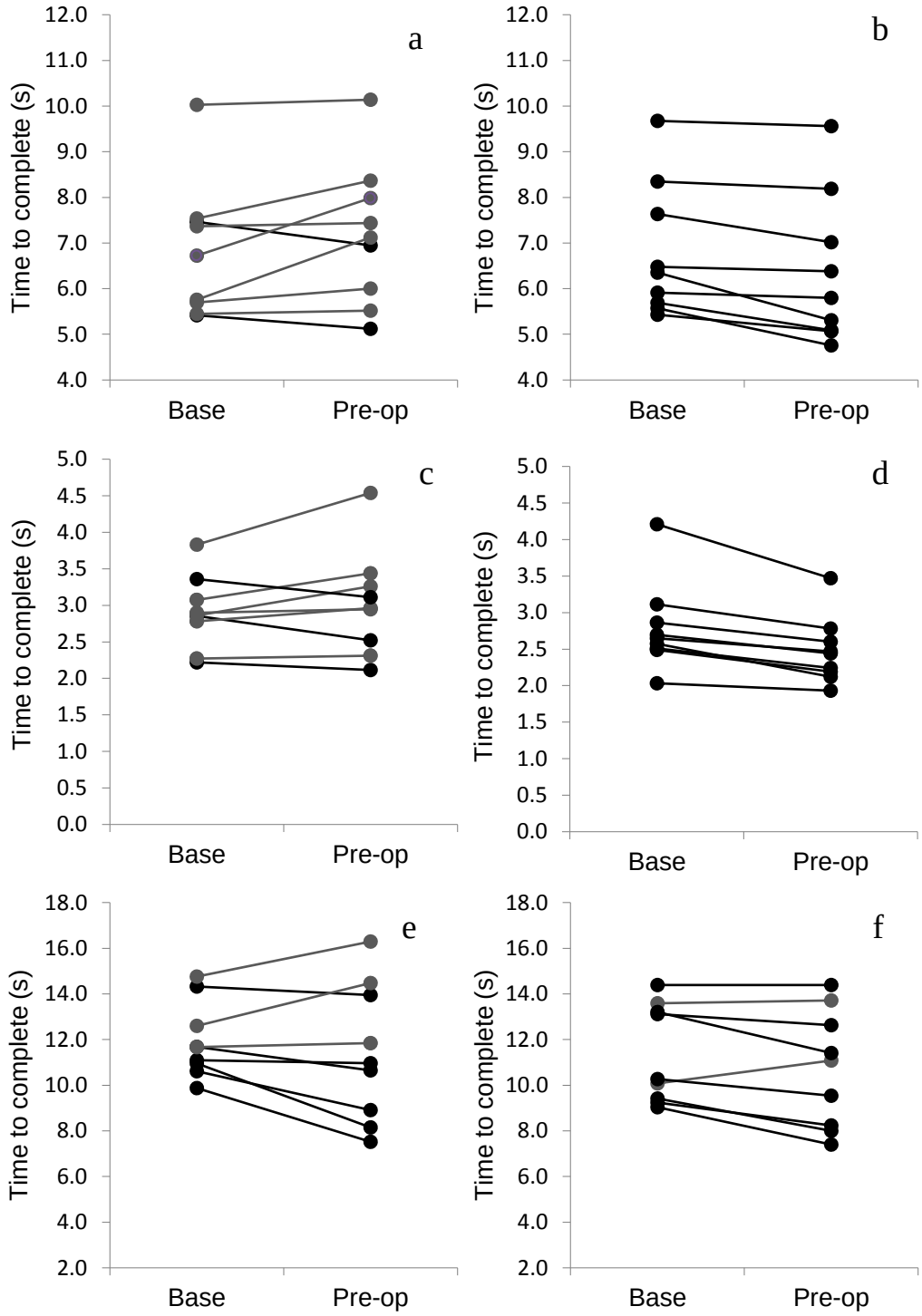


Figure 5.30: Individual performances at baseline (Base) and pre-operative reassessment (Pre-op) of the TUG (a: Control; b: PREHAB), SCT (c: Control; d: PREHAB) and FTSTS (e: Control; f: PREHAB) in both groups.

At pre-operative reassessment, distance walked in the 6MWT increased by on average 17.0 ± 9.0% (range: 3.9% to 31.2%) in the PREHAB group with all nine participants improving (Table 5.6 & Figure 5.31 b). This resulted in a significant median improvement of 68 metres [IQR: 56.5] (Z = -2.666; p = 0.008). In contrast,

distance walked in the 6MWT increased by 1.9 ± 10.1% (-9.2% to 21.2%) in the control group with only four patients improving performance and five experiencing a decline in performance (Figure 6.4 a; median difference: -6.0 [IQR: 67.0]; Z = -0.59; $p = 0.953$). Furthermore, the PREHAB group median difference (baseline to pre-operative reassessment) was significantly different to the control group (Z = -2.650; $p = 0.008$). In both groups, the percentage of HR_{max} at the end of the 6MWT was significantly higher at pre-operative reassessment compared to baseline however there was no significant change in SaO_2 between trials in either group (Table 5.7).

Table 5.6: Differences in 6MWT performance between the control and PREHAB groups.

		Baseline	Pre-op	Base vs. Pre-op (*p* value)[a]	Median difference	Between group comparison (*p* value[b])
6MWT (m)	Control (n = 9)	390.0 (184) [303; 578]	400.0 (179) [275; 588]	0.953	-6.0 (67.0) [-35; 70]	0.008
	PREHAB (n = 9)	399.0 (94) [246; 523]	501.0 (133) [298; 600]	0.008	68.0 (56.5) [19; 136]	
Baseline comparison (*p* value)[b]		0.863				

Median (Interquartile range)[Range]; [a]Wilcoxon Signed Ranks Test; [b]Mann Whitney U Test

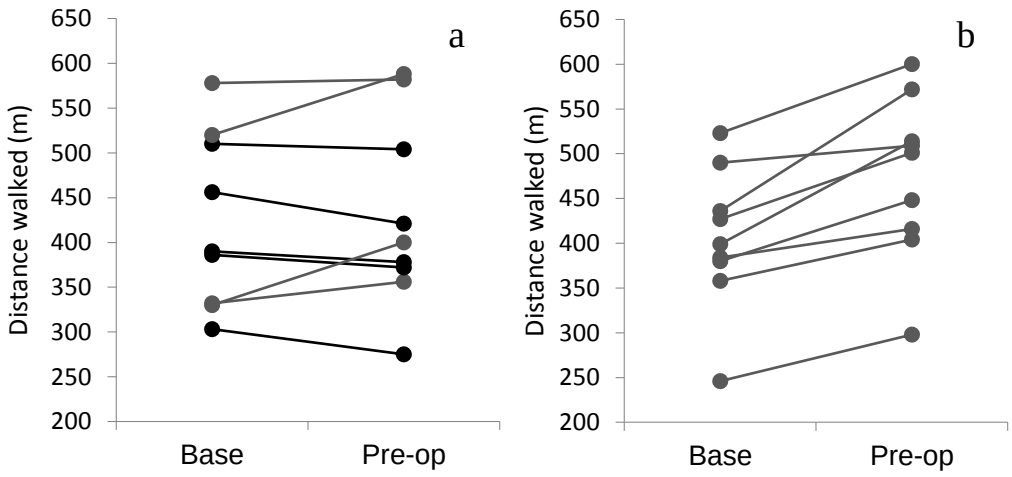


Figure 5.31: Individual changes in performances of the 6MWT between baseline (Base) and pre-operative (Pre-op) assessment in the control (a) and PREHAB (b) groups.

Table 5.7: Heart rate and SaO_2 data from the 6MWT in the control and PREHAB groups.

	Control (n = 9)			PREHAB (n = 9)		
Heart rate (bpm)	**Pre 6MWT**	**Post 6MWT**	**%HR_{max}**	**Pre 6MWT**	**End of 6MWT**	**%HR_{max}**
Baseline	74 (13) [79; 132]	108 (28) [79; 132]	71.2 (21.8) [50.3; 82.2]	81 (25) [69; 98]	109 (34) [91; 134]	71.1 (11.9) [52.4; 84.3]
Pre-operative reassessment	73 (20) [63; 94]	112 (24) [100; 136]	79.6 (14.9) [60.1; 84.9]	88 (19) [66; 98]	126 (21) [100; 150]	81.2 (8.7) [70.9; 91.2]
p value[a]	0.514	0.008	0.008	0.398	0.008	0.008
SaO_2 (%)	**Pre 6MWT**	**Post 6MWT**		**Pre 6MWT**	**Post 6MWT**	
Baseline	97 (3) [94; 100]	97 (3) [94; 99]		97.5 (1) (96; 99)	96.5 (4) [86; 98]	
Pre-operative reassessment	97 (1) [96; 98]	96 (3) [95; 99]		98 (1) [96; 98]	97 (4) [87; 99]	
p value[a]	0.317	0.861		0.705	0.726	

bpm: Beats per minute; %HR_{max}: percentage of heart rate max; Median (Interquartile range)[Range]; [a]Mann Whitney U Test

There was no significant difference between baseline and pre-operative reassessment performance of the HGD in either the control or PREHAB groups using their dominant or non-dominant hand (Table 5.8). For both their dominant and non-dominant hands, five patients increased grip strength at pre-operative reassessment in the PREHAB group whilst four had decreased grip strength (Figure 5.32 b & d). In the control group the results were similar with five out of nine patients increasing grip strength at pre-operative reassessment using their dominant hand and whilst six improved with their non-dominant hand (Figure 5.32 a & c).

Table 5.8: Differences in dominant and non-dominant handgrip dynamometry performance between the control and PREHAB groups.

		Baseline	Pre-op	Base vs. Pre-op (*p* value)[a]	Median difference	Between group comparison (*p* value[b])
HGD (kg) Dominant	Control (n = 9)	28.2 (14.0) [17.2; 48.7]	26.8 (17.3) [16.3; 50.3]	0.767	0.2 (6.2) [-7.3; 3.5]	0.289
	PREHAB (n = 9)	21.5 (16.1) [15.7; 52.8]	25.0 (17.3) [16.3; 50.3]	0.286	0.7 (4.8) [-2.5; 7.7]	
Baseline comparison (*p* value) [b]		0.340				
HGD (kg) Non-dominant	Control (n = 9)	25.3 (9.9) [17.2; 36.3]	25.7 (12.4) [17.0; 37.2]	0.075	0.2 (4.1) [-3.8; 4.0]	0.825
	PREHAB (n = 9)	21.3 (15.85) [16.8; 46.3]	21.5 (16.6) [15.5; 44.3]	0.678	0.5 (4.05) [-3.8; 4.0]	
Baseline comparison (*p* value) [b]		0.566				

Median (Interquartile range)[Range]; [a]Wilcoxon Signed Ranks Test; [b]Mann Whitney U Test

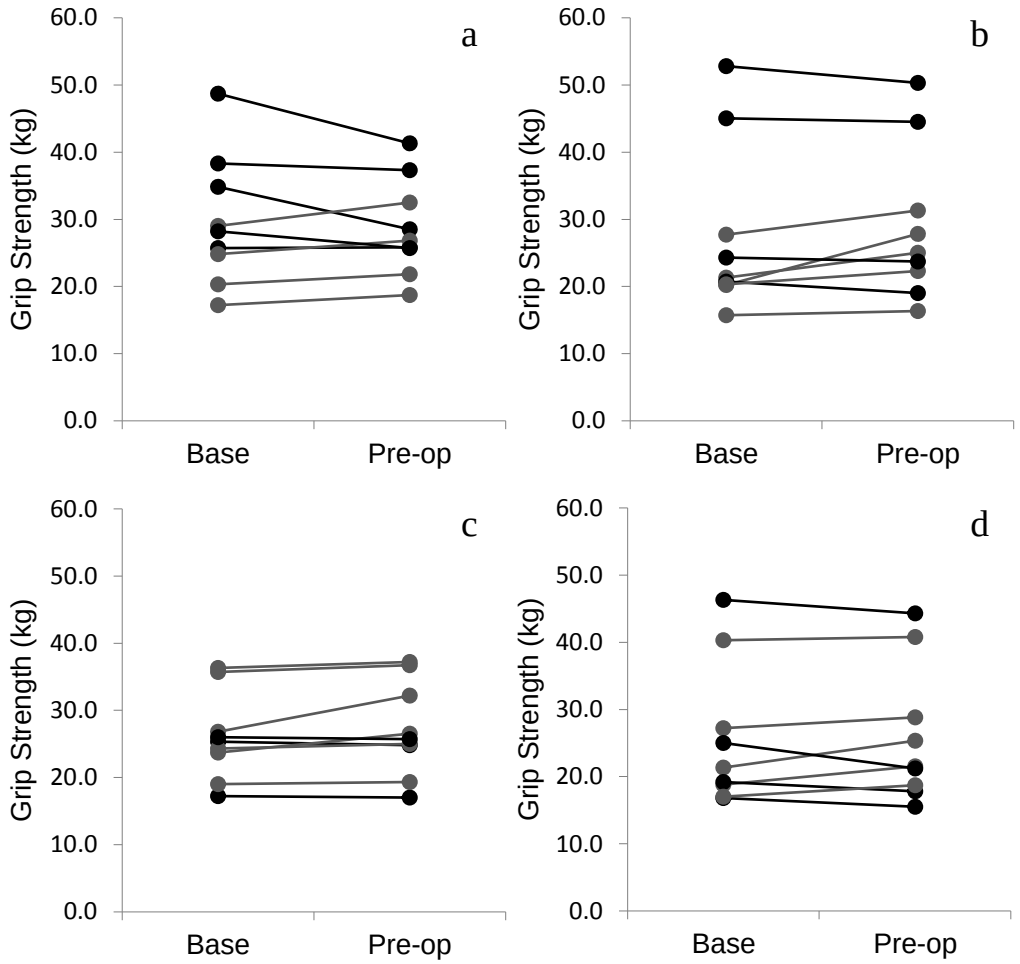


Figure 5.32: Individual changes in grip strength between baseline and pre-operative assessment for each group in the dominant (Control: a; PREHAB: b) and non-dominant hand (Control: c; PREHAB: d).

6.3.6. HRQOL

Table 5.9 shows the median (IQR) for the EORTC QLQ-C30 and HADS for both the control and PREHAB groups. On the EORTC QLQ-C30, median composite score for all components of the functional scale, with the exception of role functioning, were lower in the PREHAB group compared to the control group at both baseline and pre-operative reassessment. The difference was only significant however at baseline for physical functioning (Z= -2.136; $p = 0.033$) and social functioning (Z = -2.046; $p = 0.041$); and at pre-operative reassessment for social functioning only (Z= -2.535; $p = 0.011$). On the symptom scale, the PREHAB group had significantly higher scores for pain (Z = -2.861; $p = 0.004$) at pre-operative reassessment. At baseline, median score for GHS was lower in the control group compared to the PREHAB however the difference was not significant (Z = -0.951; $p = 0.342$). There was no evidence of a consistent directional change between baseline and pre-operative reassessment for any of the components of the EORTC functional or symptom scales in either the control or PREHAB group (Table 5.10). GHS improved between baseline and pre-operative reassessment in 7 out of 9 patients in the control group (Z = -2.375; $p = 0.018$) compared 5 out of 9 in the PREHAB group (Z = -0.511; $p = 0.610$). On the HADS, there was no difference in median depression score between the groups at baseline however median anxiety score was 2 points higher in the PREHAB group compared to the control group. The difference however was not significant (Z = -0.978; $p = 0.328$). Anxiety score increased in 5 patients (no change: 4 patients) in the PREHAB at pre-operative reassessment resulting in a 4 point increase in median anxiety score (Z = -2.032; $p = 0.042$). Although median anxiety increased overall by 1 point in the control group (Z = 0.000; $p = 1.000$), only 3 patients reported increased anxiety at pre-operative follow-up whilst anxiety decreased in 4 patients. At the three month follow-up, only four questionnaires were returned from the control group and five from the PREHAB group. Given the low response rate, statistical analysis was therefore not performed.

Table 5.9: HRQOL as measured by the EORTC QLQ-C30 and HADS

	Control (n = 9)			PREHAB (n = 9)		
	Baseline	**Pre-op**	***p* value[a]**	**Baseline[b]**	**Pre-op[b]**	***p* value[a]**
EORTC						
Functional scale						
Physical functioning	100.0 [86.7; 100]	100.0 [53.3; 100]	0.655	86.7* [66.67; 100]	93.3 [60; 100]	0.588
Role functioning	100.0 [83.3; 100]	100.0 [83.3; 100]	0.317	100.0 [66.67; 100]	100.0 [66.7; 100]	0.180
Emotional functioning	100.0 [50; 100]	100.0 [58.3; 100]	0.655	83.3 [58.3; 100]	75.0 [50; 100]	0.416
Cognitive functioning	100.0 [83.3; 100]	100.0 [83.3; 100]	0.564	83.3 [83.3; 100]	83.3 [83.3; 100]	0.317
Social functioning	100.0 [66.7; 100]	100.0 [100]	0.180	83.3 [33.3; 100]	66.7** [16.7; 100]	1.000
Symptom scale						
Fatigue	11.0 [0; 44.3]	0.0 [0; 66.7]	0.854	22.3 [0; 33.3]	11.00 [0; 55.7]	0.396
Nausea & vomiting	0.0 [0; 16.7]	0.0 [0]	0.317	0.00 [0; 16.7]	0.00 [0; 16.7]	0.564
Pain	0.0 [0; 33.3]	0.0 [0; 0]	0.317	0.00 [0; 33.3]	16.67** [0; 100]	0.102
Dyspnoea	0.0 [0; 33.3]	0.0 [0; 33.3]	0.317	0.00 [0; 33.3]	0.00 [0]	0.317
Insomnia	0.0 [0; 66.7]	0.0 [0; 66.7]	1.000	0.00 [0; 66.7]	0.00 [0; 66.7]	0.581
Appetite loss	0.0 [0; 33.3]	0.0 [0]	0.317	0.00 [0; 33.3]	0.00 [0; 100]	0.705
Constipation	0.0 [0; 66.7]	0.0 [0; 66.7]	0.317	0.00 [0; 66.7]	0.00 [0; 66.7]	1.000
Diarrhoea	0.0 [0; 66.7]	33.3 [0; 33.3]	0.655	0.00 [0; 66.7]	0.00 [0; 66.7]	0.180
Financial difficulty	0.0 [0]	0.0 [0; 33.3]	0.317	0.00 [0]	0.00 [0; 66.7]	0.317
Global health status/Quality of life scale						
HRQOL	66.7 [16.7; 83.3]	83.3 [33.3; 100]	0.018	83.3 [33.3; 91.7]	83.3 [33.3; 100]	0.610
Hospital anxiety and depression scale						
Anxiety	2.0 [0; 7]	3.0 [0; 10]	1.000	4.0 [0; 10]	8.0** [0; 11]	0.042
Depression	2.0 [0; 6]	1.0 [0; 4]	0.157	2.0 [0; 7]	2.0 [1; 9]	0.399

Data are expressed as Median [range]; [a] Wilcoxon Signed Ranks Test; [b] Mann Whitney U Test

* Significantly different to control group at baseline ($p < 0.05$)

** Significantly different to control group at pre-operative reassessment ($p < 0.05$)

Table 5.10: Directional change in HRQOL between baseline and pre-operative reassessment for control and PREHAB groups.

	Control (n = 9)			PREHAB (n = 9)		
	+	-	N/C	+	-	N/C
EORTC						
Functional scale						
Physical functioning	1	1	7	3	2	4
Role functioning	1	0	8	2	0	7
Emotional functioning	1	1	7	2	3	4
Cognitive functioning	2	1	6	1	3	5
Social functioning	2	0	7	2	2	5
Symptom scale						
Fatigue	2	2	5	3	4	2
Nausea & vomiting	0	1	8	1	2	6
Pain	0	1	8	5	2	2
Dyspnoea	0	1	8	0	1	8
Insomnia	0	0	9	2	2	5
Appetite loss	0	1	8	1	3	5
Constipation	1	1	7	2	2	5
Diarrhoea	1	1	7	0	2	7
Financial difficulty	1	0	8	1	0	8
Global health status/Quality of life scale						
HRQOL	7	0	2	5	2	2
Hospital anxiety and depression scale						
Anxiety	3	4	2	5	0	4
Depression	1	4	4	3	3	3

+: Increased score at pre-operative reassessment; -: Decreased score at pre-operative reassessment; N/C: No change at pre-operative reassessment

When all data was combined and analysed based on gender, median anxiety and depression scores were higher in females compared to males both at baseline and at pre-operative reassessment (Table 5.11). At pre-operative reassessment, median anxiety was significantly higher in females than in males (median difference 7.5 points; Z= -2.555; p = 0.011) whilst at baseline the difference was 2.5 points, however, this was not significant (Z= -1.745; p = 0.081). Furthermore, despite median depression being 1.5 points higher at baseline and 2.5 points higher at pre-operative reassessment in females compared to males, neither difference was significant (Z= -1.536; p = 0.125 and Z= 1.257; p = 0.333 respectively). Anxiety increased in seven out of the 10 females at pre-operative reassessment compared to baseline whilst two had no change (Table 5.12).

This resulted in a median 5 point increase (Z= -2.113; $p = 0.035$). In contrast, at pre-operative reassessment, both anxiety and depression scores had only increased in one male and had decreased in three males (anxiety median difference: 0 points; Z= -0.557; $p = 0.577$; depression median difference: -0.5 points; Z = -1.000; $p = 0.317$). There was no directional change in depression present in females at pre-operative reassessment.

Table 5.11: Comparison of the HADS and EORTC QLQ-C30 global health status scores based on patient gender.

	Males (n = 8)			Females (n = 10)		
	Baseline	**Pre-op**	***p* value[a]**	**Baseline**	**Pre-op[b]**	***p* value[a]**
Global health status/Quality of life scale						
HRQOL	79.2 [58.3; 83.3]	83.3 [66.7; 91.7]	0.039	66.7 [16.7; 91.7]	66.7 [33.3; 100]	0.342
Hospital anxiety and depression scale						
Anxiety	1.5 [0; 7]	1.5 [0; 8]	0.577	4.0 [1; 10]	9.0* [0; 11]	0.035
Depression	1.5 [0; 4]	1.0 [1; 3]	0.317	3.0 [0; 7]	3.5 [0; 9]	0.609

Data are expressed as Median [Range]; [a] Wilcoxon Signed Ranks Test; [b] Mann Whitney U Test
* Significantly different to males at pre-operative reassessment ($p < 0.05$)

Table 5.12: Directional change in HRQOL between baseline and pre-operative reassessment based on gender.

	Males (n = 8)			Females (n = 10)		
	+	**-**	**N/C**	**+**	**-**	**N/C**
EORTC						
GHS/QOL scale	5	0	3	7	2	1
Hospital anxiety and depression scale						
Anxiety	1	3	4	7	1	2
Depression	1	3	4	3	4	3

+: Increased score at pre-operative reassessment; -: Decreased score at pre-operative reassessment; N/C: No change at pre-operative reassessment

5.4. Discussion

The aim of this chapter was to investigate whether the inclusion of a short PREHAB programme in the period of time that precedes major elective colorectal surgery was feasible (both in terms of the delivery of the intervention and recruitment)

and whether the intervention resulted in trends towards beneficial changes in patient physical functioning, HRQOL or clinical outcomes. Consistent with previous research (Jones et al., 2007; Li et al., 2013; Sawatzky et al., 2014), the PREHAB group experienced improvements in the performance of the 6MWT as well as the TUG and SCT between baseline and pre-operative reassessment unlike the control group (Table 5.5). This improvement however did not translate into a shorter LOS, reduced post-operative complications or improved HRQOL for the PREHAB patients.

PREHAB has been proposed as a means of improving the patients' functional capacity prior to surgery (Carli & Zavorsky, 2005). Whilst CPET remains the gold standard measure of functional capacity in terms of objectively measuring physical fitness (Struthers et al., 2008), field tests such as the 6MWT provide an inexpensive alternative in clinical settings (Agnew, 2010). Although lacking the precision of CPET, the 6MWT has been validated in a number of clinical populations including cardiac and pulmonary disease patients (ATS, 2002), cancer patients (Schmidt, Vogt, Thiel, Jager, & Banzer, 2013) and more specifically colorectal surgery patients (Moriello et al., 2008). Furthermore, positive correlations have been made between the distance walked in the 6MWT and CPET parameters such as VO_{2peak} (Lee et al., 2013) and AT (Sinclair, Batterham, Davies, Cawthorn, & Danjoux, 2012).

Previous literature involving colorectal cancer patients (Carli et al., 2010; Li et al., 2013) have used an improvement of 20 metres as the threshold for identifying true change in 6MWT performance. This was based on the estimated measurement error identified in community dwelling older adults by Kervio et al. (2003). In the current study, eight out of nine patients improved by greater than 20 metres (9^{th} participant improved by 19 metres). Even the more conservative MDC_{95} distance of 37 metres identified in chapter 4 was exceeded by seven out of nine participants in the PREHAB group. In comparison, only three out of nine patients (33.3%) improved in the control group by more than 20 metres whilst two patients (22.2%) actually declined by greater than 20 metres. Currently, there is no recognised minimal clinically important difference established for the 6MWT change in colorectal patients awaiting surgery. Perera, Mody, Woodman, and Studenski (2006) reported an increase of 50 metres equated to a substantial meaningful change in 6MWT performance in older adults whilst 25 metres was reported to be the minimal clinically important difference in coronary heart disease patients (Gremeaux et al., 2011). The improvements observed in

the PREHAB group were therefore comparable to the change values seen within different clinical populations.

The possibility that the improvement in 6MWT distance at post-operative reassessment occurred as a result of a learning effect cannot be ignored as increased walking distance between the first and second trial has previously been documented (Hanson et al., 2012; Kervio et al., 2003). Consistent with chapter 4, post-6MWT HR was higher in the pre-operative reassessment compared to baseline for both the PREHAB and control groups; potentially reflecting a greater effort exerted in the pre-operative reassessment (Table 5.7). Although this coincided with an improved performance by all nine patients in the PREHAB group, in the control group only three patients improved by greater than 10 metres whilst the remaining six remained the same (±10 metres of baseline; n = 4) or decreased (>10 metres of baseline; n = 2). Whilst all pre-operative reassessments were conducted between 4-5 pm on the day prior to surgery, the baseline assessments were performed at different times of the day based around the patients' pre-existing hospital appointments meaning circadian variations may have contributed to the differences in HR between trials. Increased HR when performing the 6MWT in the afternoon compared to the morning have previously been reported in both healthy elderly participants and chronic heart failure patients (Kervio et al., 2003; Kervio et al., 2004).

The improvements seen in 6MWT were complemented by similarly improved performance by the PREHAB group in the TUG and SCT but not by the control group (Table 5.5 & Figure 5.30). Viewed independently, the clinical importance of the changes observed in TUG and SCT performance in the current study is difficult to determine however when combined with the improved 6MWT performance, an overall improvement in pre-operative physical functioning following PREHAB is further supported. As colorectal cancer patients are typically an ageing population (mean age in this study: 63.8 ± 11.3 years [range: 37 to 83 years]), a number of common movement deficits were potentially evident in some of the participants recruited to this study. Unlike previous PREHAB literature, the intervention in this thesis adopted the joint-by-joint approach of using functional exercises to address these common areas of muscular dysfunction and movement impairment, and improve movement throughout the body's whole kinetic chain (Boyle, 2010; Cook & Burton, 2010). Rather than focus on specific muscle groups independently, this systematic approach aimed to increase mobility in

the ankles, hips and thoracic spine, enhance the activation of the gluteus medius and gluteus maximus muscles and improve core and scapulae stability, all of which is likely to have been important factors in the improvements observed in the physical functioning tests.

In ageing populations a reduction in ankle mobility and hip extension has been reported resulting in impaired balance, increased falls risk and a reduction in gait speed (Alcock et al., 2013, Menz et al., 2005). Although speculative as no gait analysis was included, this raises the possibility that the improvement in 6MWT performance observed in the PREHAB group was not solely due to improved cardiorespiratory fitness but also as a result of addressing these issues. Improved ankle mobility as a result of ankle mobilisation exercises combined with increased range of motion in the hips due to exercises targeting hip flexor ROM as well as gluteal and medial gluteal activation potentially contributed to the faster gait speed observed in the 6MWT. Similarly, the increased range of motion in the ankles and hips, along with improved core and knee stability are likely to have had a beneficial role in the performance of the TUG and SCT. Given that early mobilisation after surgery is an important component of the ERAS approach taken within most modern colorectal surgery (Gustafsson et al., 2012; Lassen et al., 2009), a pre-operative improvement in physical functioning may be translated into a quicker return to normal functioning post-surgery.

It was originally intended to repeat the five physical functioning tests at three months post-surgery as a measure of recovery however arranging appointments with patients unfortunately proved too difficult. By that time point, patients where either receiving further treatment (further CRT, stoma reversal surgery), had returned to work or were simply unable/unwilling to attend an additional appointment. Using a pre- and post-intervention trial design with a separate cohort control group, a pilot study by Li et al. (2013) reported following a trimodal PREHAB programme (median duration 33 days [range: 21 to 46 days]) consisting of aerobic (30 minutes, 3 x week at 50% HR_{max}) and resistance exercises (3 x week to volitional exhaustion), whey protein supplementation (increasing daily protein intake of 1.2g/kg body weight) and a 90 minute session with a psychologist learning anxiety reduction techniques, 6MWT distance returned to similar pre-operative levels (-5 metres) by eight weeks post-surgery in colorectal surgery patients (n = 42). In contrast, 6MWT distance at eight weeks post-surgery remained below (-25 metres) those achieved at pre-operative assessment in the

cohort control group (n = 45) conducted prior to the PREHAB intervention. A subsequent follow-up RCT by the same research group (Gillis et al., 2014), compared the same trimodal intervention as both PREHAB and rehabilitation (n = 39) against rehabilitation alone (n = 38). Following PREHAB and rehabilitation, a greater proportion of patients (82% vs. 62%) returned to baseline 6MWT distance by eight weeks post-surgery probably aided by the higher compliance (53% vs. 31%) to the post-operative rehabilitation (Bordes et al., 2015). Gillis et al. (2014) however has argued that the benefits of PREHAB include promoting compliance to post-operative recovery interventions by educating and preparing patients pre-operatively. Moreover, increased participation in post-surgery CR by the PREHAB groups in both Arthur et al. (2000) and Sawatzky et al. (2014) compared to their respective control groups (Arthur et al: PREHAB: 70%; Control: 57%; Sawatzky et al: PREHAB: 100%; Control: 43%) tend to support this point.

The sample size achieved (n = 23) meant the current study was underpowered to find a significant change in LOS or post-operative complications. Only the RCT by Arthur et al. (2000), whose sample size was 246 CABG patients, have reported a reduced LOS following PREHAB. Implementing a twice weekly hospital based programme (mean duration 8.2 weeks) consisting of ROM exercises, stretching and a minimum of 30 minutes of aerobic exercise at 40-70% functional capacity, a one day reduction in median post-operative LOS (PREHAB: 5 days; Control: 6 days) was observed in the PREHAB group. Subsequent smaller (and underpowered) studies have failed to find a reduction in LOS following PREHAB (Carli et al., 2010; Dronkers et al., 2010; Li et al., 2013; Sawatzky et al., 2014). In a pilot RCT (Sawatzky et al., 2014), a twice weekly 60 minute PREHAB programme in CABG patients consisting of aerobic, resistance and stretching exercises (mean duration: 8.2 ± 2.2 weeks) displayed no reduction in LOS in the PREHAB group (n = 8; 5.1 ± 1.4 days) compared to the control (n = 7; 5.3 ± 1.0 days; $p = 0.81$). In colorectal surgery patients, Carli et al. (2010) reported no significant difference in LOS between the daily bike and strengthening group (n = 58; 20-30 minutes cycling at 50% $HR_{max;}$ 10-15 minutes resistance exercise; LOS: 11.9 ± 34.6 days) and the sham walking and breathing (WB) group (n = 54; 30 minutes walking plus 5 minutes breathing exercises daily; LOS: 6.6 ± 3.6 days; $p > 0.05$). Furthermore, no studies to date have reported a reduction in post-operative complications following a period of PREHAB. This is despite associations having previously been made between pre-operative physical fitness and subsequent post-

operative outcomes (Dronkers, Chorus, van Meeteren, & Hopman-Rock, 2013; Snowden et al., 2013; Wilson et al., 2010).

Reductions of LOS as well as post-operative complications remain popular metrics by which to measure the effectiveness of an intervention in clinical research. NHS reference costs for 2014-2015 (Department of Health, 2015) estimated the average cost of each excess hospital bed day for elective surgery inpatients at ~£359 therefore a one day reduction in the average LOS post-surgery for each would reduce the financial burden on the NHS as well as bring obvious benefits to the patient. The duration of LOS however can be affected by a diverse range of factors including those of a surgical, medical or social nature, some of which are beyond the control of the medical care team (Reddy et al., 2003). Equally, the occurrence of post-operative complications which are multifactorial can dramatically increase post-operative LOS. In studies of small sample sizes, including the current study, significant changes in either LOS or post-operative complications are unlikely to be found therefore the inclusion of additional measures of post-operative recovery such as time to mobilisation and grip strength may represent more appropriate secondary outcomes in future PREHAB research.

In the current study, there was no evidence of a beneficial effect of PREHAB on reducing symptoms of anxiety or depression prior to surgery. In the PREHAB group, symptoms of anxiety actually increased in five of the nine patients between baseline and pre-operative reassessment whilst it did not change in the remaining four patients (Table 6.9). There was however no obvious directional change in anxiety or depression in the control group or depression in the PREHAB group. This is in contrast to a number of previous studies where both reduced anxiety and depression has been reported following PREHAB (Carli et al., 2010; Coats et al., 2013; Li et al., 2013). In Li et al. (2013) (PREHAB group: n = 42), where anxiety reduction training was included in their trimodal intervention, a point decrease in both anxiety and depression was observed. Furthermore, the exercise only studies by Carli et al. (2010) (PREHAB group: n = 58) and Coats et al. (2013) (PREHAB group: n = 13), reported significant reductions of 0.8 and 1.5 points respectively in the HADS depression scale following a period of PREHAB. However, it is worthy of note that in all these studies mean baseline anxiety and depression scores were below the lower cut-off point score of 8 previously identified as the threshold for possible depressive or anxiety disorders on the HADS (Terluin et al., 2009).

In the current study, by pre-operative reassessment, six of the nine patients in the PREHAB group had an anxiety score of eight or greater (median anxiety score: 8; Tables 5.9 & 5.10); a level of possible anxiety disorder. This was influenced by the fact that anxiety increased from baseline in five of the nine PREHAB ($p < 0.05$) patients to complete both questionnaires, a point not replicated in the control group. Whilst the small sample size means the potential of this being a chance finding as a result of a type 1 error cannot be dismissed, an alternative explanation may exist. It is possible involvement in the PREHAB programme actually kept the future surgery at the forefront of the patients' mind, increasing anxious thoughts. An alternative explanation however would relate to the greater percentage of females in the PREHAB group compared to the control group. Previously, it has been reported that both females from the general healthy population as well as those with a recent cancer diagnosis are more likely to display higher symptoms of anxiety and depression (Linden, Vodermaier, Mackenzie, & Greig, 2012; Piccinelli & Wilkinson, 2000). This appears consistent with the current study, where anxiety had increased in seven out of the 10 female patients by pre-operative reassessment compared to increasing in just one out of eight male patients (Table 5.12). This appears to suggest the increase in anxiety was more related to gender rather than specific study group.

The construct of HRQOL is multidimensional, encompassing many interlinking domains. In cancer patients, these may include aspects of physical (e.g. ability to perform everyday tasks, pain and symptoms), psychological (e.g. anxiety, depression, enjoyment), social (engaging with family and friends) and spiritual well-being (maintaining hope) (Ferrell, Dow, & Grant, 1995). It has been suggested however that due to an overreliance on the use of generic questionnaires such as the HADS and the EORTC QLQ-C30, key aspects of these domains are often missed, especially when assessing patient experiences to a specific situation such as cancer treatment (Burke et al., 2013). In the current study, the only component of the EORTC QLQ-C30 to change between baseline and pre-surgery was the global health score which improved in 12 out of 18 patients (Control: 7/9 patients; $p = 0.018$; PREHAB: 5/9 patients; $p = 0.610$) regardless of study group. Whilst the limited sample size of the current study more than likely influenced the absence of significant findings in the other domains of HRQOL, the administration of the questionnaires on the day prior to surgery could mean important changes in HRQOL during the pre-operative period may not have been

captured. The incorporation of qualitative interviews to measure QOL potentially could have identified important changes in HRQOL which were beyond the scope of the questionnaires used and should be considered in future work.

Two recent embedded studies (Burke et al., 2013; Burke, West, Grocott, Brunet, & Jack, 2015) qualitatively investigated patient perspectives to QOL and experiences of adherence during a 6 week interval training programme in advanced rectal cancer patients (West et al., 2015). Increased vitality, a positive attitude, social connection and a strong sense of purpose were all identified as positive themes present following participation in PREHAB (Burke et al., 2013). Furthermore, through adhering to the PREHAB programme, patients developed a sense of camaraderie and peer support with other patients, gained a sense of structure to their day and felt safe and encouraged by the presence of supervision (Burke et al., 2015). Many of these themes appeared to be present in the informal conversations that occurred with patients during the course of exercise sessions held in the current study. One patient in the current study expressed that participating in the study:

> *"Allowed them to feel like they were "taking a proactive role in their own treatment"*

Whilst another patient stated attending the exercise sessions:

> *"Provided a welcome distraction from the surgery ahead"* and *"provided an incentive to get up and do more things during the day"*

One patient even expressed the belief that they:

> *"Felt physically and mentally stronger even after only a couple of sessions"*

These were all in keeping with the themes of positive attitude, promoting a sense of purpose and structure and increased vitality as identified by Burke et al. (2013; 2015). It is acknowledged that the inclusion of anecdotal evidence based upon conversations rather than structured interviews is lacking in scientific rigour and therefore difficult for conclusions to be drawn from. The apparent presence of these themes within the conversations however reinforces the potential need to incorporate a

more qualitative approach to assessing QOL in future research rather than relying purely on questionnaires.

Despite beneficial changes in physical functioning being evident following PREHAB, the feasibility of implementing the intervention into standard clinical practice in its current form is questionable. For an intervention such as a PREHAB programme to be adopted by the NHS, not only does there need to be a patient demand for the service but also it needs to be cost-effective. In the current study, the participant consent rate was relatively low with around only 19% of those approached consenting to participate. Many of the obstacles highlighted in chapter 3 and within research relating to uptake and adherence to CR, were evident for non-participation including other commitments, lack of time, lack of interest and reluctance to travel. It could be argued therefore that those who consented to participate in the study were a self-selecting highly motivated population from which improvements were more likely to be achieved. The randomised controlled design of the study therefore is an important strength in controlling for any such bias that may have existed.

The issues with the low consent rate to this study were further compounded by 42% (84/198) of the patients being ineligible for the study as insufficient time (<2 weeks) existed between diagnosis and scheduled surgery. In line with NHS targets for the treatment of cancer (Baker & Nakatudde, 2015), the hospital where the current study was based were on occasions scheduling patients for surgery as soon as one week after being listed at the MDT meeting. As the duration of the PREHAB programme was determined by surgical wait time alone, many patients were therefore ineligible to participate. Levett and Grocott (2015) have suggested a more appropriate target for PREHAB programmes would be in those patients receiving NACRT prior to surgery given the 6-12 week recovery period that tends to exist between the conclusion of treatment and surgery. Furthermore, the potential benefits gained through targeting this specific population may be greater given that undergoing NACRT has been found to result in a decline in objectively measured cardiorespiratory fitness (Jack et al., 2014; West et al., 2014a).

A recent (non-randomised) controlled study by West et al. (2015) supported this having found a 6 week interval training programme (three times a week, 6 x 3 minutes at 80% workload at AT and 6 x 2 minutes at 50% difference between work rate at

VO_{2peak} and VO_2 at AT) returned post-NACRT AT and VO_{2peak} to pre-NACRT levels by six weeks post treatment. This was still maintained at 14 weeks post treatment. In comparison, AT in the control group continued to decrease up until week 14 post-NACRT. Such an intervention is therefore appropriate providing access is available to the patient immediately following NACRT however in patients not receiving NACRT, the time scale to surgery is much shorter. In the current study, as the recruitment was open to patients scheduled for either colon or rectal surgeries of a benign or malignant histology, two-thirds of the patients recruited had not received NACRT prior to beginning the PREHAB programme. Furthermore, the median duration of 21 days between entry to the study and surgery in the PREHAB group is considerably less than that the 14 week period observed in West et al. (2015), making a similar intervention to their study unsuitable for the current study.

Once recruited to the PREHAB programme, the mean adherence rate of ~90% attendance was promising along with the fact over half of the patients were fully adherent and no patient failed to complete the PREHAB intervention. Furthermore, of all the missed sessions, none were the result of the patient simply not attending. Other hospital/facility based PREHAB programmes (Dronkers et al., 2010; Timmerman et al., 2011; West et al., 2015) have reported similar high adherence rates and low dropout numbers (chapter 2; Table 2.6). As mentioned previously, the social support and reassurance gained through performing the exercise under supervision potentially promotes adherence and motivation to continue with participation (Burke et al., 2015), an element potentially lost in home-based interventions. Although all patients were aware the study was investigating exercise in preparing patients for surgery and no restrictions were placed on the physical activity the control group could perform, only two of the 11 control patients actually reported increased physical activity during the pre-operative period (as monitored through the use of self-report physical activity diaries). This raises the question that had the intervention been home-based without supervision, would an adherence rate of 89% still have been achieved and is therefore an aspect to be considered in future investigations.

An absence of a consensus remains regarding the optimal intervention for PREHAB (O'Doherty et al., 2013). Whilst an intervention similar to the interval training utilised by West et al. (2015) may represent the best opportunity for improving aerobic fitness (as determined by CPET), it is dependent on two key points; firstly, sufficient

time being available for the intervention to elicit positive changes and secondly in the case of West et al. (2015), the patient being physically capable of cycling for 40 minutes at a time. Improvements in VO_{2peak} (57.3 ± 2.6 ml.kg.min to 63.8 ± 3.0 ml.kg.min) have been observed following two weeks of daily high intensity training (2-7 reps of 15 seconds all out cycling with 45 seconds rest periods followed by 2-7 x 30 seconds sprints separated by 12 minutes rest periods) in young healthy male adults (Rodas, Ventura, Cadefau, Cusso, & Parra, 2000). The evidence of similar improvements being observed in clinical populations in such a short period of time is less clear. High intensity interval training has been reported to be both safe and effective in clinical populations such as heart failure (Wisloff et al., 2007) and coronary artery disease patients (Tschentscher et al., 2016) although the interventions have typically been over more prolonged time periods (> 6 weeks) than those available for patients with colorectal cancer. It has also been proposed that the inclusion of more intensive exercise programmes (both in terms of intensity and volume) may prove intimidating to patients' therefore affecting initial uptake and subsequent adherence to the programme (Carli et al., 2010; Li et al., 2013).

An important strength of the current study therefore was the application of individualised exercise programmes to each participant. The joint-by-joint approach adopted within the PREHAB programme placed emphasis on improving physical functioning rather than focusing purely on improving aerobic fitness. This allowed the intervention to be adapted to each participant's own physical capabilities. Although the aerobic exercise intensity of the current study was of low to moderate intensity (40-60% HRR), it still exceeded the minimum threshold of 35% VO_2 reserve/HRR identified as being required to improve cardiorespiratory fitness in individuals of lower baseline fitness (Swain & Franklin, 2002). Furthermore, certain exercises (e.g. push press, kettlebell swing) used within the resistance circuits resulted in transient increases in HR in excess of 80% HRR (Table 5.3), representing further periods of increased physiological demand on the patient. Within the study population recruited, a diverse range of physical capabilities existed. Out of the 11 patients randomised to the PREHAB group, two patients were unable to perform continuous cycling for a duration of greater than 10 minutes, a factor that potentially would have resulted in exclusion from a number of previous studies (Carli et al., 2010; West et al., 2015). Therefore, the approach taken within potentially represents a more inclusive format for PREHAB in colorectal cancer patients.

Despite the limited periods of structured cycling completed by these patients, potential beneficial effects in terms of physical functioning were still evident (Table 5.5). Whether a pre-surgery improvement in patient physical functioning alone, without an accompanying decrease in LOS and/or a reduction in post-surgical complications are sufficient to justify the implementation of PREHAB into standard care is hard to determine. Without the financial savings associated with a reduction in LOS, the cost of implementing a supervised PREHAB intervention would need to be found from other sources at a time of restricted spending and cuts within the NHS (Campbell, 2016). It is therefore unlikely that the PREHAB intervention used in this study would be adopted into standard care in its current form, however, through modifying into a home based programme, thus minimising the need for ongoing supervision and associated facility costs, a more cost-effective alternative may exist.

In summary, participation in an individualised PREHAB intervention consisting of as few as five sessions appeared to be sufficient to induce an improvement in physical functioning, and in particular walking capacity, in patients scheduled for major elective colorectal surgery. Whether this change in physical functioning is translated into either short term improvements in post-surgery recovery or has longer term benefits to the patient remains unclear. Whilst the study has further demonstrated the application of pre-surgery exercise is safe in patients awaiting colorectal surgery, the feasibility of implementing facility-based sessions on a wider scale is questionable. A shorter than expected time to surgery (< 2 weeks) and the subsequent poor recruitment uptake of eligible participants raises doubts as to the cost-effectiveness of adopting facility-based PREHAB into the standard care pathway for colorectal surgery alone without benefits beyond improved physical functioning being evident. Adoption as a modified home-based programme or as a service encompassing alternative surgical population may represent a more cost-effective and appropriate approach in the future.

Chapter 6: The effects of PREHAB on circulatory markers of cellular stress in elective colorectal surgery patients prior to surgery.

6.1. Introduction

In chapter 5, it was demonstrated that consistent with previous literature (Jones et al., 2007; Li et al., 2013; Sawatzky et al., 2014), undertaking a short period of PREHAB appears to be sufficient to facilitate an improvement in physical functioning prior to surgery. It is plausible that alongside these improvements, beneficial adaptations on a cellular level may have also occurred. The acute trauma caused when undergoing a major surgical procedure is associated with the stimulation of a number of endocrinological, immunological and haematological responses (Desborough, 2000), one of which is the generation of oxidative stress (Pappas-Gogos et al., 2013). Two protective mechanisms the body has against oxidative damage are a group of stress proteins known as Hsps as well as the antioxidant defence system.

Heat shock protein 72 and Hsp32 have both been reported to have important roles in providing protection when the body is exposed to cellular stress. It has been proposed that the prior induction of Hsp72 by exposure to a non-lethal stressor such as heat shock or hypoxic conditions, potentially can provide protection from a subsequent second more severe stress such as that caused by exercise or heat exposure (Madden et al., 2008; Taylor et al., 2012). Following 3-10 days of heat acclimation, with or without concurrent exercise training, an elevation in leukocyte Hsp72 has been reported to portray increased thermotolerance against a subsequent stress in healthy young male adults (Magalhaes Fde et al., 2010; McClung et al., 2008). It remains unclear however whether a short period of exercise training alone can elicit a similar upregulation in Hsp72 expression in humans. Conversely, long term training (>8 weeks) along with lifelong participation in physical activity has been reported to decrease basal Hsp72 expression compared to more sedentary controls (Bautmans et al., 2005; Beltran Valls et al., 2014; Simar et al., 2007). Given that the Hsp72 response has been reported to be inversely proportional to basal content (Vince et al., 2010), should PREHAB actually decrease pre-surgical levels of Hsp72, there may be greater potential for increased expression in response to surgical stress. Although limited research exists relating to its response to exercise training, the ability of Hsp32 to function as a mediator of antioxidant activity during oxidative assault (Otaka et al., 2006) means elevating basal expression prior to surgery may be beneficial.

As the primary endogenous antioxidant in the body, glutathione serves an important role scavenging oxygen free radicals through the oxidation of GSH to GSSG. A shift in the TGSH/GSSG balance towards a more oxidative state therefore has been reported to be a sensitive measure of oxidative stress (Elokda & Nielsen, 2007). Similarly, a shift towards a more reduced state of TGSH/GSSG could provide greater protection against surgery induced oxidative stress. Six weeks of either aerobic or circuit weight training is reported to have increased erythrocyte GSH and GSSG in sedentary healthy volunteers (Elokda & Nielsen, 2007), thus highlighting one of the beneficial adaptations that may be induced by a period of PREHAB.

The primary aim of this chapter was therefore to investigate whether a short period of PREHAB would upregulate basal leukocyte Hsp72 and Hsp32 expression in colorectal patients prior to surgery. The effect PREHAB had on other markers of circulatory stress (plasma Hsp27, plasma cortisol and whole blood glutathione) was also investigated along with any relationships that may exist between the respective markers.

6.2. Methods

6.2.1. Ethical Approvals and participants

As described in chapter 5, sections 5.2.1 and 5.2.2.

6.2.2. Experimental design

The general experimental design was as described in chapter 5 (section 5.2.3). Specifically for this chapter, venous blood samples (2 EDTA and 1 sodium citrate plasma tubes) were taken at baseline and pre-operative reassessment prior to the participant performing the five physical functioning tests. From the samples taken, the expression of Hsp32 and Hsp72 in monocytes, lymphocytes and granulocytes was measured via flow cytometry whilst plasma Hsp27, Hsp72, total glutathione, oxidised glutathione and cortisol were measured using commercially available assay or ELISA kits. Peripheral blood mononuclear cells were isolated via density gradient centrifugation for future analysis (chapter 7). Full detailed descriptions of all the procedures used will now be described in sections 6.2.3 to 6.2.9.

6.2.3. Blood collection and preparation

Venous blood samples were taken via standard venepuncture on entry to the study and on the morning of surgery into two potassium EDTA tubes and one tri-sodium citrate coagulation tube (Vacuette®, Greiner BIO-one, UK). Whilst every attempt was made to ensure all blood samples were taken at the same time of the day to account for circadian variation in biochemical markers (Blanco et al., 2007; Sandstrom et al., 2009), the logistics of timing blood samples around patient hospital appointments and scheduled surgery times meant that this was not always possible. The baseline samples were taken at varying times during the day according to patients' hospital appointment time. All pre-operative blood samples were taken at approximately 7:30 am on the morning of the scheduled surgery.

One potassium EDTA tube was used for the immediate analysis of Hsp72 and Hsp32 in leukocytes by flow cytometry (Section 6.2.5) and the isolation of PBMCs (Section 6.2.4) whilst the second tube was centrifuged at 2500 x gravity (*g*) for 10 minutes for the separation of plasma. The plasma was then aliquoted into 1 ml cryovials before undergoing controlled freezing to -80°C prior to being transferred to liquid nitrogen for later analysis of Hsp27, Hsp72 and cortisol (sections 6.2.7 – 6.2.9). From the tri-sodium citrate coagulation tube, 1 ml of whole blood was mixed thoroughly with 4 ml of ice cold 5% metaphosphoric acid (Sigma-Aldrich Company Ltd., Dorset, England) and stored on ice for 15 minutes. The 5% metaphosphoric acid removed any proteins which may interfere with the glutathione assay described in section 6.2.6. Following the 15 minute period, samples were subsequently centrifuged at 14,000 x *g* for 14 minutes in a temperature controlled microcentrifuge set at 4°C. The clarified supernatant was aliquoted into 1 ml cryovials and immediately frozen at -80°C for later analysis.

6.2.4. Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated using density gradient centrifugation (lymphocyte separation medium, PAA, UK). EDTA treated whole blood was mixed with phosphate buffered saline (PBS) to a 1:1 ratio before being carefully layered on top of an equal amount of lymphocyte separation medium and centrifuged at 400 x *g* for 30 minutes. Following centrifugation, the suspended PBMC layer was

carefully extracted, washed in 10 ml of PBS and centrifuged at 400 x *g* for 10 minutes. The washing procedure was performed twice more and the cells were resuspended in 2 ml of freeze media (90% Fetal Bovine serum [FBS]; 10% Dimethyl Sulfoxide [Sigma-Aldrich]) and underwent controlled freezing to -80°C before being transferred to liquid nitrogen for storage and subsequent use in chapter 7.

6.2.5 Intracellular Hsp72 and Hsp32 Assay

The analysis of intracellular Hsp72 (leukocytes and tumour cell lines) and Hsp32 (leukocytes only) was performed via flow cytometry; a method reported to provide a quantitative measurement of heterogeneous cell populations that is more sensitive than Western blotting (Bachelet et al., 1998). The method utilised in this thesis is an optimised method for determining Hsp expression that has been well established in the literature (Peart, Kirk, Hillman, et al., 2012; Peart et al., 2011; Sandstrom et al., 2009; Taylor et al., 2012; Vince, Oliver, Midgley, McNaughton, & Madden, 2010).

For the analysis of Hsp32 and Hsp72 from freshly taken blood, 100 µl of EDTA treated whole blood was mixed with 2 ml red blood cell lysing buffer (Erythrolyse, AbD Serotec, Oxford, UK; diluted 1/10 with distilled water) and incubated at room temperature for 15 minutes. Cells were twice washed in 2 ml PBS and centrifuged at 400 x *g* for 3 minutes. Following red blood cell lysis, cells were incubated in 100 µl of fixation medium (Leucoperm Reagent A, AbD Serotec) for 15 minutes, washed in 2 ml PBS and then the cell pellet resuspended in 150 µl of permeabilsation buffer (Leucoperm Reagent B, AbD Serotec). The sample was then divided into three 50 µl samples and 4 µl of either monoclonal Hsp72:FITC (C92F3A-5) antibody (Enzo Life Sciences, Exeter, UK), Hsp32:FITC (HO-1-2) antibody (Enzo Life Sciences) or corresponding negative control (IgG1 and IgG2b; AbD Serotec) added and then incubated for 30 minutes in the dark. Following incubation, samples were washed in 2 ml PBS before being re-suspended in 300 µl of PBS ready for analysis via flow cytometry.

Flow cytometry was performed on a BDFACSCalibur® (BD Biosciences, Oxford, UK) running CELLQuest software. Monocyte, lymphocyte and granulocyte populations were gated by forward/side scatter properties with a total of 25,000 events counted (Figure 6.1). Results were calculated as the median fluorescence ratio (MFR)

based in the median fluorescence intensity gained with the anti-Hsp antibody compared to that of the isotype matched negative control (Figure 6.2).

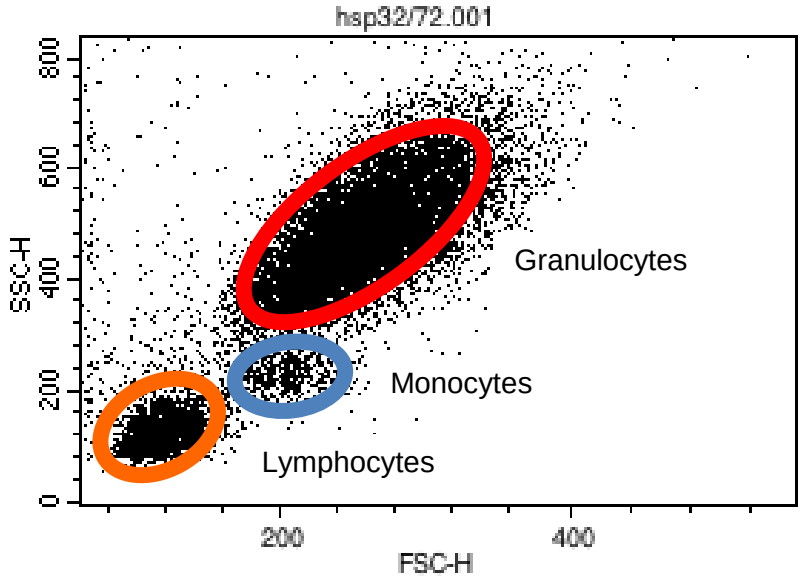


Figure 6.1: Typical representation of monocyte, lymphocyte and granulocyte populations based on their FSC/SSC properties.

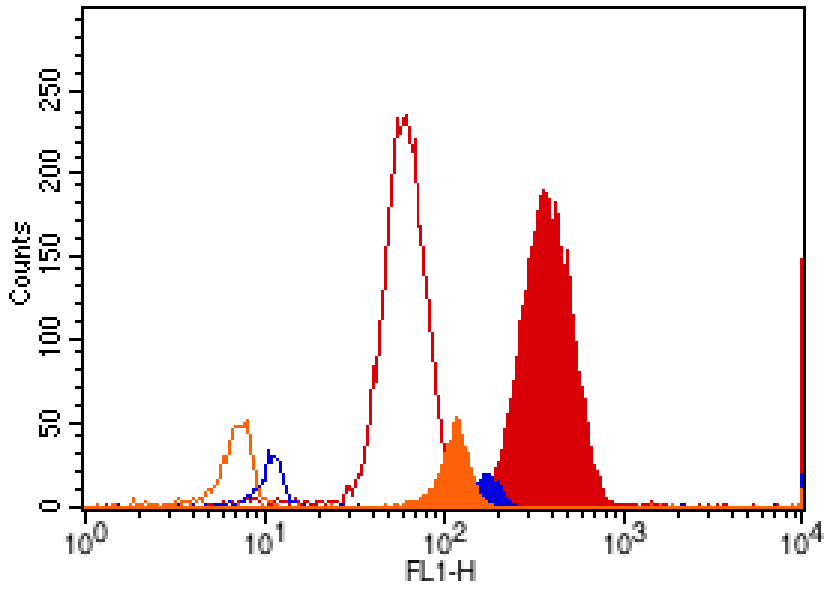


Figure 6.2: Typical representation of MFI for monocytes (blue), lymphocytes (orange) and granulocytes (red) as determined for the isotype matched negative control (solid lines) and Hsp antibody (filled).

6.2.6. Glutathione (Total and oxidised glutathione) Assay

Whole blood total (TGSH) and oxidised (GSSG) glutathione were measured in duplicate in the collected supernant (described in section 3) using a commercially available assay kit (Glutathione detection kit, Enzo Life Sciences). For the measurement of TGSH, samples were diluted 1:50 in 1X assay buffer before 50 µl of each sample was transferred to a 96 well microtiter plate. To each well on the plate, 150 µl of reaction mix, which contained 5,5'-dithiobis-2-nitrobenozoic acid (DTNB, Ellman's reagent) and 10 µl glutathione reductase, was added. The resulting reaction with the samples formed the yellow coloured 5-thio-2 nitrobenzoic acid, with the rate of production read at 405 nm every minute for 10 minutes in a microplate reader (Biotek Synergy HT-R, Biotek Instruments, USA). A standard curve (100 pmols to 12.5 pmoles) for determination of TGSH concentration was produced by the serial dilution of 50 µl assay buffer (diluted 1/25 with distilled water) and 50 µl of 4 µm GSSG standard. The process for determining GSSG was the same TGSH with exception that each 50 µl sample as well as the GSSG standards were treated with 1µl of 2M 4-vinylpyridine (Sigma-Aldrich) and incubated for one hour at room temperature prior to analysis. The purpose of this reagent was to block free thiols present in the reaction which may result in overestimation of GSSG.

6.2.7. Plasma Hsp27 ELISA

Hsp27 expression was measured in plasma using a commercially available enzyme linked immunosorbent assay (ELISA) (Enzo Life Sciences) according to the manufacturers' protocol. Standards prepared through the serial dilution of 2.5 µl of Hsp27 standard (5 µg/ml recombinant Hsp27) with sample diluent. In duplicate, 100 µl of each standard or sample (diluted 1:4 in sample dilute) was added to a 96 well clear microtiter plate pre-coated with monoclonal mouse Hsp27 antibody, covered with an adhesive plate sealer and incubated at room temperature for 1 hour. Following incubation, the wells were washed six times with 300 µl of wash buffer using an automated microplate washer and 100 µl of diluted anti-Hsp27 antibody (1:500 with antibody diluent) added to each well before being covered and incubated for a further hour. Again, the wells were washed six times with 300 µl of wash buffer and 100 µl of diluted horseradish peroxidase (HRP) conjugate (1:500 with HRP conjugate diluent) added to each well before being covered and incubated for 30 minutes. The wash

procedure was repeated before 100 µl of TMB (tetramethylbenzidine and H_2O_2) substrate was added to each well and left to incubate in the dark to allow for colour development. After 15 minutes, the reaction was terminated through the addition of 100 µl of stop solution (1 N solution of hydrochloric acid and H_2O). Absorbance was read immediately at 450nm on a microplate reader.

6.2.8. Plasma Hsp72 ELISA

Hsp72 expression was measured in plasma using a commercially available high sensitivity ELISA (Enzo Life Sciences) according to the manufacturers' protocol. Standards were prepared through the serial dilution of 5 µl of Hsp72 standard (10 µg/ml of recombinant human Hsp72) with assay buffer (PBS containing bovine serum albumin and detergent). In duplicate, 100 µl of each standard or sample (diluted 1:2 in sample diluent) was added to a 96 well clear microtiter plate pre-coated with monoclonal mouse Hsp72 antibody, covered with an adhesive plate sealer and incubated on a plate shaker at room temperature for 2 hours. Following incubation, the contents of the wells were aspirated and washed by the addition of 400 µl of wash buffer (Tris buffered saline) using an automated microplate washer. This was repeated a further three times before adding 100 µl of Hsp72 antibody to each well, covering and incubating for one hour on the shaker. The wells were washed again as previously described and 100 µl of HRP conjugate added to each well and incubated for a further hour. The wash procedure was repeated before 100 µl of TMB substrate was added to each well and plate left to incubate for 30 minutes before the reaction was terminated through the addition of 100 µl of stop solution. Absorbance was read at 450 nm on a microplate reader.

6.2.9. Plasma Cortisol ELISA

Plasma cortisol was measured by ELISA (Alpco, USA) according to manufacturers' instructions. In duplicate, 20 µl of each calibrator (0, 0.5, 2, 5, 10, 30 and 60 µg/dl cortisol), control (containing cortisol in a human serum-based buffer with a non-mercury preservative) and sample was added to an anti-cortisol antibody coated microtiter plate. Aliquots of 100 µl cortisol-HRP conjugate (diluted 1:100 with a protein-based assay buffer) were then added to each well and the plate incubated on a plate shaker (200 rpm) for 45 minutes at room temperature. Following incubation, three separate washes with 300 µl of diluted wash buffer (containing non-ionic detergent and

a non-mercury preservative) per well were performed using a microplate washer in order to remove any unbound material. Between each wash, the plate was firmly tapped against absorbent paper to ensure it was dry. Following the third wash, 150 µl of TMB substrate was added to each well and the plate was incubated for 15 minutes on a plate shaker after which the reaction was stopped through the addition of 50 µl of 1M sulfuric acid stop solution. Absorbance was read at 450 nm on a microplate reader.

6.2.10. Statistical Analysis

Statistical analysis was performed using IBM SPSS statistics version 20 (SPSS Inc., Chicago, IL). As in chapter 5, primary analysis focused on descriptives as per recommendations for pilot studies (Lancaster, 2015; Lancaster et al., 2004) with significance testing used as supporting secondary analysis. Normal distribution of data was assessed through visual inspection of histograms, quantile-quantile plots and box plots in conjunction with statistics for skewness and kurtosis. Where the data was normally distributed, results are reported as means ± standard deviation and range, with median (IQR) and range used for non-normally distributed data. Attempts to transform the data that violated the assumptions were unsuccessful in most instances therefore non parametric tests were used. Between group differences was compared using Independent Sample T-tests for normally distributed data and the Mann-Whitney U test for data that violated the assumption of normal distribution. Within group changes between baseline and pre-operative reassessment for both groups was assessed using either Paired Sample T-tests or the Wilcoxon Rank-Sum test. Relationships between variables was analysed using the Spearman rho correlation. Statistical significance was set at $p < 0.05$.

6.3. Results

Following analysis of blood samples for leukocyte expression of Hsp32 and Hsp72, complete baseline and pre-operative data was available from a total of 16 participants (Control: n = 8; PREHAB: n = 8). For the subsequent analysis of plasma Hsp72, Hsp27, glutathione and cortisol, complete data was available for 19 out of the 22 participants described in chapter 5. Participant descriptive data for those with complete data in each category of blood analysis is detailed in Table 6.1.

Table 6.1: Descriptive information of study participants.

	Whole blood		Plasma	
	Control (n = 8)	**PREHAB (n = 8)**	**Control (n = 9)**	**PREHAB (n =10)**
Age (Years)	62.3 ± 11.8 [37; 77]	63.8 ± 11.5 [46; 79]	62.8 ± 11.2 [37; 77]	64.1 ± 10.5 [46; 79]
Gender ratio (M:F)	5:3	3:5	6:3	4:6
Height (cm)	169.3 ± 10.7 [147.5; 181.7]	159.6 ± 8.2 [145.5; 170.4]	169.4 ± 10.0 [147.5; 181.7]	160.9 ± 9.2 [145.5; 176.6]
Body mass (kg)	80.2 ± 24.8 [44.7; 119.0]	76.9 ± 12.8 [57.4; 101.4]	79.1 ± 23.5 [44.7; 119.0]	78.4 ± 13.1 [57.4; 101.4]
BMI (kg/m^2)	27.4 ± 6.1 [19.9; 38.1]	30.2 ± 4.9 [24.4; 38.6]	27.1 ± 5.9 [19.9; 38.1]	30.3 ± 4.3 [24.4; 38.6]
Smoker	Yes 1 No 7	Yes 0 No 8	Yes 1 No 8	Yes 0 No 10
Previous smoker	Yes 3 No 5	Yes 2 No 6	Yes 3 No 6	Yes 3 No 7
Drinks alcohol	Yes 3 No 5	Yes 6 No 2	Yes 4 No 5	Yes 8 No 2
Histology at surgery (TNM classification of malignant tumours)	T2N0M0 1 T3N0M0 2 T3N1M0 1 T3N2M0 1 T3N1M1 1 T4N0M0 1 Benign 1	T3N0M0 4 T3N1M1 2 No residual malignancy 1 Benign 1	T2N0M0 1 T3N0M0 3 T3N1M0 1 T3N2M0 1 T3N1M1 1 T4N0M0 1 Benign 1	T3N0M0 6 T3N1M1 2 No residual malignancy 1 Benign 1
Length of stay (days)	8 (1.5) [6; 20]	8 (6.8) [5; 12]	8 (7) [6; 27]	10 (7) [5; 12]
PREHAB sessions attended		6.6 ± 2.5 [3; 10]		6.9 ± 2.3 [3; 10]
6MWT distance (m)	400 (210) [#] [356; 588]	501.0 (110) [#] [298; 600]	410.5 (189.0) [##] [356; 588]	501.0 (132.5) [##] [298; 600]

TNM: Tumour, Nodes, Metastasis
[#] Control (n = 7); PREHAB (n = 7) [##] Control (n = 8); PREHAB (n = 9)

6.3.1. Hsp32 and Hsp72

No significant difference in Hsp32 expression was seen in monocytes ($_{m}$Hsp32), lymphocytes ($_{l}$Hsp32) or granulocytes ($_{g}$Hsp32) between baseline and pre-operative reassessment for either the control group or the PREHAB (Figure 6.3 a, c and e). Although median $_{m}$Hsp32 increased at pre-operative reassessment in the PREHAB group, six out of eight actually had decreased $_{m}$Hsp32 at pre-operative reassessment

compared to baseline (Figure 6.4 b) although this was not statistically significant (Z = -1.680; $p = 0.093$). Furthermore, of those to decrease, the percentage change was less than 7% in four of the six participants. No other leukocyte cell type displayed a consistent directional change between baseline and pre-operative reassessment in participant Hsp32 expression (Figures 6.4 a, c-f). No significant difference in mean difference was present between baseline and pre-operative reassessment for the control and PREHAB groups (Table 6.2).

Median $_{m}$Hsp72 expression was lower at pre-operative reassessment compared to baseline in both the control (median difference: -2.29 [IQR: 1.76] MFR; Z = -2.240; $p = 0.025$) and PREHAB groups (median difference: -1.49 [IQR: 5.05] MFR; Z = -1.680; $p = 0.093$) (Figure 6.3 b, d and f) although the difference was only significant for the control. Within the separate groups, basal $_{m}$Hsp72 expression was decreased in seven out of eight participants in the control group and in six out of eight of the PREHAB group at pre-operative reassessment compared to baseline (Figure 6.5 a and b). As for Hsp32 expression, neither lymphocyte nor granulocytes displayed a consistent directional change in Hsp72 between baseline and pre-operative reassessment for either group (Figures 6.5 d-f). This was reflected by the absence of a significant difference between time points in either group for $_{l}$Hsp72 or $_{g}$Hsp72. Median difference between groups was not significantly different for any of the leukocyte cell types (Table 6.2).

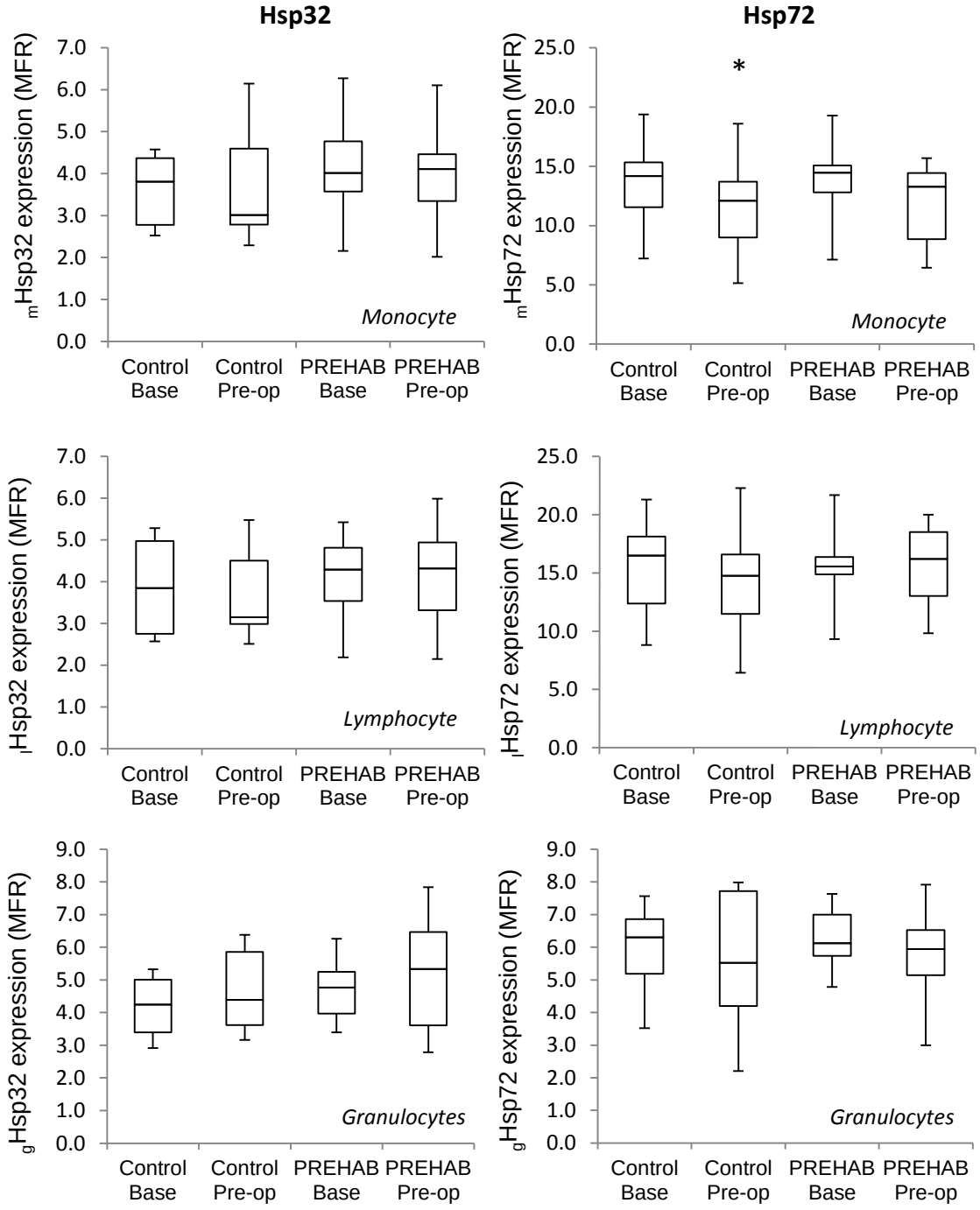


Figure 6.3: Alterations in leukocyte Hsp32 and Hsp72 expression in the control and PREHAB groups between baseline and pre-operative reassessment. Displayed as Median (IQR) [Range]: *Significantly different to baseline ($p < 0.05$); MFR: median fluorescence ratio

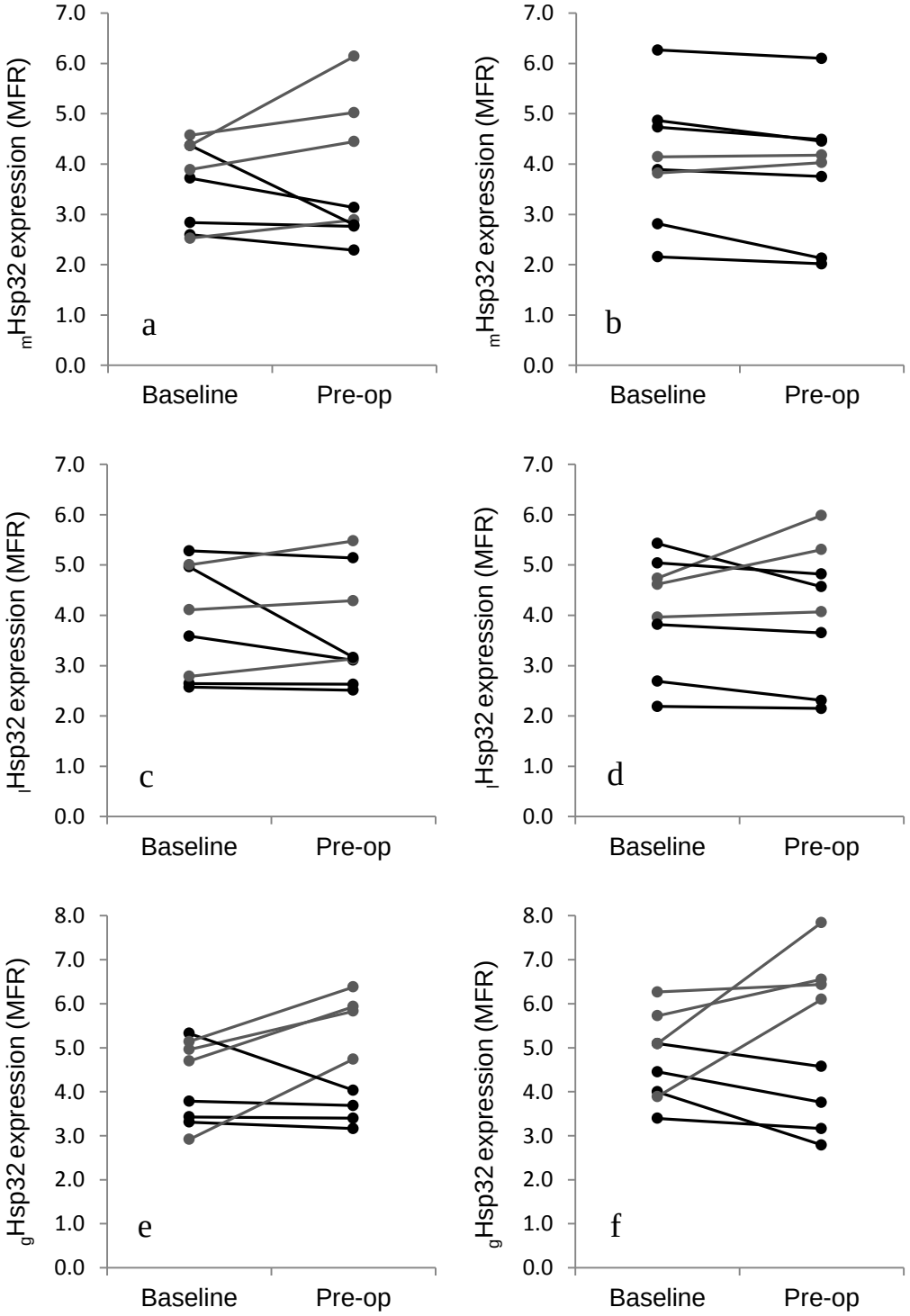


Figure 6.4: Individual participant changes in leukocyte Hsp32 expression between baseline and pre-operative reassessment for the control (a: monocyte; c: lymphocyte; e: granulocyte) and PREHAB (b: monocyte; d: lymphocyte; f: granulocyte) groups. MFR: median fluorescence ratio

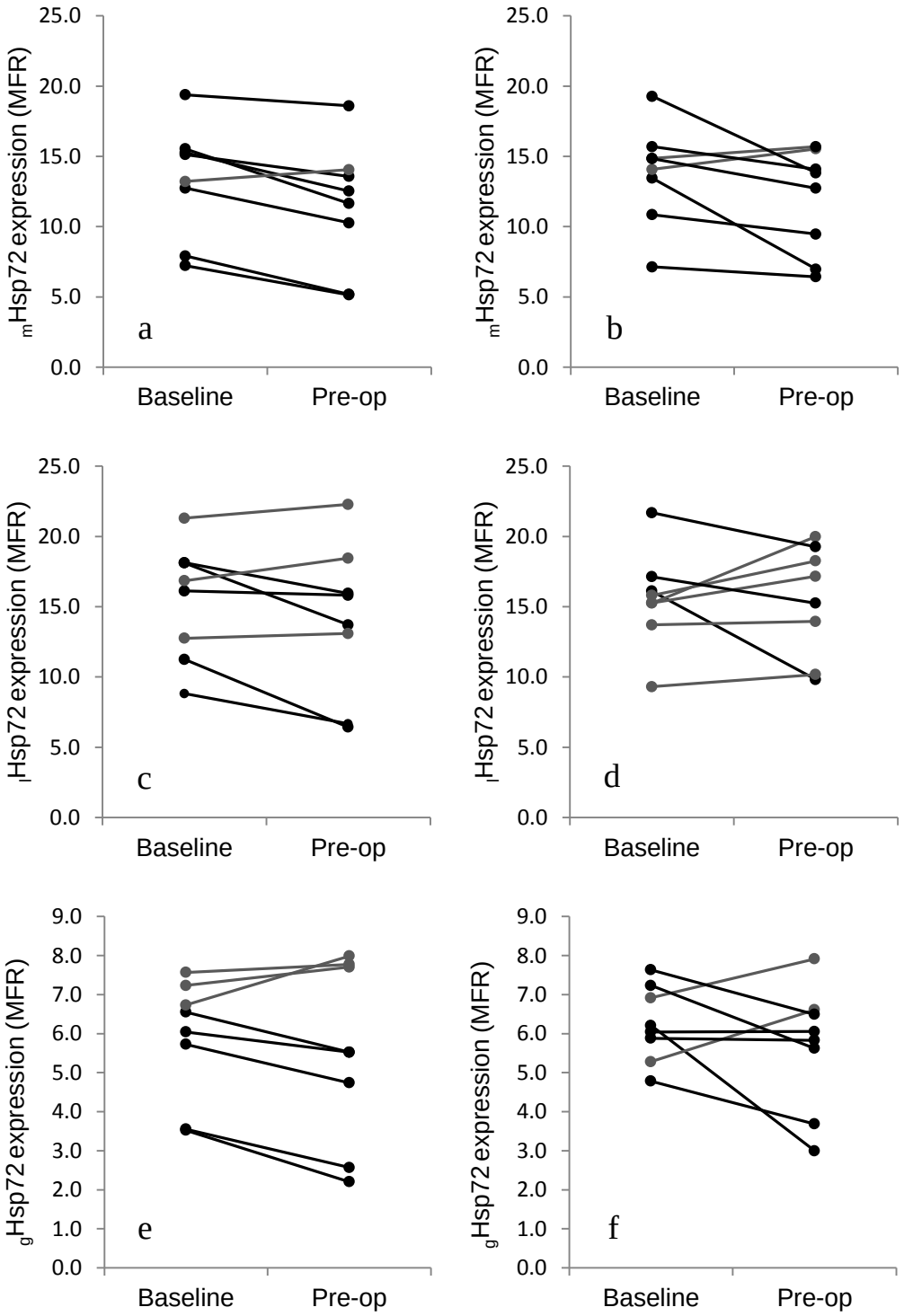


Figure 6.5: Individual participant changes in leukocyte Hsp72 expression between baseline and pre-operative reassessment for the control (a = monocyte; c = lymphocyte; e = granulocyte) and PREHAB (b = monocyte; d = lymphocyte; f = granulocyte) groups. MFR: median fluorescence ratio

Table 6.2: Mean/median difference (MFR) for leukocyte Hsp32 and Hsp72 expression between baseline and pre-operative assessment in the control and PREHAB groups.

	Control (n = 8)	PREHAB (n = 8)	Between group comparison (*p* value)
$_{m}$Hsp32	0.07 ± 0.98	-0.19 ± 0.72	0.478[a]
	[-1.58; 1.77]	[-0.68; 0.21]	
$_{l}$Hsp32	-0.04 (0.70)	-0.11 (0.89)	1.000[b]
	[-1.80; 0.48]	[-0.86; 1.25]	
$_{g}$Hsp32	0.42 (1.38)	-0.03 (2.51)	0.721[b]
	[-1.30; 1.82]	[-1.21; 2.75]	
$_{m}$Hsp72	-2.29 (1.76)	-1.49 (5.05)	0.600[b]
	[-3.89; 0.84]	[-6.48; 1.46]	
$_{l}$Hsp72	-1.22 (4.66)	0.56 (4.60)	0.345[b]
	[-4.82; 1.60]	[-6.29; 4.74]	
$_{g}$Hsp72	-0.75 (1.42)	-0.58 (2.25)	0.599[b]
	[-1.32; 1.26]	[-3.21; 1.33]	

Mean ± Standard deviation [range]; Median (IQR) [range]; [a]Independent Sample T-Test; [b] Mann-Whitney U Test

When analysed with both PREHAB and control groups combined, only $_{m}$Hsp72 displayed a significant difference in expression between baseline and pre-operative reassessment with 13 out of 16 participants displaying a lower MFR (Table 6.3). The pattern of individual changes in Hsp32 and Hsp72 expression at pre-operative reassessment for the remaining leukocyte cell type in all participants were as follows: $_{m}$Hsp32: 6 increased; 10 decreased; $_{l}$Hsp32: 6 increased; 10 decreased; $_{g}$Hsp32: 8 increased; 8 decreased; $_{l}$Hsp72: 8 increased; 8 decreased; $_{g}$Hsp72: 6 increased; 10 decreased. The difference was not significant between any of these.

Table 6.3: Leukocyte Hsp32 and Hsp72 expression (MFR) at baseline and pre-operative reassessment with data from both groups combined.

	Baseline	Pre-op	Median difference	Between group comparison (*p* value[b])
$_{m}$Hsp32	3.89 (1.71)	3.89 (1.71)	-0.14 (0.71)	0.501[b]
	[2.16; 6.27]	[2.02; 6.14]	[-1.58; 1.77]	
$_{l}$Hsp32	4.04 (2.28)	3.86 (2.31)	-0.05 (0.65)	0.660[b]
	[2.19; 5.43]	[2.15; 5.99]	[-1.80; 1.25]	
$_{g}$Hsp32	4.57 (1.61)	4.66 (2.84)	0.07 (1.69)	0.277[b]
	[2.92; 6.26]	[2.79; 7.84]	[-1.30; 2.75]	
$_{m}$Hsp72	14.46(4.15)	12.64 (6.47)	-1.84 (2.14)	0.004[b]
	[7.14; 19.38]	[5.14; 18.59	[-6.48; 1.46]	
$_{l}$Hsp72	15.48 ± 3.67	14.77 ± 4.64	-0.71 ± 2.94	0.349[a]
	[13.52; 17.44]	[12.29; 17.24]	[-2.27; 0.86]	
$_{g}$Hsp72	6.13 (1.76)	5.72 (3.48)	-0.75 (1.54)	0.179[b]
	[3.53; 7.63]	[-3.21; 1.33]	[-3.21; 1.33]	

MFR: Median fluorescence ratio; Mean ± Standard deviation [range]; Median (IQR) [range]; [a]Independent Sample T-Test; [b]Mann-Whitney U Test.

As the data was not normally distributed when combined, the relationships between monocyte, lymphocyte and granulocyte Hsp32 and Hsp72 expression was examined using Spearman's rho correlations. Significant moderate to strong positive relationships were observed for both Hsp32 (r_s = 0.806 to 0.921, all $p < 0.001$) and Hsp72 (r_s = 0.742 to 0.877, all $p < 0.001$) expression between monocytes, lymphocytes and granulocytes (Figure 6.6 a-f). Moderate to strong positive correlations (r_s= 0.517 to 0.631, all $p < 0.01$; Figure 6.6 g, h, i) were also observed between the expression of Hsp32 and Hsp72 in each of the respective cell types.

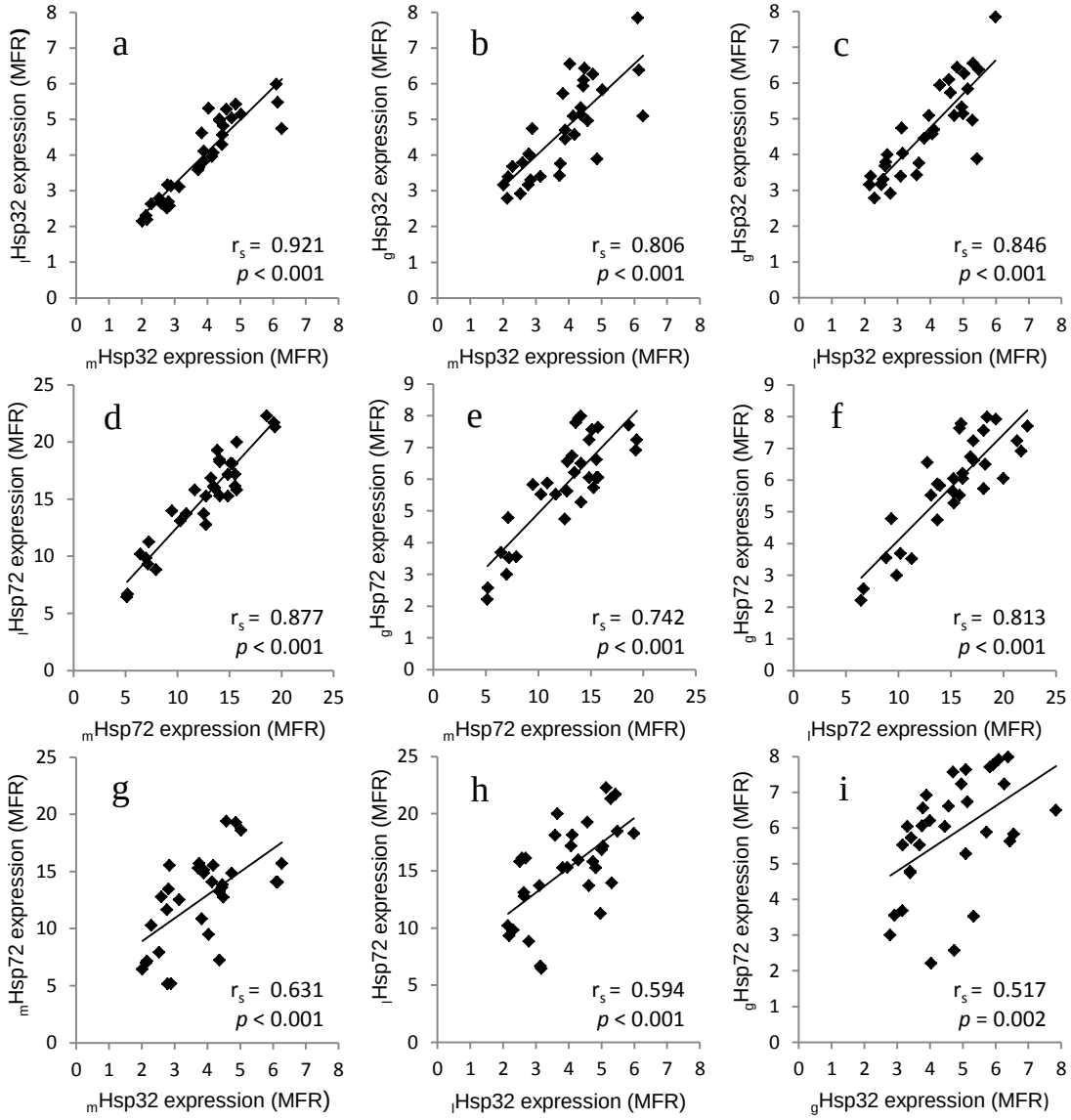


Figure 6.6: Relationships between total Hsp32 and Hsp72 expression in monocytes, lymphocytes and granulocytes. MFR: median fluorescence ratio

6.3.2. Glutathione

Median total glutathione was lower in the PREHAB group at both baseline (5717.54 vs. 5367.95 pmol; Z = - 0.817; $p = 0.414$) and pre-operative reassessment (5781.74 vs. 4970.19 pmol; Z = -0.490, $p = 0.624$) compared to the control group however the difference was not significant (Figure 6.7). Total glutathione increased in seven out of 10 participants with a median difference of 337.76 pmol (IQR: 1975.75 [range -4234.46 to 2701.57 pmol]) in the PREHAB group whilst four out of nine increased in the control group (median difference: -668.00 pmol; IQR: 1975.7 [range: -3066.64 to 1012.81 pmol]; Figure 6.8). The change from baseline to pre-operative reassessment was not significant for either group (PREHAB: Z = -0.968; $p = 0.333$ and control: Z = -0.652; $p = 0.515$). Median difference between groups was not significantly different (Z = -1.470; $p = 0.142$).

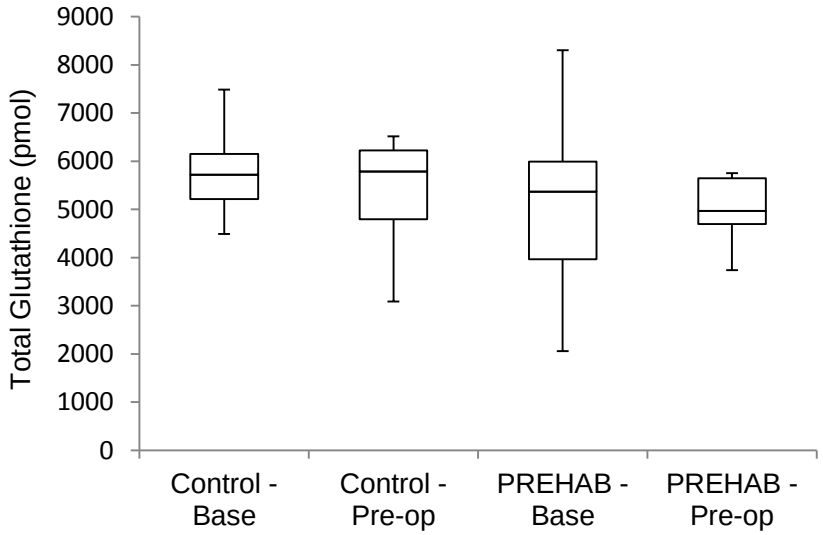


Figure 6.7: Total glutathione at baseline (Base) and pre-operative (Pre-op) reassessment in the control and PREHAB groups. Displayed as Median (IQR) [Range]

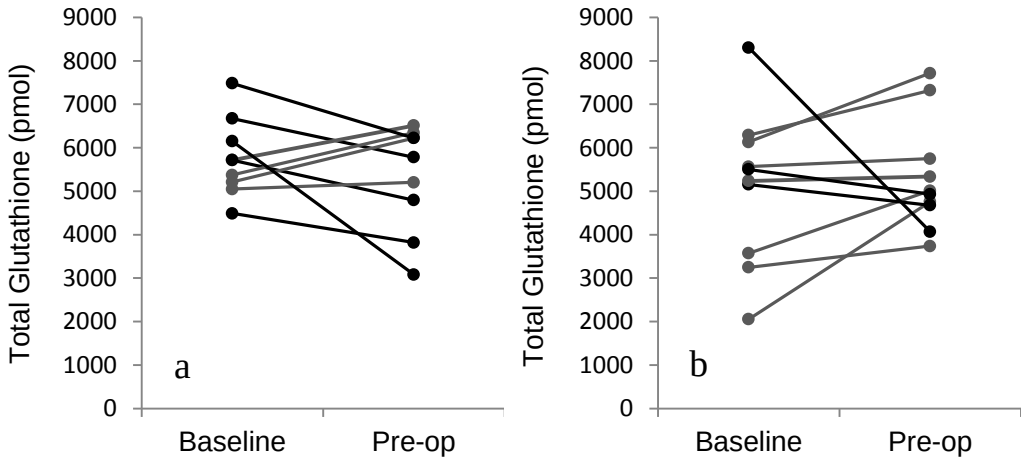


Figure 6.8: Individual participant changes in total glutathione between baseline and pre-operative reassessment for the control (a) and PREHAB (b) groups.

Median GSSG/GSH was ~5% higher in the control group at pre-operative reassessment compared to baseline (Z = 0.560, p = 0.575) and 7% higher than the PREHAB group at pre-operative reassessment (Z = 0.327, p = 0.744), although the difference was not significant for either (Figure 6.9). Individual participant changes in GSSG/GSH between baseline and pre-operative reassessment were similar in both groups. In the control group, GSSG/TGSH had increased in four participants at pre-operative reassessment, decreased in four and remained the same in one, whilst in the PREHAB group, GSSG/GSH increased in five participants and decreased in the remaining five (Figure 6.10). When both groups were combined, gender had no influence on median GSSG/GSH levels at either baseline (Z = -1.184, p = 0.236) or pre-operative reassessment (Z = -0.653, p = 0.549).

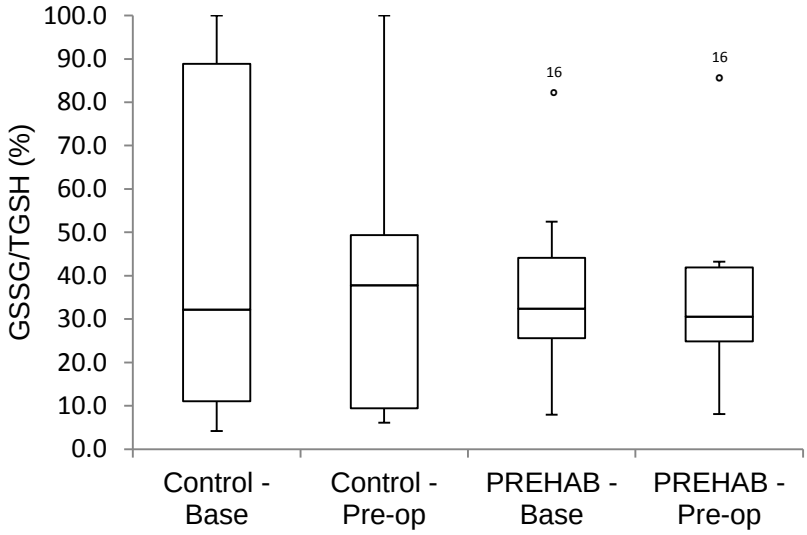


Figure 6.9: GSSG/TGSH at baseline (Base) and pre-operative reassessment (Pre-op) in the control and PREHAB groups. Displayed as Median (IQR) [Range]

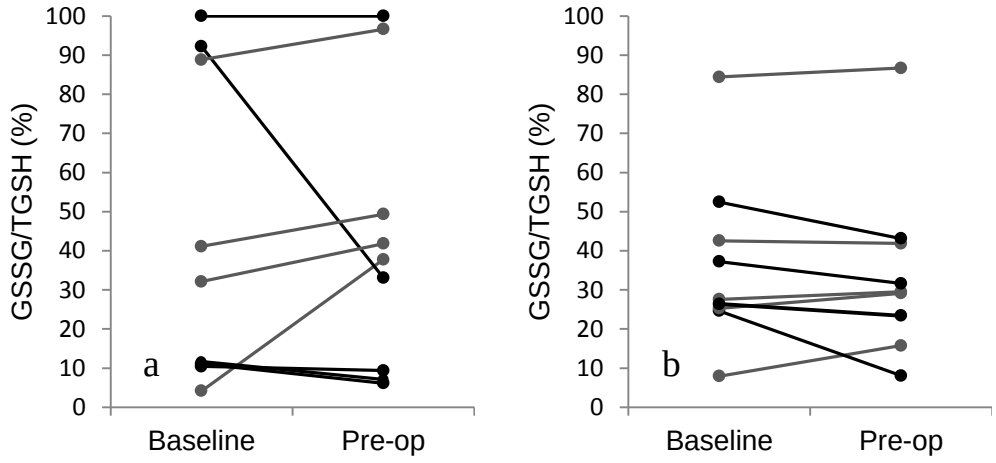


Figure 6.10: Individual participant changes in GSSG/TGSH between baseline and pre-operative reassessment for the control (a) and PREHAB (b) groups.

6.3.3. Cortisol

There was no significant difference in cortisol between the two groups at either baseline (Z = 0.221, p = 0.825) or pre-operative reassessment (Z = 0.622, p = 0.508) (Figure 6.11). A significant within group increase in plasma cortisol between baseline and pre-operative reassessment was seen in the control group (median + 3.95 µg/dL, Z = 2.192, p = 0.028), the PREHAB group (median +9.34 µg/dL, Z = 2.429, p = 0.015) and when combined (mean difference + 9.47 µg/dL, t = 3.515, p = 0.003). In both groups, eight out of nine participants had elevated cortisol levels at pre-operative

reassessment compared to baseline (Figure 6.12). The results for one participant in the PREHAB group exceeded the upper limits of the ELISA therefore were not included in the analysis.

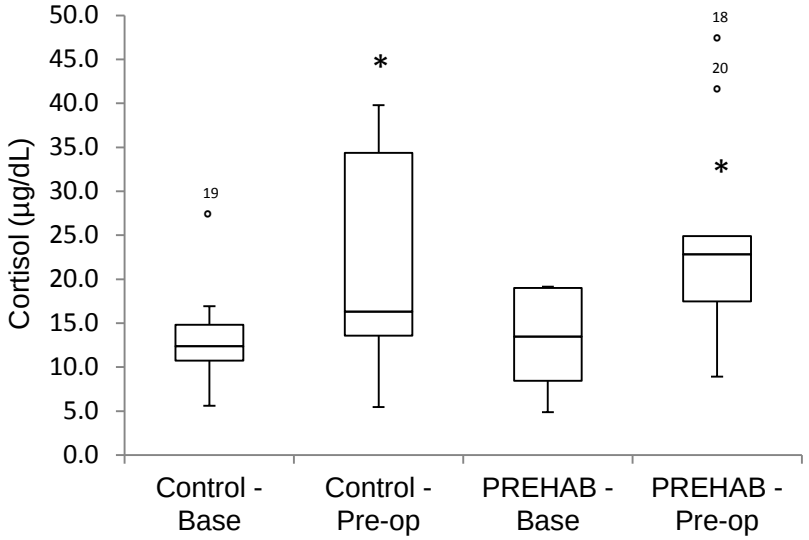


Figure 6.11: Plasma cortisol at baseline (Base) and pre-operative (Pre-op) reassessment in the control and PREHAB groups. Displayed as Median (IQR) [Range]; *Significantly different to baseline ($p < 0.05$)

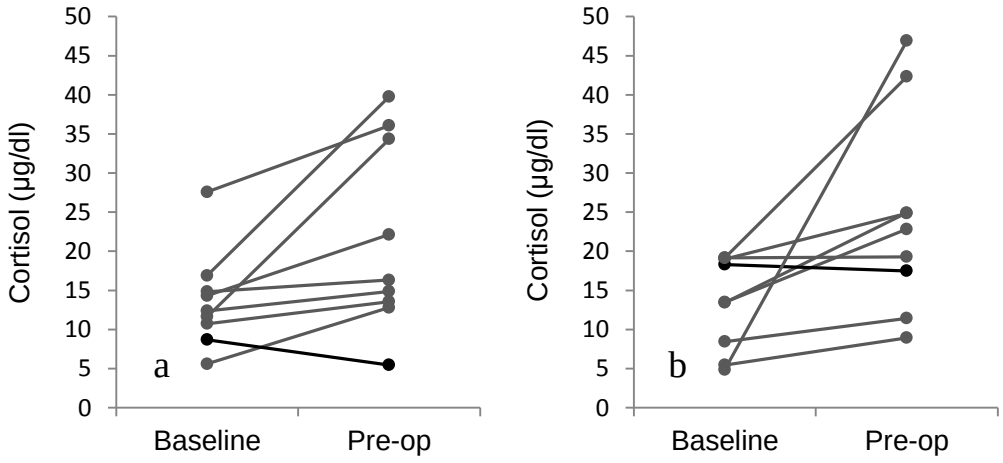


Figure 6.12: Individual participant changes in plasma cortisol between baseline and pre-operative reassessment for the control (a) and PREHAB (b) groups.

6.3.4. Hsp27 and Hsp70

A total of eight out of 10 participants in the PREHAB group had decreased plasma Hsp27 at pre-operative reassessment compared to baseline resulting in a

significant median decrease of -37.25 [IQR: 124.77] ng/ml (Z = -1.988; p = 0.047) (Figures 6.13 and 6.14 b). In the control group, plasma Hsp27 decreased in two participants, increased in five participants with negligible change (< 7 ng/ml) seen in the remaining two participants (Figure 6.14 a). The change between baseline and pre-operative reassessment in the control group was not significant (median difference: +15.04 [IQR: 96.27]; Z = -1.125; p = 0.260) however between group comparisons of median difference was (Z = -1.960; p = 0.050).

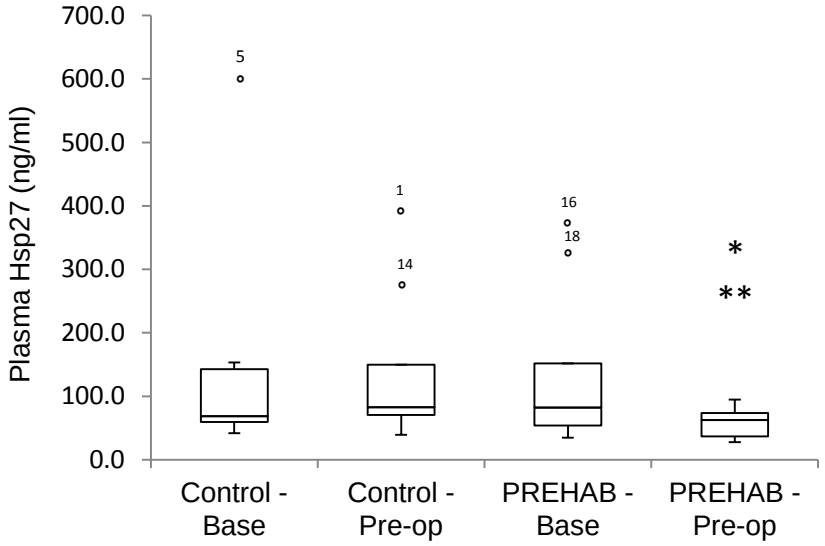


Figure 6.13: Plasma Hsp27 at baseline (Base) and pre-operative reassessment (Pre-op) in the control and PREHAB groups. Displayed as Median (IQR) [Range]; *Significantly different to baseline (p < 0.05), **Significantly different to control pre-operative reassessment (p < 0.05).

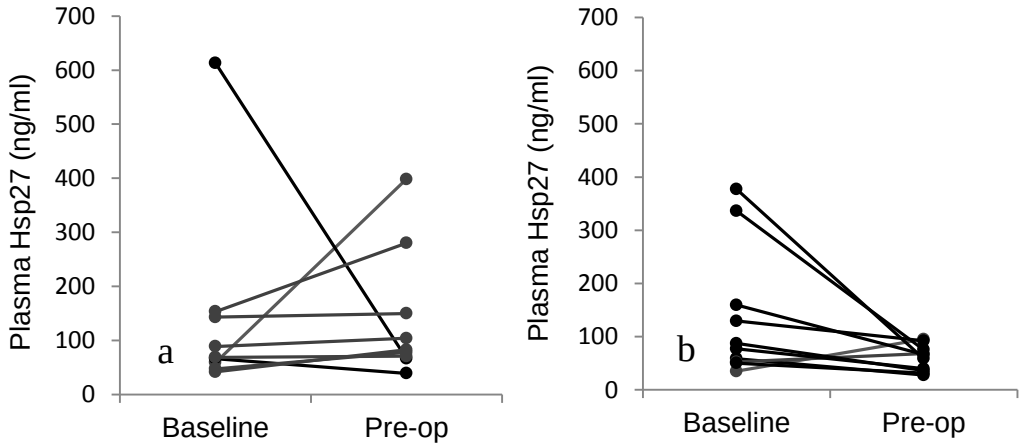


Figure 6.14: Individual participant changes in plasma Hsp27 between baseline and pre-operative reassessment for the control (a) and PREHAB (b) groups.

No detectable level of plasma Hsp70 was found in either group at baseline or at pre-operative reassessment.

6.3.5. Relationships between blood markers, 6MWT distance and length of stay

When all baseline and pre-operative reassessment data was pooled, no relationship was evident between leukocyte Hsp32 or Hsp72 expression and plasma levels of cortisol, Hsp27 or whole blood glutathione ($n = 32$; $r_s = 0.302$ to -0.290; $p > 0.05$). Furthermore, no relationship existed between plasma cortisol, Hsp27 and whole blood glutathione ($n = 36$; $r_s = 0.114$ to -0.269; $p > 0.05$). Finally, there was no relationship between any of the blood markers at pre-operative reassessment and pre-operative 6MWT distance or hospital LOS.

6.4. Discussion

Despite the improvements in physical functioning that were seen in chapter 5, participating in a short period of PREHAB prior to major elective colorectal surgery appears to be insufficient to induce adaptive changes in basal Hsp32 and Hsp72 expression (Figures 6.3 to 6.5). To the author's knowledge, this was the first study to investigate the effect a short period of PREHAB has on the expression of leukocyte Hsp32 and Hsp72 prior to colorectal surgery. Given the protective function Hsps play in defending against systemic and cellular stress (Kregel, 2002), manipulating their expression prior to surgery could aid in providing important protection against surgery induced stress (Madden et al., 2008). A short period of exercise training represents one preconditioning stimulus with the potential to induce such an adaptation to occur.

In individuals who have a long-term history of participation in physical activity and exercise, lower basal leukocyte Hsp72 levels have been reported compared to sedentary individuals (Fehrenbach et al., 2000b; Shastry et al., 2002), even in elderly populations (Simar et al., 2007). The presence of a downregulated oxidative potential and/or a subsequent improvement in antioxidant status following chronic exercise training have both been suggested as potential mechanisms behind these reductions (Fehrenbach et al., 2000b; Simar et al., 2007). In the current study, whilst monocyte Hsp72 expression decreased in six of the eight participants in the PREHAB groups at

pre-operative reassessment, seven out of eight participants in the control group also had decreased Hsp72 expression (Figure 6.5 a & b). The change therefore was probably related to something different than the PREHAB intervention with the presence of a time of day effect one of a number of possible contributors.

In human monocytes, diurnal variation in the expression of basal Hsp72 has previously been reported with Hsp72 expression positively correlated with core temperature (Sandstrom et al., 2009). In the current study, it was not feasible to ensure that baseline and pre-operative reassessment blood samples were taken at the same time of day. The time of the initial baseline assessment was varied between participants (Earliest: ~9:30 am; Latest: ~4:30 pm) as they were scheduled to coincide around their hospital clinic appointments. In contrast, all pre-operative blood samples were taken at approximately 7:30 am on the morning of surgery whilst participants were still in bed. The minimal amount of prior physical activity available on the morning of surgery is therefore likely to have limited any increases in Hsp72 expression due to elevations in core temperature. The consistent pattern of decreased Hsp72 expression at pre-operative reassessment in both groups however was only evident in monocytes and not lymphocytes or granulocytes (Figure 6.5 c-f). This potentially can be explained as the Hsp72 response to stress is reported to be more sensitive in monocytes than the other leukocyte cell types (Bachelet et al., 1998; Hillman et al., 2011; Peart, Kirk, Madden, Siegler, & Vince, 2012).

It may have been expected that rather than a decrease as observed in the current study, basal monocyte Hsp72 expression following PREHAB would have been elevated. Studies relating to thermal preconditioning have reported elevated basal levels of leukocyte Hsp72 expression following 3-10 days of heat acclimation with or without concurrent exercise training in healthy young adults (Magalhaes Fde et al., 2010; McClung et al., 2008; Yamada, Amorim, Moseley, Robergs, & Schneider, 2007). Furthermore, when exposed to a secondary stressor such as a heat shock, the inducibility of Hsp72 is blunted in heat acclimated individuals (Magalhaes Fde et al., 2010; McClung et al., 2008). It is proposed therefore that the presence of elevated basal leukocyte Hsp72 levels following heat acclimation provides increased cellular protection reducing the need for additional protein synthesis when exposed to a subsequent stressor (Morimoto, 1998). To date however, studies characterising whether a similar response occurs following a short term period of exercise training are absent.

Research relating to short term changes in basal Hsp32 and Hsp72 following exercise training is limited. Acutely, leukocyte Hsp32 and Hsp72 expression has been reported to be induced following exercise, although to what extent is thought to be intensity and duration dependent, with greater increases evident following high intensity exercise/prolonged periods of endurance exercise (Fehrenbach et al., 2003). The number of human studies identified relating to changes in basal leukocyte Hsp72 expression following exercise training is limited to three studies (Bautmans et al., 2005; Beltran Valls et al., 2014; Simar, Malatesta, Mas, Delage, & Caillaud, 2012) whilst the author has been unable to identify any existing studies relating to changes in Hsp32.

Bautmans et al. (2005) and Beltran Valls et al. (2014) both reported that a period of resistance training reduced basal Hsp70/72 expression in leukocytes. In Bautmans et al. (2005), 6 weeks of progressive resistance training (3 x 10 reps at 50% 1RM progressing to 3 x 10 reps at 70-80% 1RM of leg press, leg abductor, leg adductor, vertical traction, chest press and shoulder press) in elderly individuals (10 males [69.3 ± 5.6 years] and 21 females [68.0 ± 6.0 years]) was sufficient to induce a significant ~20% decrease ($p < 0.01$) in lymphocyte Hsp72 expression post-intervention but not monocytes (10% decrease; $p > 0.05$). Subsequent heat shock treatment of PBMCs isolated at the same time for 1 hour at 37°C and 42°C revealed increased Hsp72 expression post training compared to baseline for both monocytes and lymphocyte. This demonstrates a training effect on Hsp72 stress response existed. Beltran Valls et al. (2014) reported the basal expression of leukocyte Hsp70 and Hsp27 (as measured by Western blot) decreased by ~32% and ~50% respectively following 12 weeks of explosive-type resistance training (40-70% 1RM, 3-4 sets, 10-15 reps) compared to no change in the control group. The considerable differences in the intensity and interventions used in the aforementioned studies compared to the present study make direct comparisons difficult; however, they do demonstrate that a decrease in Hsp72 may have occurred following PREHAB.

In contrast, Simar et al. (2012) found eight weeks of aerobic training and vitamin C and E supplementation in elderly septuagenarians failed to decrease $_{m}$Hsp72 and $_{g}$Hsp72 expression, as determined by flow cytometry any more than vitamin C and E supplementation alone. The lack of either a placebo or training alone group does impact however on the interpretation of the study as it remains unclear whether exercise alone would impact on Hsp72 expression in the absence of supplementation. As none of

the aforementioned studies (Bautmans et al., 2005; Beltran Valls et al., 2014; Simar et al., 2012) measured Hsp72 after two to four weeks of training it remains unclear whether the decrease in Hsp72 was preceded by an initial increase as is seen following heat acclimation (Magalhaes Fde et al., 2010; McClung et al., 2008; Yamada et al., 2007).

If a change in basal expression of leukocyte Hsp32 and Hsp72 following a period of PREHAB was to be expected, the absence could be as a result of insufficient training stimulus having been present to promote physiological adaptations to occur. Identifying which specific stressors actually contribute to the induction of Hsps during exercise is difficult as many of the proposed stimuli are often present during acute exercise (Kregel, 2002). Elevations in core temperature, energy depletion, oxidative stress, disruption to acid base balance and ischaemia can all occur during exercise, all potentially contributing to Hsp32 and Hsp72 expression being induced. As a large proportion of the resistance exercises used within intervention were low intensity function based movements, it unknown whether the presence of up to 25 minutes of moderate intensity aerobic exercise (up to 60% HRR) alongside some higher intensity resistance exercises (e.g. push press, kettlebell swing: HR_{peak} reaching in excess of 80% HRR; Table 5.3) produced the required stimulus to induce Hsp expression. Ideally blood samples would have been taken before and after training at the start of the intervention, at the midpoint and the end of the intervention for each participant in the current study. This would have allowed it to be established firstly whether the PREHAB programme stimulated a sufficient disturbance to acutely induce Hsp32 and/or Hsp72 expression and secondly, whether training resulted in more chronic adaptations to occur.

As well as the time of day effect discussed earlier, the potential influence of the slight gender imbalance between groups, in addition to within participant differences in dietary intake and prior physical activity on the days of sampling cannot be dismissed as confounding variables as they have all been shown to potentially influence Hsp72 expression (Febbraio et al., 2004; Voss et al., 2003; Whitham, Walker, & Bishop, 2006). It is therefore acknowledged that an inability to control for these factors clearly represents a major limitation of the study. Unfortunately, these ultimately proved unfeasible to control when restricted by the need to arrange baseline and pre-operative testing around hospital appointments. This does mean any possible changes in basal

Hsp72 expression that had occurred as a result of the PREHAB intervention may have been masked within the decrease that was observed in both groups.

It has been proposed the adaptive response of Hsp72 expression following heat acclimation is biphasic; with an initial increase in basal Hsp72 followed by the development of an altered threshold at which subsequent Hsp72 transcription occurs (Maloyan et al., 1999). As the activity of Hsp72 mRNA was not measured in the current study whether any protection gained as a result of altered mRNA levels and transcription rates remains unknown. Fehrenbach et al. (2000a) did report that elevated levels of Hsp72 mRNA existed in trained individuals compared to those who are untrained. This supported previous heat acclimation studies (Horowitz et al., 1997; Maloyan et al., 1999), in that elevated basal Hsp72 mRNA potentially allows for more immediate transcription in the presence of a stressor (Fehrenbach et al., 2000a).

Whilst the potential for any changes having occurred as a result of PREHAB cannot be excluded, it seems less likely given that there was also no accompanying changes in either glutathione (total or GSSG/TGSH) or Hsp32 in leukocytes (Figures 6.3, 6.7 and 6.9). Changes in the GSSG/TGSH status have been reported to be a sensitive measure of oxidative stress within an individual, with high levels of GSSG representative of greater stress (Elokda & Nielsen, 2007). Therefore, increasing basal levels of glutathione and/or inducing a shift in GSSG/TGSH ratio towards a more reduced state prior to major surgery is an attractive proposition given the increased capacity to scavenge for free radicals that may exist.

Elokda and Nielsen (2007) reported a 6 week exercise intervention (3 x 40 minutes per week) consisting of either AET (40 minutes at 65-75% HR_{max}), CWT (five exercises, two sets of 15 repetitions at 50% 1RM) or CWT+AET all increased erythrocyte glutathione levels and decreased GSSG levels in sedentary healthy volunteers (aged 32.1 ± 7.3 years) compared to the control group. Furthermore, the combined CWT+AET programme had a greater effect on the GSSG/TGSH ratio than either the CWT or AET programmes alone. This was not replicated in the current study, despite similarities existing with the CWT+AET intervention used in Elokda and Nielsen (2007), as no change in whole blood total glutathione or GSSG/TGSH ratio was observed at pre-operative reassessment compared to baseline in either group. Differences in the source of glutathione (whole blood vs. erythrocyte), methodology and

assay used as well as participant age and health status are all possible reasons for the difference between studies. In Elokda and Nielsen (2007) any participants who failed to attend a minimum of 15 out of 18 sessions in the six week period were excluded from the study. In contrast, within the current study, the mean number of sessions attended was considerably lower (6.9 ± 2.3 session; Table 6.1) meaning a sufficient training stimulus may not have been present to elicit a meaningful change in this population.

Interestingly, plasma Hsp27 expression decreased in eight out of ten participants in the PREHAB group by pre-operative reassessment (Figure 6.13 b) resulting in a significant median reduction of -37.25 [IQR: 124.77] ng/ml. This was not replicated however in the control group (Figure 6.13 a). Whether the difference was related to the PREHAB intervention however is unclear as research relating to changes in plasma Hsp27 expression following exercise training is limited. Acute elevations in plasma Hsp27 were observed following both maximal cycling exercise (Jammes et al., 2012) and maximal static handgrip to exhaustion (Brerro-Saby et al., 2010), potentially reflecting Hsp27 translocation to the cytoskeleton in response to exercise induced stress (Mymrikov et al., 2011). In respects to more prolonged exercise, Beltran Valls et al. (2014) did observe a ~50% decrease in leukocyte Hsp27 expression (as measured by Western blot) following 12 weeks of resistance training, a figure similar to that seen in plasma Hsp27 (median: -50.7%) in the present study. Beltran Valls et al. (2014) attributed this decreased basal expression to repeated exposure to exercise stress facilitating physiological adaptations.

Of course, it is conceivable the change was unrelated to the PREHAB intervention as the tumour stage, status and previous treatment can all affect circulatory Hsp27. The presence of both elevated Hsp27 levels in tumour tissues (Bauer et al., 2012; Zhao et al., 2012) and in the serum (Schweiger et al., 2015; Zhao et al., 2014) of certain cancer patients has previously been documented. Furthermore, raised serum Hsp27 has been observed in patients with metastatic cancer compared to non-metastatic tumours (Zhao et al., 2014). In the current study, only three participants (Control: n = 1; PREHAB: n = 2) had metastatic cancer at the time of surgery, of which two had decreased expression (Control: n = 1; PREHAB: n = 1) and one elevated expression (PREHAB: n = 1) of Hsp27. It was also reported in Zhao et al. (2014) that the expression of serum Hsp27 was reduced in ovarian cancer patients following chemotherapy. Of the five participants (Control: n = 1; PREHAB: n = 4) to receive

NACRT prior to surgical treatment in the current study, three had decreased Hsp27 expression (all PREHAB) whilst two displayed increased levels (Control: n = 1; PREHAB: n = 1) post-treatment. The imbalance in participants receiving NACRT may have contributed to the decreased Hsp27 in the PREHAB group; however, due to the low sample size available, performing covariate models of analysis incorporating elements such as metastatic status and tumour stage as predictors of Hsp27 was beyond the scope of this pilot study.

The presence of these aforementioned confounding variables therefore make the clinical significance of the decreased Hsp27 expression in the PREHAB group difficult to interpret and highlights one of the problems of measuring extracellular Hsp expression in plasma or serum. Although changes in Hsp expression may be detected, the specific origin of its expression remains unknown (Madden et al., 2008). Finally it does need acknowledging that the limited sample size and influence of outliers in both groups (Figure 6.13) means the potential that results are a chance finding cannot be dismissed and therefore need interpreting with caution.

The significant increase in plasma cortisol levels at pre-operative reassessment that was observed in both groups in the current study (Figures 6.11 and 6.12) was consistent with the presence of the cortisol awakening response (CAR). Wilhelm, Born, Kudielka, Schlotz, and Wust (2007) reported a steep rise in serum cortisol level within 30 minutes of the participant being awoken existed with similar results having also been seen for salivary cortisol levels (Wust et al., 2000). As discussed earlier, the time of the early morning pre-operative samples (~7.30 am) therefore are likely to have coincided with this 30 minute time period of increased cortisol release. Furthermore, given that the CAR is reported to be heightened by the presence of psychological stress (Schlotz, Hellhammer, Schulz, & Stone, 2004), the response may have further been exaggerated given the stress that was likely caused by the imminent major surgery.

In the current study, when all the data was pooled from both the control and PREHAB group, no relationship was seen between the expression of any of the blood markers and either hospital LOS or 6MWT distance at pre-operative reassessment. This analysis however is obviously limited by the sample size and the presence of other confounding variables that were not controlled for. Significant positive relationships were observed between the different leukocyte cell populations for Hsp32 and Hsp72

expression (Figures 6.6 a-f). An apparent strict correlation has been reported in total Hsp72 expression in monocytes and lymphocytes from both apparently healthy male volunteers at rest and following exercise (Peart et al., 2012) as well as in chronic lymphocytic leukaemia and chronic myelomonocytic leukaemia patients (Madden et al., 2012). In Madden et al. (2012), it was proposed that this may have demonstrated that similar stress and tumour-associated pathways exist in the expression of Hsp72 between the different leukocyte cells. In the current study, this finding was extended with a positive monotonic relationship seen not only between total Hsp32 and Hsp72 in monocytes and lymphocytes but also with granulocytes (Figure 6.6 g-i). Furthermore, positive monotonic relationships were observed between Hsp32 and Hsp72 expression in all three cell types suggesting that the expression of both stress proteins were linked by common regulatory mechanisms within this sample population (Njemini et al., 2007). There was no relationship between any of the remaining blood markers.

In summary, undergoing a two to six week period of PREHAB prior to major elective surgery appears insufficient to elevate basal levels of Hsp32 and Hsp72 in leukocytes or whole blood total glutathione levels. Interestingly though, plasma Hsp27 levels had decreased significantly by pre-operative reassessment in the PREHAB group whilst no change was evident in the control group. The potential clinical relevance of this finding is unclear.

Chapter 7: Inducibility of Hsp72 expression to heat shock in cancerous and non-cancerous cell lines and in PBMCs from colorectal cancer patients.

7.1. Introduction

In chapter 6, an increase in the basal expression of Hsp72 in leukocytes following a short period of PREHAB compared to a control group prior to major elective colorectal surgery was not evident. Despite this, it is plausible that participation may have improved the inducibility of Hsp72 expression when exposed to a subsequent stressor. The transcription of Hsp72 mRNA in human leukocytes following 2 hours heat shock at 42°C has previously been reported to be higher in trained endurance runners (mean age: 32.3 ± 9.3 years) compared to sedentary controls (mean age: 45.4 ± 11.4 years) (Fehrenbach et al., 2000a). These elevated transcription levels of Hsp72 mRNA are postulated to facilitate the immediate translation of Hsp72 in response to a subsequent stressor (Fehrenbach et al., 2000a). Furthermore, although the inducibility of Hsp72 has been reported to be attenuated in heat stressed (42°C) human monocytes and lymphocytes of individuals of older age (mean age: 75 ± 3.4 years) compared to those of younger age (mean ages: 31.4 ± 6.1 years and 50.2 ± 7.2 years respectively) (Njemini et al., 2002), this decline in the ability to induce Hsp72 is potentially counteracted by maintaining physical activity levels during ageing (Simar et al., 2004). Whether such changes in the inducibility of Hsp72 can occur following a short period of PREHAB however is unknown.

Any changes in the inducibility of Hsp72 however are likely to be influenced by the basal expression levels already present. Vince et al. (2010) demonstrated the ability to induce further Hsp72 expression was proportional to pre-existing basal levels in the monocytes of healthy male volunteers. Moreover, the stress response has been reported to be attenuated in human leukocytes with elevated basal Hsp72 expression following a period of heat acclimation (Lee, Mackenzie, Cox, James, & Thake, 2015; McClung et al., 2008). The raised Hsp72 is therefore suggested to be providing increased tolerance to a subsequent stressor negating the need for further expression (Madden et al., 2008). Whilst in healthy cells the expression of Hsp72 is reported to play an important protective role against a variety of different stressors including heat (McClung et al., 2008) and oxidative stress (Taylor et al., 2012), the same protective mechanisms mean the presence of elevated Hsp72 levels within tumour cells can conveys an anti-apoptotic and anti-senescent effect on the tumour increasing its resistance to cancer therapies such as chemo radiotherapy (Grivicich et al., 2007; Rashmi et al., 2004).

Therefore two main aims existed within this chapter. The first aim was to investigate whether participation in PREHAB increased the inducibility of Hsp72 expression in monocytes exposed to a subsequent period of non-lethal heat shock. The second aim of the chapter was to further examine the effect of basal Hsp72 expression on subsequent induction following non-lethal heat shock (40°C) for 90 and 180 minutes. In order to have a variety of basal levels of Hsp72, the heat shock was performed on a variety of cancer (Colo30, A2780, Jurkats) and non-cancer (HUVEC) cell lines as well as human colorectal cancer patient PBMCs.

7.2. Methods

7.2.1. Ethical Approvals and participants

The ethical approvals and participant details for the PBMCs used in this chapter were as described in chapter 5, sections 5.2.1 and 5.2.2.

7.2.2. Culture and maintenance of cell lines

Three different cancer cell lines and one non-cancerous cell line were used to investigate the in vitro Hsp72 response to heat stress. The cancer cell lines were:

- A2780 – a human ovarian cancer cell line established from tissue obtained from an untreated patient (Behrens et al., 1987).
- Colo320 – a moderately undifferentiated human colon adenocarcinoma cell line originating from the tumour of a 55 year old Caucasian female (Quinn, Moore, Morgan, & Woods, 1979).
- Jurkat – a human leukemic T-lymphocyte cell line established from the peripheral blood of a 14 year old boy (Schneider, Schwenk, & Bornkamm, 1977).

All cancer cell lines were maintained in 20 ml of RPMI1640 medium (Lonza, Cambridge, UK) supplemented with 1% penicillin/streptomycin, 1% $_{L}$-glutamine and 10% (v/v) FBS (all Lonza) using 75cm^2 tissue culture flasks (Sarstedt, Leicestershire, UK). Cells were stored at 37°C in a 5% CO_2 humidified incubator with complete growth medium changed three times per week. All cell lines were sub-cultured in the

log phase when 70-80% confluent in order for continued growth to occur. Cells were physically detached from the flask surface using a cell scraper (Sarstedt) into the media and centrifuged at 300 x *g* for 5 minutes to pellet the cells. The supernatant was discarded and the cells resuspended in fresh RPMI1640 medium (1 ml per new flask) and transferred to new 75cm^2 tissue culture flasks containing 20 ml of media each).

Human umbilical vein endothelial cells (HUVEC), which were used as a non-cancer cell line, were maintained in 5 ml endothelial cell growth medium, supplemented with 0.02 ml.ml FBS, 0.004 ml.ml endothelial cell growth supplement, 0.1 ng.ml epidermal growth factor, 1 ng.ml basic fibroblast growth factor, 90 µg.ml heparin and 1 µg.ml hydrocortisone (all Promocell, Heidelberg, Germany) using 25cm^2 tissue culture flasks for sensitive adherent cells (Sarstedt). As with the cancer cell lines, HUVEC were incubated at 37°C in a 5% CO_2 humidified incubator with complete growth medium changed three times per week. The detachment of the HUVEC from the flask surface was performed using trypsinisation. The medium was removed from the flask and the cell layer rinsed with 2 ml of PBS. Trypsin-EDTA solution (2 ml; Sigma-Aldrich) was added to the flask which was then gently swirled around ensuring all cell were covered. The flask was incubated for 5-10 minutes at 37°C in 5% CO_2 incubator whilst the cells disassociated before 2 ml of complete growth medium was added to neutralise the actions of the trypsin. Samples were centrifuged at 300 x *g* for 5 minutes to pellet the cells and the supernatant discarded. Cells were then re-suspended in the required volume of fresh endothelial cell growth medium (1 ml per flask) and transferred to new flasks, each containing a further 5 ml of medium.

7.2.3. Experimental design

The heat shock procedure in this chapter involved incubating the cell lines and PBMCs at either 37°C or 40°C for either 90 or 180 minutes. Incubation times of 90 and 180 minutes were chosen as this period represented the typical range of duration for most colorectal resection surgeries at Castle Hill Hospital (personal communication). The use of 37°C represented at normal homeostatic core temperature in the humans whilst 40°C has been shown to induce the maximal heat shock response immediately following exposure in leukocytes (Lovell, Madden, Carroll, & McNaughton, 2007) whilst being non-fatal. The 90 and 180 minute heat shock interventions were performed on different days for the cancer cell lines (Colo320, A2780 and Jurkat cell lines) whilst

both conditions were performed on the same day for the HUVEC and PBMCs. Experiments on A2780, Colo320 and Jurkats cell lines were performed in triplicate whilst the HUVEC experiment was performed in duplicate. Due to financial constraints, cell Hsp72 expression was not measured prior to incubation therefore the 37°C condition was the control condition.

i. Heat shock procedure for A2780, Colo320 and Jurkats,

On the morning of the experiments, the cell lines (Colo320, A2780 and Jurkat) were removed from their cell culture flasks centrifuged at 400 x *g* for 3 minutes and resuspended in 3 ml of RPMI medium pre-heated to 37°C. From the Colo320, A2780 and Jurkat solution, 2 ml was added to a 75 cm^2 tissue culture containing fresh prepared media and returned to the incubator for further growth. The remaining 1 ml was divided into two 500 µl aliquots and transferred into 2 ml round bottom polypropylene tubes (Sarstedt) before being added to a preheated heating block (Stuart SBH130DC, SciLabware, Staffordshire, UK) and incubated in either of the following conditions:

- 90 minutes at either 37°C (± 0.1°C) or 40°C (± 0.1°C)
- 180 minutes at either 37°C (± 0.1°C) or 40°C (± 0.1°C)

Following incubation, the expression of $_{m}$Hsp72 was measured via flow cytometry using the methods described in section 7.2.5.

ii. Heat shock procedure for HUVEC and PBMC

On the morning of use, PBMCs isolated from colorectal patients in chapter 6 (section 6.2.4) were removed from the liquid nitrogen and slowly defrosted at room temperature. Once defrosted, cells were transferred to a 50 ml tube and 10 ml of RPMI 1640 media slowly drip added. Cells were then centrifuged at 400 x *g* and resuspended in 2 ml of RMPI 1640 medium pre-heated to 37°C. Cell viability was assessed using the trypan blue exclusion test (section 7.2.3). The HUVEC were removed from their cell culture flasks as described in section 3.7.9 and resuspended in 2 ml of RPMI 1640 medium pre-heated to 37°C. Each sample was divided into four 500 µl aliquots and transferred into 2 ml round bottom polypropylene tubes (Sarstedt). One of each aliquot

was then added to the preheated heating block and incubated in the following conditions:

- 90 minutes at either 37°C (± 0.1°C) or 40°C (± 0.1°C)
- 180 minutes at either 37°C (± 0.1°C) or 40°C (± 0.1°C)

Following incubation, the expression of $_{m}$Hsp72 was measured via flow cytometry using the methods described in section 7.2.5.

7.2.4. Cell viability

Cell viability was measured at different times in the cell lines and the PBMC using a trypan blue exclusion assay test. In the cell lines, cell viability was performed prior to and following heat shock for 90 and 180 minutes in order to establish whether cell death occurred during the heat shock procedure. In the PBMC, viability was measured following the thawing of samples prior to heat shock. This was to check the extent that the freeze/thaw cycle had resulted in cell death.

The trypan blue exclusion assay test works on the principle that viable cells have intact membranes which cannot be permeated by the dye whilst the damaged membrane of dead cells allows the cytoplasm to be dyed blue (Strober, 2001). To perform the trypan blue exclusion test, 10 µl of cell suspension (cell lines/PBMC) was transferred to a 0.5 ml polypropylene tube (Sarstedt) and 10 µl of 0.4% trypan blue (Sigma-Aldrich) added. Following a gentle mixing to obtain a homogenous cell suspension, 10 µl of the suspension was added to the counting chamber under the cover slip (via capillary action) on a Hawksley haemocytometer (Hawksley, Sussex, UK). Percentage viability was determined by counting the viable and dead cells located in the large central gridded square using the 10X objective of a phase contrast microscope.

7.2.5. Heat shock protein 72 analysis

For the analysis of Hsp72 in the PBMC and the cell lines, cells were separated from the media by centrifuging them at 400 x *g* for 3 minutes. Cells were then washed twice in 2 ml PBS and centrifuged at 400 x *g* for 3 minutes. Cells were incubated in 100 µl of fixation medium (Leucoperm Reagent A, AbD Serotec) for 15 minutes, washed in

2 ml PBS and then the cell pellet resuspended in 100 µl of permeabilsation buffer (Leucoperm Reagent B, AbD Serotec). The sample was then divided into two 50 µl samples and 4 µl of either monoclonal Hsp72:FITC (C92F3A-5) antibody (Enzo Life Sciences) or negative control (AbD Serotec) added and then incubated for 30 minutes in the dark. Following incubation, samples were washed in 2 ml PBS before being re-suspended in 300 µl of PBS ready for analysis via flow cytometry. All cells were gated by forward scatter/side scatter properties and with a total of 10,000 events counted. Results were calculated as MFR gained with the anti-Hsp antibody compared to that of the isotype matched negative control.

7.2.6. Statistical analysis

When normal distribution could not be assumed due to small samples, central tendency and dispersion are displayed as both mean ± standard distribution and median (IQR). Between groups analysis was completed using either Independent Sample T-Tests for normal distribution or Mann-Whitney U Tests for non-normally distributed data. Within cell changes following heat shock was performed using the Wilcoxon Rank-Sum test due to the non-normal distribution of the relevant data. The relationship variables were assessed using either Pearsons r or Spearman rho correlations depending on normality of distribution. Statistical significance was set at $p < 0.05$.

7.3. Results

7.3.1. Hsp72 stress response to heat shock in cancer and HUVEC cell lines

Cell viability remained consistent for all cell lines after both 90 and 180 minutes exposure at 37°C and 40°C. Mean viability following 90 minutes incubation was 92.2 ± 4.4% at 37°C and 92.0 ± 4.4% at 40°C. Following 180 minutes incubation, viability was 91.0 ± 5.0% at 37°C and 90.0 ± 6.8% at 40°C.

Following a 90 minute incubation period, seven out of the 11 experiments conducted (A2780: 2 out of 3 experiments, Colo320: 3 out of 3 experiments, Jurkat: 1 out of 3 experiments, and HUVEC: 1 out of 2 experiments) reduced in Hsp72 expression in the 40°C condition compared to 37°C whilst three increased (Jurkat: 1 out of 3 experiments, HUVEC: 1 out of 2 experiments) and one did not change (Jurkat: 1

out of 3 experiments) (Figure 7.1). In the two lowest Hsp72 expressing cell lines (Jurkat & HUVEC), individual changes in MFR at 40°C ranged between -0.28 (-5.9%) to + 0.30 (6.4%) of the MFR at 37°C resulting in a mean MFR almost identical in both conditions (Table 7.1). In contrast, in the two cell lines (A2780 and Colo320) with the highest Hsp72 expression at 37°C, mean MFR decreased by 0.67 MFR (7.1%) and 1.25 MFR (13.7%) respectively at 40°C with five out of six experiments displaying reduced expression. When all data was combined, median MFR was approximately 4.2% lower for the 40°C condition compared to 37°C condition however the median difference in MFR of -0.27 (IQR: 0.87) was not significant (Z = -1.580; $p = 0.114$). A significant negative correlation was seen between MFR at 37°C and change in MFR at 40°C ($r_s = -0.755$; $p = 0.007$; Figure 7.1).

Table 7.1: Hsp72 expression (MFR) for A2780, Colo320, Jurkat and HUVEC cell lines following incubation at 37°C and 40°C for 90 minutes.

		37°C	40°C	Mean/Median difference (95% CI)	*p* value
A2780 (n = 3)	Mean ± SD	9.50 ± 2.56	8.83 ± 1.71	-0.67 (-3.40; 2.06)	0.360[a]
	Median	10.19	9.74	-0.29	
Colo320 (n = 3)	Mean ± SD	9.14 ± 3.35	7.89 ± 3.09	-1.25 (-3.64; 1.14)	0.153 [a]
	Median	8.50	6.32	-1.31	
Jurkat (n = 3)	Mean ± SD	4.12 ± 0.48	4.13 ± 0.39	0.01 (-0.47; 0.49)	0.957 [a]
	Median	4.18	4.00	0.00	
HUVEC (n = 2)	Mean ± SD	4.74 ± 0.06	4.75 ± 0.35	0.01 (-3.67; 3.69)	0.978 [a]
	Median	4.74	4.75	0.01	
Combined (n = 11)	Median (IQR)	6.15 (5.62)	5.89 (5.62)	-0.27 (1.50)	0.114[b]

Mean ± SD and median (IQR); [a]Paired Sample T-Test ($p < 0.05$); [b]Wilcoxon Signed Rank Test

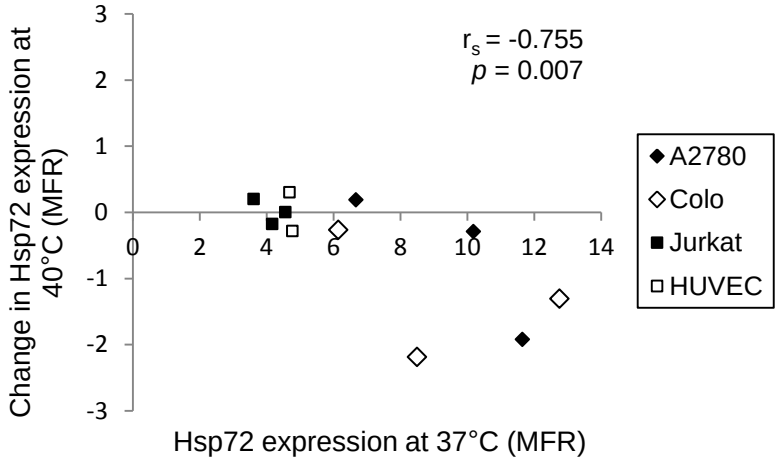


Figure 7.1: Change in Hsp72 expression for A2780, Colo320, Jurkat and HUVEC cell lines following 90 minutes of heat shock at 40°C in relation to expression following incubation at 37°C. MFR: median fluorescence ratio

When incubated at 40°C for 180 minutes compared to 37°C, Hsp72 expression increased in eight out of the11 experiments (A2780: 2 out of 3 experiments, Colo320: 3 out of 3 experiments, Jurkat: 1 out of 3 experiments, and HUVEC: 2 out of 2 experiments) (Figure 7.2). Mean MFR for the 40°C condition increased for all cell lines except the Jurkat (Table 7.2) which accounted for two of the three experiments to display a decreased Hsp72 following a 180 minute incubation at 40°C. Of the other cell lines (A2780, Colo320 and HUVEC), all displayed an increased expression at 40°C, with the exception of one A2780 experiment were MFR decreased marginally by 0.16 (2.6%) MFR. The remaining A2780 experiments increased by 1.43 (23.1%) MFR and 0.38 (4.7%) MFR respectively. When all data was combined, although median Hsp72 expression was 4.3% lower at 40°C than the 37°C condition, an increase in median difference of 0.59 (IQR: 1.23) MFR was seen at 40°C although this was not significant ($Z = -1.689$; $p = 0.091$). No significant relationship was seen between MFR at 37°C and change in MFR at 40°C when incubated for 180 minutes ($r_s = 0.300$; $p = 0.370$; Figure 7.3).

Table 7.2: Hsp72 expression (MFR) for A2780, Colo320, Jurkat and HUVEC cell lines following incubation at 37°C and 40°C for 180 minutes.

		37°C	40°C	Mean/Median difference (95% CI)	*p* value
A2780 (n = 3)	Mean ± SD	6.79 ± 1.10	7.34 ± 1.27	0.55 (-1.46; 2.56)	0.360[a]
	Median	6.21	7.64	0.38	
Colo320 (n = 3)	Mean ± SD	7.45 ± 0.64	8.50 ± 0.69	1.05 (0.92; 1.19)	0.001[a]
	Median	7.36	8.44	1.07	
Jurkat (n = 3)	Mean ± SD	5.62 ± 1.04	5.05 ± 0.68	-0.57 (-4.65; 3.52)	0.611[a]
	Median	5.72	5.42	-0.25	
HUVEC (n = 2)	Mean ± SD	4.01 ± 1.27	4.56 ± 0.49	0.56 (0.24; 0.87)	0.029[a]
	Median	4.01	4.56	0.56	
Combined (n = 11)	Median (IQR)	6.21 [2.83]	5.94 [3.52]	0.59 (1.23)	0.091[b]

Mean ± SD and median (IQR); [a]Paired Sample T-Test ($p < 0.05$); [b]Wilcoxon Signed Rank Test

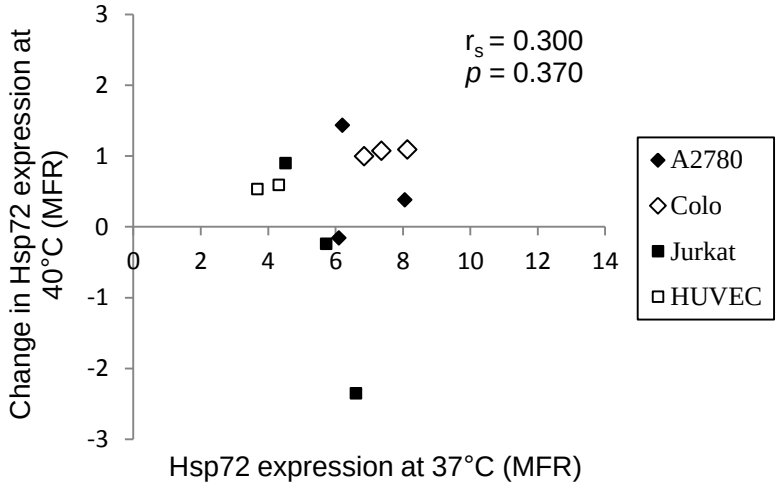


Figure 7.2: Change in Hsp72 expression for A2780, Colo320, Jurkat and HUVEC cell lines following 180 minutes of heat shock at 40°C in relation to expression following incubation at 37°C. MFR: median fluorescence ratio

Median change in MFR after the 180 minute incubation period (median: 0.53 [IQR: 1.23] MFR) was significantly greater than that of the 90 minute incubation period (median: -0.27 [IQR: 1.5] MFR; Z = -2.659; *p* = 0.008). Regardless of whether

incubated for 90 or 180 minutes, Hsp72 expression was significantly positively correlated with that at 40°C (Figures 7.3 a and b).

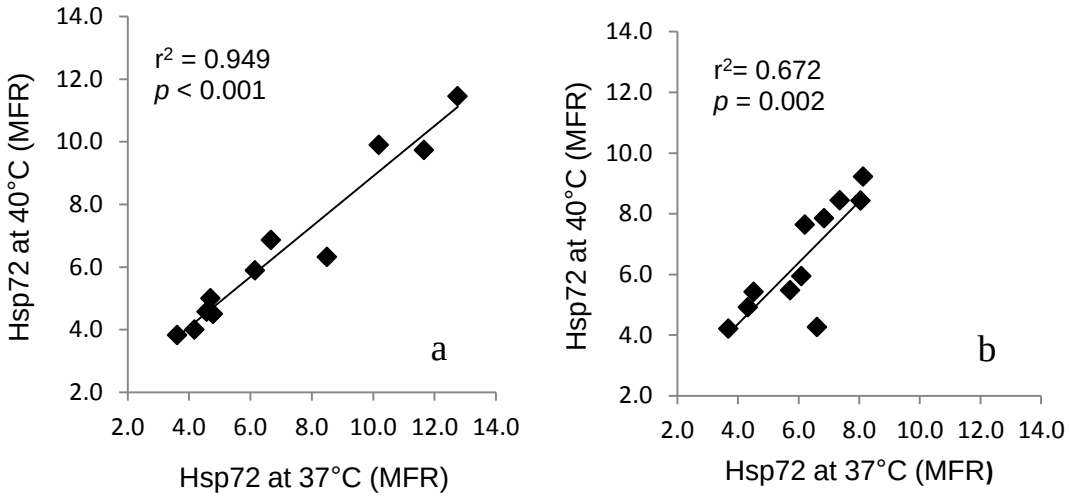


Figure 7.3: Heat shock responses of all cell lines combined at 40°C in relation to Hsp72 expression following incubation at 37°C for 90 (a) and 180 (b) minutes respectively. MFR: median fluorescence ratio

7.3.2. Effect of PREHAB on the inducibility of Hsp72 in PBMC following 90 and 180 minutes heat shock at 37° and 40°C.

Although baseline and pre-operative reassessment PBMC were collected and stored from 16 patients, following the defrosting process the number of viable samples was reduced to 9 patients (Control: n = 5; PREHAB: n = 4). In addition to these, two patients had viable baseline only samples (Figure 7.4). Of the remaining samples following thawing, cell viability was >80% prior to incubation. With the exception of the pre-op sample for the PREHAB group following 90 minutes incubation at 40°C, normal distribution of the data could not be assumed plausible therefore non-parametric analysis was used.

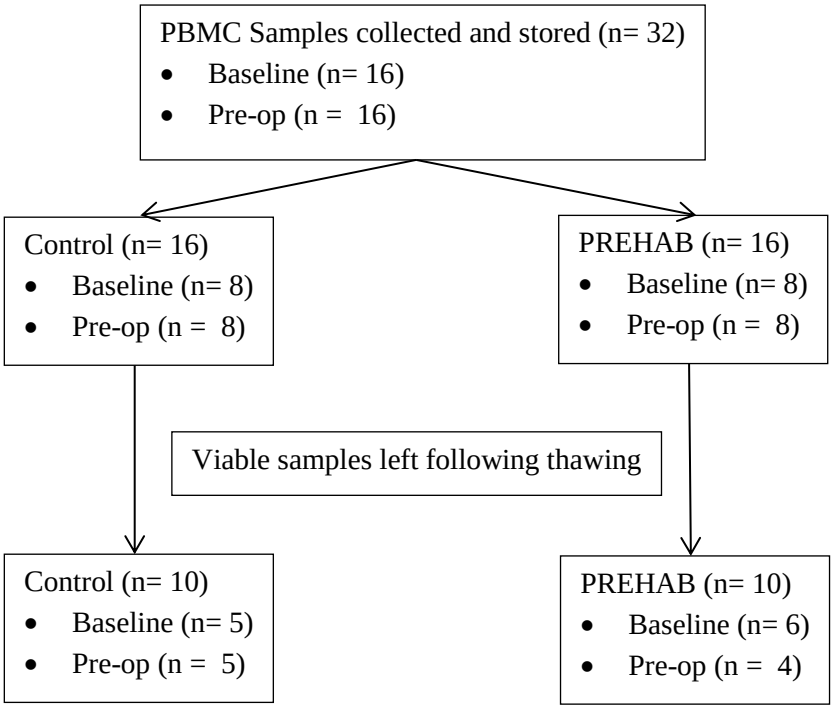


Figure 7.4: Flow of PBMCs available for analysis from the control and PREHAB groups following thawing.

In the control group, median MFR at 37°C following 90 minutes incubation was higher in four of the five patients at pre-op reassessment compared to baseline although the difference was not significant (Z = -1.753; p = 0.080; Table 7.3 and Figure 7.5 a & b). For all other comparisons of Hsp72 expression at baseline assessment compared to pre-operative reassessment following incubation at 37°C (Table 8.3 and Figures 8.5 and 8.6), the number of samples with either increased or decreased Hsp72 expression was similar (Z = -0.365 to -1.433; all $p > 0.05$). Following heat shock at 40°C, Hsp72 expression increased in four out of five patients in the control baseline 90 minute group, three out of four patients in the PREHAB pre-op 180 minute group and decreased in four out of five patients in the control pre-op 90 minute group compared to the 37°C condition. Despite this, no significant difference was observed in the expression of Hsp72 between 37°C and 40°C conditions following 90 or 180 minutes incubation in either the control or PREHAB group at baseline or pre-operative reassessment (Z = -0.365 to -1.753; all $p > 0.05$; Table 7.3).

Table 7.3: Hsp72 expression following 90 and 180 minutes incubation at either 37°C or 40°C in PBMCs from the control and PREHAB group.

90 minutes incubation period					
		At 37°C (MFR)	**At 40°C (MFR)**	**Median difference (MFR)**	***p* value**
Control	Base (n = 5)	4.07 (2.95) [1.08; 5.33]	4.68 (3.40) [2.05; 8.58]	0.97 (2.28) [-0.75; 3.25]	0.138
	Pre-op (n = 5)	5.05 (1.45) [3.52; 6.04]	4.64 (2.12) [1.69; 5.19]	-1.32 (1.53) [-1.84; 0.28]	0.080
PREHAB	Base (n = 4)	4.79 (2.37) [2.11; 5.09]	3.95 (3.84) [2.62; 7.37]	0.24 (3.15) [-1.70; 2.32]	0.715
	Pre-op (n = 4)	3.43 (2.42) [2.33; 5.38]	3.66 (2.27) [1.89; 4.66]	-0.17 (0.94) [-0.72; 0.35]	0.465
180 minutes incubation period					
		At 37°C (MFR)	**At 40°C (MFR)**	**Median difference (MFR)**	***p* value**
Control	Base (n = 5)	3.82 (1.60) [2.58; 4.66]	3.92 (0.92) [3.00; 4.53]	0.42 (1.13) [-0.73; 0.71]	0.686
	Pre-op (n = 5)	3.96 (0.48) [3.59; 4.22]	4.11 (1.61) [3.40; 5.28]	0.39 (1.26) [-0.48; 1.23]	0.345
PREHAB	Base (n = 4)	4.39 (2.74) [2.33; 5.42]	3.80 (1.68) [2.46; 4.66]	-0.23 (1.51) [-1.57; 0.20]	0.465
	Pre-op (n = 4)	4.24 (2.51) [2.07; 5.28]	4.94 (2.61) [2.03; 5.47]	0.31 (0.82) [-0.04; 0.97]	0.144

Median (IQR) [Range]; Base: Sample taken at entry to trial; Pre-op: Sample taken at pre-operative reassessment; MFR: median fluorescence ratio

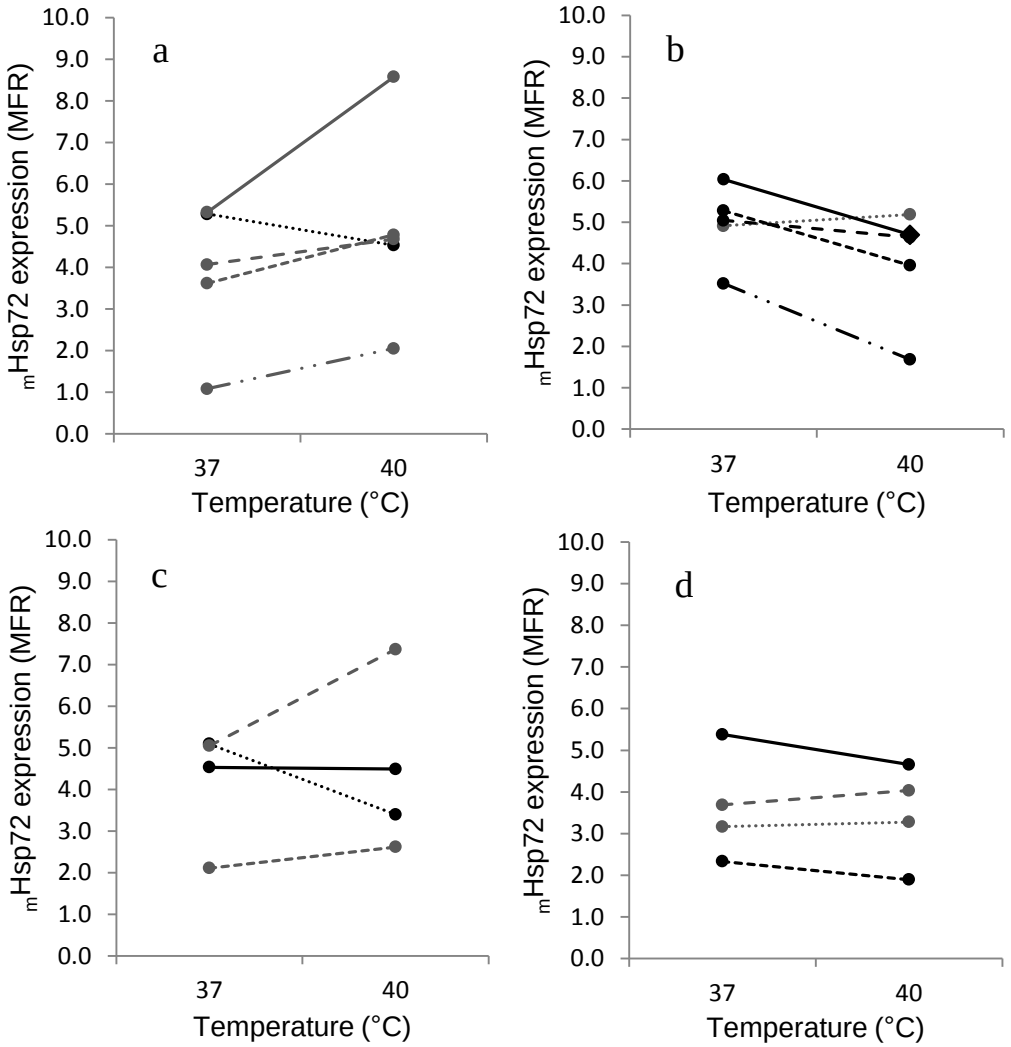


Figure 7.5: Individual participant changes in monocyte Hsp72 response following 90 minutes heat shock at 40°C compared to 37°C. a: Control baseline; b: Control pre-operative reassessment, c: PREHAB baseline, d: PREHAB pre-operative reassessment). Grey lines represent PBMC with increased expression at 40°C and black lines decreased expression.

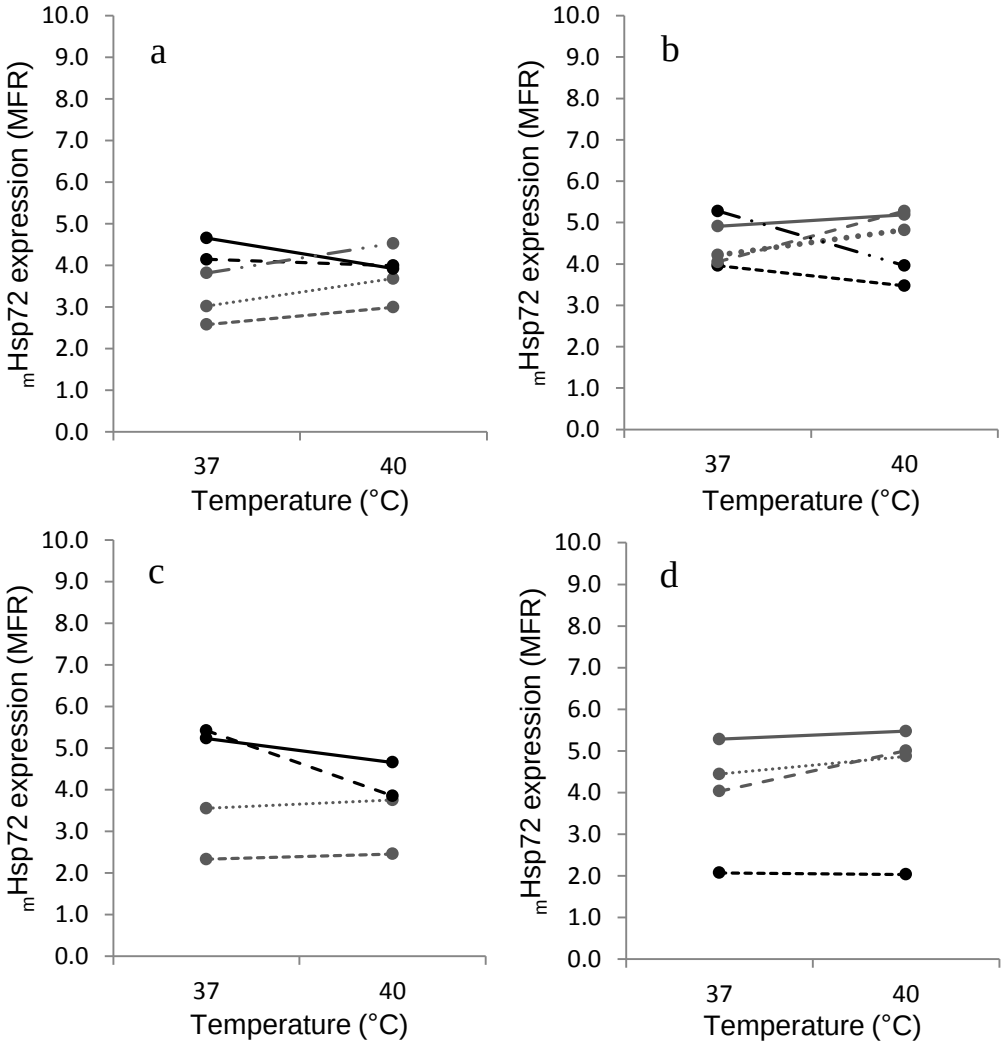


Figure 7.6: Individual participant changes in monocyte Hsp72 response following 180 minutes heat shock at 40°C compared to 37°C. a: Control baseline; b: Control pre-operative reassessment, c: PREHAB baseline, d: PREHAB pre-operative reassessment). Grey lines represent PBMC with increased expression at 40°C and black lines decreased expression.

To examine effect the role of basal Hsp72 levels on subsequent induction all samples were pooled and analysed combined. Following 90 minutes incubation, Hsp72 expression increased at 40°C in 10 samples and decreased in 10 samples compared to the 37°C condition whilst following 180 minutes incubation 12 increased and 8 decreased. This meant there was no significant difference in Hsp72 expression at 40°C compared with 37°C following incubation for either 90 and 180 minutes ($Z = -0.037$; $p = 0.970$ and $Z = -1.176$; $p = 0.240$ respectively; Table 7.4). There was no relationship evident between the change in Hsp72 expression at 40°C and expression at 37°C following either 90 or 180 minutes (Figure 7.7 a & b). Hsp72 expression at 40°C was significantly positively correlated with expression at 37°C following incubation for both 90 and 180 minutes (Figure 7.7 c & d).

Table 7.4: Hsp72 expression for control and PREHAB groups combined following incubation at 37°C and 40°C for 90 and 180 minutes.

	At 37°C (MFR)	At 40°C (MFR)	Median difference (MFR)	*p* value
90 min	4.68 (1.98)	4.51 (1.98)	0.04 (1.61)	
(n = 20)	[1.08; 6.04]	[1.32; 8.58]	[-1.84; 3.25]	0.970
180 min	4.00 (1.45)	3.96 (1.44)	0.20 (0.77)	
(n = 20)	[1.66; 5.42]	[1.58; 5.57]	[-1.57; 1.23]	0.240

Median (IQR) [Range]; MFR: median fluorescence ratio

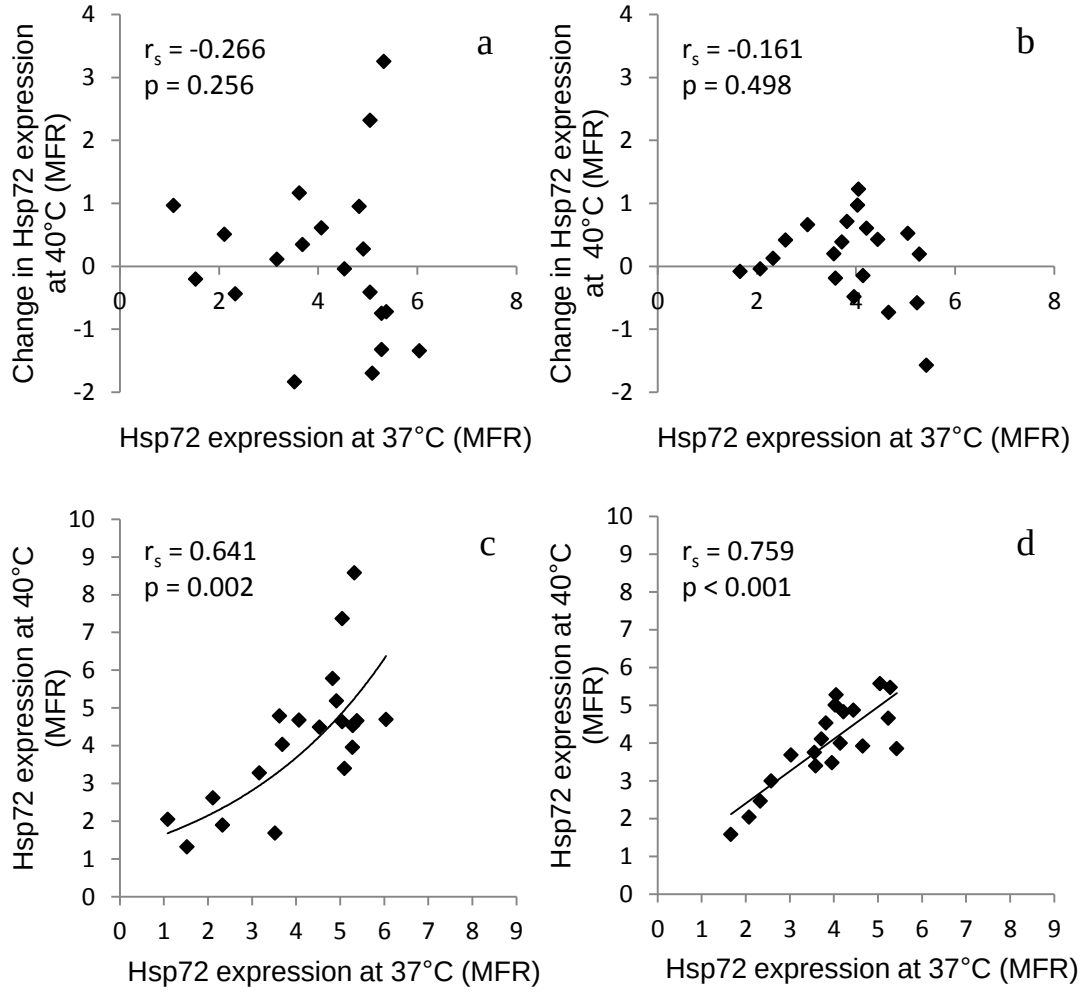


Figure 7.7: Change in Hsp72 expression in colorectal cancer patients PBMCs following 90 (a) and 180 (b) minutes of heat shock at 40°C in relation to expression following incubation at 37°C. Relationship between Hsp72 expression at 37°C and 40°C following incubation for either 90 (c) and 180 (d) minutes.

There was no significant difference in Hsp72 expression following 180 minutes incubation at 37°C compared with 90 minutes at 37°C (Z= -0.859; *p* = 0.390; Table 7.4). However, a significant negative relationship existed between the Hsp72

expression at 90 minutes and the subsequent change in expression at 180 minutes (Figure 7.8 a & b).

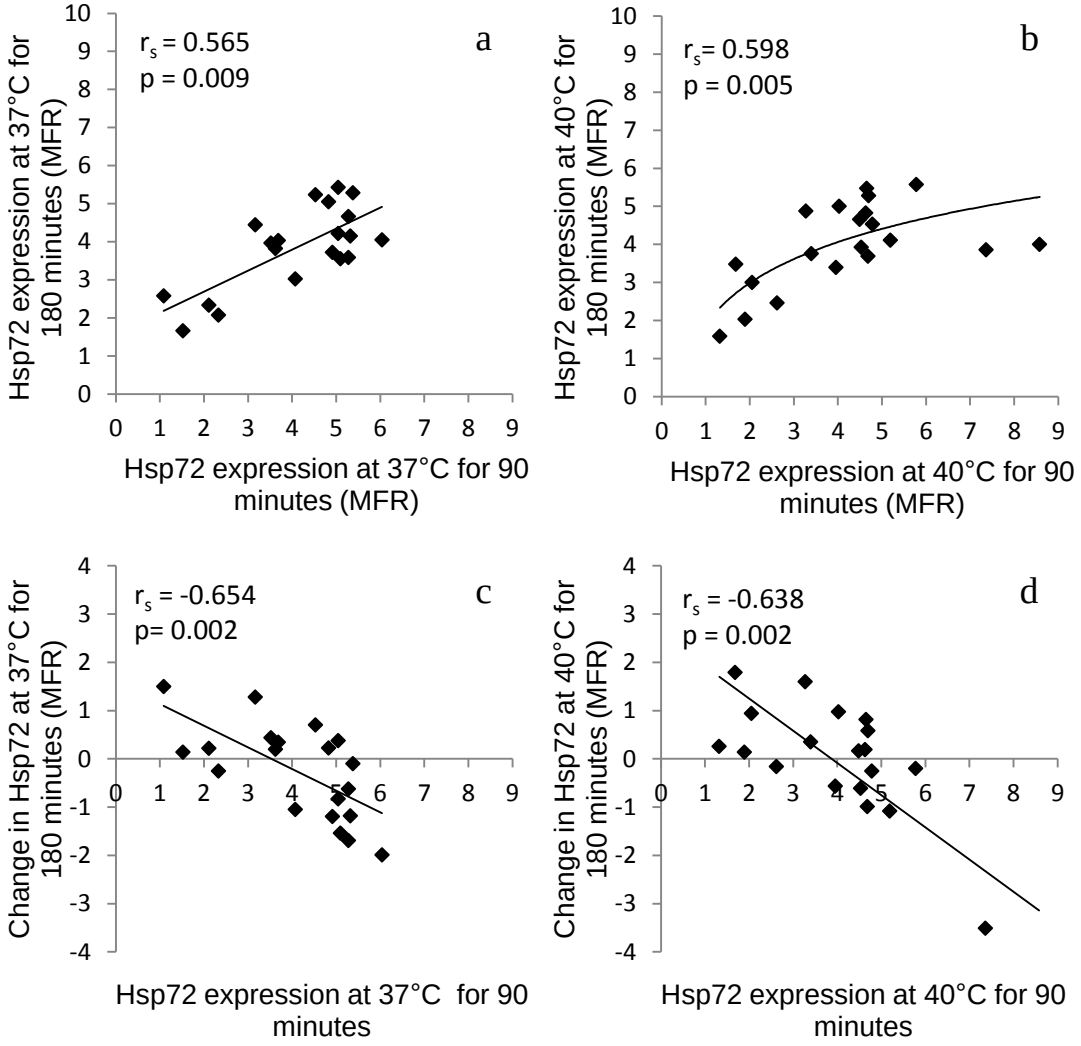


Figure 7.8: Relationship between the Hsp72 expression (MFR) (a and b) and change in Hsp72 expression (c and d) in colorectal cancer patients PBMCs following 180 minutes incubation at either 37°C or 40°C compared to 90 minutes.

7.4. Discussion

The main findings within this final experimental chapter were as follows:

1). In cancer cell lines, where Hsp72 expression is likely to be elevated, only a more sustained period of sub-lethal heat stress (40°C) appears sufficient to induce further expression above that of the control condition (37°C) (Figures 7.1 & 7.2). A similar

stress response however was not evident in the PBMC from colorectal cancer patients (Figures 7.7 a & b).

2). In PBMCs, a significant negative relationship existed between Hsp72 expression following incubation at a given temperature (37 or 40°C) for 90 minutes and the subsequent change in expression following 180 minutes at the same temperature (Figure 7.8 a & b).

3). A positive relationship between Hsp72 expression at 37°C and 40°C existed in both cell lines and PBMCs following incubation for both 90 and 180 minutes (Figures 7.3 & 7.7).

Whether participation in the PREHAB intervention used in chapters 6 and 7 altered the inducibility of Hsp72 in the PBMC from colorectal cancer patients however could not be determined.

Elevated Hsp72 expression is a characteristic reported in many cancer cells (Calderwood, Khaleque, Sawyer, & Ciocca, 2006; Gabai, Budagova, & Sherman, 2005), with the increased expression associated with providing cellular protection against apoptosis (Beere et al., 2000) and senescence (Gabai, Yaglom, Waldman, & Sherman, 2009; Yaglom et al., 2007). This ultimately can convey tumour resistance to cancer therapy treatment (Grivicich et al., 2007; Rashmi et al., 2004). In the first series of experiments therefore three different cancer cell lines (A2780 – human ovarian carcinoma; Colo320 – human colorectal adenocarcinoma and Jurkat – human acute T cell leukaemia) were used in addition to one non-diseased cell line (HUVEC) to further investigate the impact of basal Hsp72 expression on stress response.

In order for further Hsp synthesis to occur, the activation of existing Hsf is required for the transcription of Hsp mRNA to take place (Kiang & Tsokos, 1998). In cells with an overexpression of Hsp70, it has been proposed to have a repressive function on Hsf both in vivo (Mosser, Duchaine, & Massie, 1993) and in vitro (Abravaya, Myers, Murphy, & Morimoto, 1992). Consistent with this, in the current study following 90 minutes of heat shock at 40°C, Hsp72 expression decreased in seven of the 11 experiments when compared to the 37°C condition (median decrease: -5.8%; [range -2.8%; -25.7%]; Figure 7.1). Furthermore, out of the three experiments to

experience increased Hsp72 expression, the largest increase observed was just 6.4% (median increase: 5.9%; [range 2.8%; 6.4%]) of that seen at 37°C. As neither Hsp72 mRNA nor Hsf-1 was measured in this study, it can only be postulated that a repression of Hsf-1 accounted for this. Tang et al. (2016) recently reported a progressive decrease in Hsp70 expression in neonatal rat myocardial cells (as measured by Western blot) following up to 480 minutes of heat shock at 42°C compared to 37°C. Furthermore, the decrease in Hsp70 was greatest at 120 minutes onwards; a point at which both Hsf-1 expression and Hsp70 mRNA transcription appeared to increase exponentially thus potentially further demonstrating the repressive effect of Hsp70 on Hsf-1 expression.

Interestingly although not significant, increased Hsp72 expression (median increase: 14.5 % [range 4.78%; 23.1%]) was observed in eight of the 11 experiments conducted at 40°C condition compared to the 37°C following 180 minutes incubation (Figure 7.2). In a further two experiments, although Hsp72 expression decreased, the magnitude of this was less than 5% of Hsp72 expression at 37°C. It would therefore appear that in these specific cell lines, the more sustained period of simulated non-lethal stress (180 minutes of heat shock at 40°C) was sufficient to induce further Hsp72 synthesis. This is in contrast to Tang et al. (2016), where with the exception of at 20 minutes of heat stress, Hsp70 expression remained below that of the control condition in rat myocardial cells. A number of factors may explain this including basal Hsp72 expression and the presence of differing stress responses existing between cells from different species (rat vs. human) or cell origin (cardiac vs. colon vs. ovaries) both providing plausible explanations.

The second series of experiments performed in this chapter were on PBMC isolated from colorectal cancer patients in the PREHAB study in chapters 5 and 6. As with the cell lines, when the PBMC from both groups (control and PREHAB) and time points (Baseline and Pre-operative) were combined, no significant difference existed between the 37°C and the 40°C conditions for PBMC at either time point (Table 7.4). However, whereas the cell line displayed an apparent directional pattern of change at both 90 minutes (7/11decreased Hsp72 expression at 40°C) and 180 minutes (8/11 increased Hsp72 expression at 40°C), no such pattern was evident in the PBMC (90 minutes: 10 increased/10 decreased; 180 minutes: 12 increased/8 decreased; Figure 7.7 a & b).

In PBMC obtained from young healthy volunteers, a relationship has previously been reported between the extent of heat stress response achieved in PBMCs and the amount of Hsp72 already present (Lee et al., 2014; Lee et al., 2015; McClung et al., 2008; Vince et al., 2010). Vince et al. (2010) demonstrated Hsp72 induction in PBMC from healthy young males (range: 18 to 24 year) following heat shock at 40°C was proportional to basal content with the largest increase seen in cells with the lowest Hsp72 expression prior to heat shock. Furthermore, the Hsp72 stress response has been demonstrated to be attenuated following periods of heat acclimation where basal Hsp72 content is increased (Lee et al., 2015; McClung et al., 2008). Following 10 days of heat acclimation, McClung et al. (2008) reported blunted ex vivo Hsp72 induction following heat shock (1 hour at 43°C) in PBMC from healthy soldiers (age: 23 [18-29 years]). This coincided with a 17.7 ± 6.1% increase in basal Hsp72 following day 10 of heat acclimation. In the current study, however no direct relationship between Hsp72 at 37°C and change at 40°C was apparent. This could be due to a relatively high basal expression of Hsp72 in the PBMC used in this study, as previously the lower the basal expression the higher the magnitude of the stress response (Vince et al., 2010) and so a high basal expression could account for the lack of a consistent inducement of Hsp72 observed here. Whether the stress response in PBMC obtained from older patients (66.4 ± 9.7 years) with colorectal cancer differs from that of younger non-diseased populations is unknown. Therefore direct comparisons with previous research such as Vince et al. (2010), McClung et al. (2008) or Lee et al. (2015) is difficult as both the diseased condition and patient age may have had an influence on the stress response.

It is plausible that basal Hsp72 expression may be higher in the PBMCs of colorectal cancer patients compared to healthy controls, although to the author's knowledge this has not been investigated to date. Nadin, Vargas-Roig, Drago, Ibarra, and Ciocca (2007) reported elevated basal Hsp72 expression in the lymphocytes of cancer patients (mean age: 15.1 [range: 1 to 59] years; Hodgkin's lymphoma: n = 11; bone: n = 5; testicular: n = 3; other: n = 6) compared to healthy controls (mean age: 32 years [range 22-50 years]). This was potentially attributed to the individuals' tumour condition although the influence of the disparity in age between the groups cannot be dismissed. In colorectal cancer patients, increased serum levels of soluble Hsp72 have been observed with high baseline concentrations predictive of poorer mortality (Kocsis, Madaras, Toth, Fust, & Prohaszka, 2010); however it is worth noting that the quantity of Hsp72 in serum has been found not to correlate with monocyte expression in healthy

young males (mean age: 20.2 ± 1.9 years) (Taylor et al., 2010a). Human tumour cells are associated with increased production of ROS (Szatrowski & Nathan, 1991), potentially contributing to an increasingly oxidative environment sufficient to induce increased Hsp72 expression within the PBMC of cancer patients. Despite this, total Hsp72 expression was only elevated in the monocytes and lymphocytes of chronic lymphocytic leukaemia (mean age: 71.2 ± 7.6) and chronic myelomonocytic leukaemia (mean age: 66.6 ±13.6 years) compared to healthy young male controls (mean age: 22.1 ± 2.3 years) and not breast cancer patients (mean age: 58.8 ± 10.3 years) (Madden et al., 2012). This indicates an increase in Hsp72 in PBMC cannot be assumed based simply on the presence of a tumour. In respect to age, Njemini et al. (2002) reported an age-related attenuation in the ability of PBMC to induce Hsp72 expression in response to heat shock (1 hour at 42°C). Either of these factors therefore may have contributed to an altered stress response being present.

Although no relationship between Hsp72 expression at 37°C (control condition) and the change in expression at 40°C was evident, consistent with findings of Vince et al. (2010), a significant positive monotonic correlation was observed between Hsp72 expression at 37° and 40°C at both time points in cell lines (Figure 7.3) and PBMC (Figure 7.7 c & d). Interestingly in the PBMCs, a negative correlation existed between the Hsp72 expression following 90 minutes at a given temperature (37°C or 40°C) and the subsequent change in expression at 180 minutes at the same temperature. This could be argued to further support the presence of a relationship between basal expression and subsequent induction as even prolonged incubation (180 minutes) at 37°C appears sufficient to induce further Hsp72 expression over that seen at 90 minutes in lower expressing cells. Furthermore, higher expressing cells appeared to display decreased expression at 180 minutes potentially due to the repressive function of Hsp72 discussed previously. As the 90 and 180 minute experiments were only performed on the PBMCs, whether a similar relationship existed in the cell lines is unknown and requires further investigation in the future.

In chapter 6, $_{m}$Hsp72 expression decreased in the majority of patients from both the PREHAB and control groups at pre-operative reassessment indicating PREHAB had no effect on basal $_{m}$Hsp72 expression. Unfortunately conclusions as to whether participation in PREHAB altered the inducibility of Hsp72 in response to a subsequent period of non-lethal heat shock cannot be made from this study. Whilst statistical

analysis has been included to compare within group changes for completeness (Table 7.3), the low recruitment rates and subsequent lack of patients with corresponding baseline and pre-operative samples available ultimately limited the results obtained. Despite the isolation procedure being performed according to the manufacturers' instructions and an established protocol (Vince et al., 2010) for the thawing of PMBCs being adhered to, one batch of cells containing samples from 4 patients in particular had very poor viability (mean viability: 10.7 ± 5.9% compared to 85.7 ± 6.4%) following thawing and therefore were unusable. In addition to these issues with sample viability, the absence of a control group in respects to the PBMC means the possibility of different stress responses being present in cancer patients compared to healthy controls cannot be ruled out.

In summary, although it was not possible to establish whether PREHAB altered the induction of Hsp72 in the monocytes of colorectal cancer patients, this chapter appears to provide further evidence of a relationship between basal Hsp72 content and subsequent induction in response non-lethal heat shock. The presence of high basal content of Hsp72 in cancer cell lines was sufficient to prevent further induction in response to 90 minutes of heat shock, with an increase only observed after 180 minutes. Although a similar pattern was not seen in the PBMC of colorectal cancer patients, the presence of higher Hsp72 expression following 90 minutes at 37°C was associated with reduced change in expression at 180 minutes potentially reflecting the repressive function of Hsp72 on further induction.

Chapter 8: General discussion

The overall aim of this thesis was to investigate the potential implementation of PREHAB prior to colorectal cancer surgery in the current NHS structure. This included aspects such as the acceptance and uptake of the intervention by patients, the potential benefits on pre-operative physical functioning and HRQOL and subsequent impact on post-operative LOS and complication rates. The potential adaptive effects PREHAB may have on pre-operative Hsp72 basal expression and other markers of circulatory stress were also investigated. In order to discuss the combined findings of this thesis and how they contribute to the existing literature, it is important to first revisit the aims of each experimental chapter and identify the main findings from each.

Experimental chapter 1 (Chapter 3)

- To examine the attitudes of both the residents and GPs of Hull and the East Riding of Yorkshire to the potential use of PREHAB as a means of preparing patients for surgery. This included identifying factors such as preferred format and the perceived obstacles and benefits to participation.

    - *An open and receptive attitude appeared to exist within the majority of the survey population to the use of PREHAB as a means of preparing patients for surgery. Important potential obstacles to recruitment were identified such as cost, time and working commitments, and travel issues.*

Experimental chapter 2 (Chapter 4)

- To investigate the test-retest and absolute reliability of the five tests of physical functioning that were to be used in the main clinical study when performed four weeks apart (the expected approximate duration between enrolment to the study and surgery).

    - *All five tests displayed excellent test-retest reliability (ICC: > 0.90) although a potential learning effect was present in the TUG, FTSTS and SCT. Reference values for the measurement error of each test were established to help with the interpretation of results in chapter 5.*

Experimental chapter 3 (Chapter 5)

- To examine the feasibility of delivering a supervised PREHAB intervention into the standard NHS care pathway of patients awaiting major colorectal surgery.

    - *Recruitment uptake during the 18 month recruitment period was very slow with a consent rate of less than 19% (21/114). Moreover, over 40% of patients (84/198) assessed for eligibility were excluded due to having less than 2 weeks to surgery from diagnosis. Combined this raises doubts about the feasibility of implementing PREHAB.*

- To investigate the effects of PREHAB on pre-operative physical functioning, HRQOL and clinical outcomes in colorectal cancer patients.

    - *Participating in a 2-6 week period of PREHAB prior to elective surgery appeared to be sufficient to produce an improvement in physical functioning (6MWT, SCT and TUG performance) in colorectal surgery patients. These however did not translate into improved HRQOL or reduced LOS or complications in the PREHAB group albeit in a pilot study with a limited sample size.*

Experimental chapter 4 (Chapter 6)

- To investigate whether PREHAB would upregulate basal leukocyte Hsp72 and Hsp32 expression in colorectal patients prior to surgery. The effect PREHAB had on other markers of circulatory stress (plasma Hsp27, whole blood glutathione, plasma cortisol and Hsp70) was also investigated along with any relationships that may exist between the respective markers.

    - *Participation in PREHAB did not appear to upregulate basal leukocyte Hsp72 or Hsp32 expression nor alter circulatory glutathione, cortisol or Hsp70. An absence of sufficient training stimulus being produced the likely reason although the limited recruitment and issues controlling for a potential time of day effect do impact on the interpretation of the results. A significant decrease in plasma Hsp27 was observed in the*

PREHAB group at pre-operative reassessment which was not seen in the control although whether this was a result of PREHAB requires further investigation.

Experimental chapter 5 (Chapter 7)

- To investigate whether participation in PREHAB altered the induction of PBMC Hsp72 expression in response to heat shock.

    - *Insufficient samples due to poor recruitment and PBMC viability issues mean it is impossible to draw any conclusions regarding the effect of PREHAB on Hsp72 induction following heat shock.*

- To examine the effect of basal Hsp72 expression on subsequent induction in response to 90 and 180 minutes of non-lethal (40°C) heat shock.

    - *In cancer cell lines with high basal Hsp72 content, 90 minutes of non-lethal heat shock was insufficient to induce further expression with an increase only seen after 180 minutes. This supported the association between basal expression and subsequent induction previously reported. Although a similar response was not observed in the PBMC of colorectal cancer patients, the presence of a negative relationship between Hsp72 expression after 90 minutes at 37°C and change in expression at 180 minute at 37°C potentially reflects the repressive function of Hsp72 on further induction.*

8.1. Recruitment and feasibility issues with PREHAB

The first objective of this thesis was to investigate the feasibility of implementing a PREHAB intervention during the post diagnosis/pre-surgery period in colorectal cancer patients. In order to gain an insight into the public perception of the use of PREHAB prior to surgery (chapter 3), a questionnaire was conducted covering thoughts on participating in PREHAB, preferred format, perceived benefits of participation and potential barriers. Overall interest in participation was positive with 81% of respondents agreeing the concept of PREHAB was a good idea in principle,

whilst over 75% indicated they would be interested in PREHAB if they were awaiting major surgery. Preferences in regards to intervention format (e.g. preferred number of sessions, location and level of supervision; Table 3.2) were identified; many of which were reflected in the PREHAB intervention used in chapter 5. Furthermore, the five most perceived obstacles to participation in chapter 3 (Figure 3.2) were lack of time (62.1%), cost (46.2%), work commitments (43.2%), family commitments (36.4%) and travel (30.3%); all issues for which strategies (flexible appointments for exercise sessions, travel expenses paid, arranging of taxis) were in place to try minimise their impact on recruitment in chapter 5. Despite this, when the PREHAB intervention was applied to a clinical population (colorectal patients) in chapter 5, the actual participant uptake to the trial was disappointing with only 19% (21/114 patients) of those approached consenting to participate.

Problems with the recruitment in clinical RCTs is not a new issue with various participant-related (e.g. age, gender, lack of interest), environmental (e.g. culture, geographical issues) and research-related factors (e.g. time constraints, travel demands) all reported to impact on recruitment (Ridda, MacIntyre, Lindley, & Tan, 2010; Townsley, Selby, & Siu, 2005). In this thesis, various factors may explain the disparity between the perceived interest in chapter 3 and actual uptake in chapter 5, not least the fact responses to the questionnaire used in chapter 3 were based on theoretical need for surgery rather than when faced with a life-threatening condition such as colorectal cancer. Although no formal method of recording the reasons for non-participation were taken, the doctor responsible for recruiting to the trial in chapter 5 reported numerous potential participants declined participation in order to spend time with their family. Therefore when actually faced with a life-threatening condition such as colorectal cancer, attitudes may change with greater priority placed on other aspects of their lives such as spending time with family.

To the author's knowledge, the trial that formed chapters 5 and 6 of this thesis was the first to pragmatically assess the application of a PREHAB intervention into a UK secondary care setting. The design of the trial meant that the length of time available to perform PREHAB was dictated purely by the surgical wait time, and not vice versa. As a consequence, during almost 18 months of recruitment for the main study in this thesis (chapters 5 and 6), only 114 out of 198 (58%) of colorectal cancer patients met the minimum requirement of at least two weeks between being identified at

the MDT meeting and their scheduled surgery date to be eligible for the study. This was far fewer than had been envisaged during discussions with the surgical care team at the developmental stage of the trial. The NHS requirement for consultants to initiate cancer treatment within 31 days of the decision to treat (Baker & Nakatudde, 2015) was no doubt a decisive factor in this, although a tendency for medical investigators to overestimate the number of eligible patients available to recruit has previously been reported (van der Wouden et al., 2007). Eight out of the twelve patients not to receive NACRT prior to surgery actually had less than 3 weeks between baseline assessment and surgery (median: 18 days; [range: 13 to 27 days]) further illustrating the limited window of opportunity that exists to implement PREHAB. Considering these points discussed above, one of the most pertinent findings of this thesis is that at least within the NHS trust which hosted this trial, the application of PREHAB into the surgical care pathway would be difficult for colorectal cancer patients not receiving NACRT prior to surgery without procedures in place to delay the start of surgery, a circumstance that is not favourable to either the patient or surgical team.

8.2. Effect of PREHAB on pre-operative physical functioning, HRQOL and clinical outcomes

Having established that an interest existed for the potential use of PREHAB in chapter 3, it was important to determine the test-retest reliability and reference values of measurement error for the five physical functioning tests when separate by the expected time period of PREHAB (~4 weeks). All five tests used (TUG, FTSTS, SCT, HGD and 6MWT) were simple field tests chosen as they that had previously been applied to different clinical populations (Hanson et al., 2012; Rejeski et al., 1995; Ries et al., 2009; Steffen et al., 2002) and were suitable for a clinical setting where the availability of space and equipment was limited. All five tests displayed excellent test-retest reliability with ICCs in excess 0.90 when analysed regardless of gender (Tables 4.2, 4.4 and 4.6) whilst the SEM% and MDC_{95}% ranged from as low as 2.3% and 6.3% for the 6MWT to 5.2% and 16.1% for the FTSTS. This would subsequently aid in interpreting the effectiveness of the PREHAB intervention used in chapter 5.

When the PREHAB intervention was applied in chapter 5, despite the issues with recruitment and eligibility discussed in section 8.1, an improvement in 6MWT, TUG and SCT performance appeared evident in the PREHAB group but not the control

group. Moreover, this improvement was even present after only 2 weeks (~6 sessions) of training suggesting there is a potential role for PREHAB prior to surgery to elicit worthwhile changes in physical functioning. Although improvements in 6MWT performance have been reported previously prior to both colorectal cancer surgery (Li et al., 2013) and other types of surgery (Jones et al., 2007; Sawatzky et al., 2014) this is the first study in colorectal cancer patients to suggest such improvements in physical functioning can be achieved in less than 4 weeks (median duration of PREHAB: 23 (IQR: 14) days [range: 13 to 35 days]). This was likely a reflection of the joint-by-joint approach adopted in the PREHAB intervention used in this thesis. The functional resistance exercises used addressed common movement deficits such impaired muscle activation and reduced joint ROM which was likely to impair the performance of activities of daily living, especially in individuals with pre-existing functional deficits. The intervention therefore was potentially more inclusive than those which have previously placed the emphasis firmly on cardiorespiratory fitness alone (Kim do et al., 2009; West et al., 2015) or combined with resistance training based on sets and repetitions in isolated muscle groups which have lacked functional purpose (Carli et al., 2010; Gillis et al., 2014; Li et al., 2013).

Whilst this thesis did not place an emphasis on improving cardiorespiratory fitness prior to surgery, understandably there is a growing interest in HIT training as a potential effective and time-efficient method of improving cardiorespiratory fitness prior to colorectal cancer surgery (Weston, Weston, Prentis, & Snowden, 2016). Observational data supports the presence of an association between pre-operative cardiorespiratory fitness and post-operative recovery (Snowden et al., 2013) therefore any intervention that can improve fitness in a short period of time (<4 weeks) is an attractive proposition. Two recent papers (Boereboom, Phillips, Williams, & Lund, 2016b; Dunne et al., 2016; Table 8.1) demonstrated improved AT and VO_{2peak} following 4 weeks of HIT in healthy older participants and liver resection patients respectively (Table 8.1), whilst currently in the UK, at least two further clinical trials are registered to investigate the effects of four weeks of HIT (3 sessions per week) as a means of improving physical fitness prior to colorectal surgery (ClinicalTrials.gov, 2016a; 2016b). Whilst these findings are promising, whether such interventions would prove tolerable in patients of low starting fitness or those unaccustomed to cycle-based exercise is unknown. In fact, the ability to partake in cycle-based exercise was listed as

an inclusion criterion in Dunne et al (2016), potentially excluding older patients who may have greater mobility issues yet may benefit the most from PREHAB.

As discussed in chapter 5, the physical limitations of at least two patients in the PREHAB group of this study (unable to complete more than 10 minutes low to moderate intensity cycling) would likely have excluded them from the trials such as those conducted by Boereboom et al. (2016b) and Dunne et al. (2016). Anecdotally speaking, it may be argued however that despite the limited cycling they could perform, it was one of these patients who benefited most from participating in PREHAB with improved performance in four of the five physical functioning tests. Whilst there were also elements of the resistance programme that the patient was unable to perform (e.g. whole kinetic chain exercises), by applying a more inclusive and adaptive intervention, completion of five sessions over two weeks resulted in a 20% (+52 metres) improvement in 6MWT performance, in part due to a reduced reliance on a walking aid. Informal discussions during the five training sessions seemed to elaborate on this improvement in physical functioning, indicating that increased confidence gained through participating in the PREHAB had allowed the patient to 'go walking around town without their walking stick for the first time in years'. Whilst this suggests the improvement in 6MWT performance was unlikely to be as a result of improved cardiorespiratory fitness, an improved ability to perform simple tasks such as walking is likely to impact on the individual's day to day functioning and overall HRQOL, an important point not identified within the HRQOL questionnaires used. It is also worth considering n respect to the potential use of HIT, that based on median time until surgery (18 days) observed in chapter 5, without procedures being put in place to delay surgery up to the maximum of 31 days available (a situation most unlikely to be favoured by the patients), in many cases insufficient time to implement a HIT intervention would exist.

Table 8.1: PREHAB studies published since January 2016

Authors, Country, Design and study population	Number of patients (Females) and Mean age [range]	Intervention	Outcomes
Boereboom et al. (2016b) UK Observational Healthy elderly	N = 21 (13) 67 [62-73] years	12 x 16.5 minutes sessions of HIT over 4 weeks consisting of: • 2 minutes unloaded warm-up • five intervals of 60 seconds cycling at 100-110% Wmax as determined by CPET followed by 90 seconds unloaded cycling • 3.5 minutes cool-down	**VO_{2peak} (ml.kg.min)** 23.9 ± 4.7 vs. 26.2 ± 5.4* **AT (ml.kg.min)** 17.9 ± 4. 5 vs. 20.2 ± 4.1* **CPET time to failure (seconds)** 737 (IQR 95) vs. 772 (IQR 155)*
Dunne et al. (2016) UK RCT Liver resection	N = 37 (11) P: 20 (7) 61 [56-66] years C: 17 (4) 62 [56-72] years	12 x 40 minutes of HIT over 4 weeks consisting of: • 7 minutes warm-up (50% workload at AT) • 6 x 5 minute intervals (120-160 seconds at >90% VO_{2peak} followed by 180-140 seconds at 60% VO_{2peak}) • 3 minutes recovery (60% workload at AT)	**VO_{2peak} (ml.kg.min)** P: 17·6 ± 2·3 vs. 19·6 ± 3·8*$^{+}$ C: 18·6 ± 3·9 vs. 18·7 ± 4·1 **AT (ml.kg.min)** P: 11·2 ±1·5 vs. 12·2 ± 2·4^{+} C: 11·4 ± 1·8 vs. 11·0 ± 2·1 **SF-36® scores (Overall)** P: 65 ± 23 vs. 77 ± 18*$^{+}$ C: 71 ± 20 vs. 71 ± 22
Jenson et al. (2015 & 2016) Denmark RCT Radical cystectomy	N = 107 (28) P: 50 (11) 69 [66-72] years C: 57 (17) 71 (68-73) years	Twice daily endurance and strength training for two weeks. Each session consisted of: • 15 minutes step training • Six different muscle strength exercises Followed by post-op physiotherapy for 7 days	**Length of stay** 8 days both groups **Complications** No between group difference for incidence or severity **Mobilisation** (distance walked during the first 7 days postoperatively) P: 4806 m C: 2906 m^{+} **Median PADL (days)** P: 3 days C: 4 days^{+} **Pre-op muscle power (W.kg)** P: 2.00 vs. 2.35*$^{+}$ C: 1.98 vs. 2.01

*Significantly different to baseline, $^{+}$Significantly different to control group; VO_{2peak}: peak oxygen consumption; AT: anaerobic threshold; P: Prehab; C: Control; PADL: Personal activities of daily living; W.kg: watts per kg body mass

It had been hoped that in chapter 5, the potential impact of PREHAB on HRQOL and clinical outcomes such as post-operative LOS or complication rates could be investigated however with such low numbers it was not possible to draw any inferences with regards to its effect on these factors. In chapter 3, improved mental well-being (~72%), reduced depression (~40%) and reduced anxiety (~36%) were all identified as potential benefits of participating in PREHAB (Figure 3.1); however none of these were evident in chapter 5, with anxiety in the PREHAB group actually

increasing although detailed possible explanations for this are provided in the discussion of the relevant chapter (section 5.4). Potentially more important though were the possible HRQOL benefits that were experienced by the participants that went beyond the scope of the questionnaires used. Anecdotally, although formal qualitative analysis in the form of interviews was not completed, further interesting points became apparent during the delivery of sessions. Unlike in the people who declined participation due to preferring to spend time with family, discussions with a number of participants in the PREHAB group appeared to suggest participation in the trial allowed them to feel like they were taking an active role in trying to overcome their diagnosis. This feeling of taking control may in part explain the high adherence rates that were seen in the PREHAB group. The potential insights gained during informal discussions with the participants mean that in hindsight, rather than the priority being placed on the physical, biochemical and clinical aspects of the trial as it was at the inception of the thesis, a qualitative section would have added further breadth to the thesis.

Whilst improvements in physical functioning observed in this thesis following PREHAB are positive, for such an intervention to be implemented into standard clinical practice, not only does there need to be clinical benefits evident (e.g. shorter length of post-operative hospital stay, reduced post-operative complications) which were not seen in this thesis due to the limited recruitment but also these benefits must exceed the financial cost of the intervention. Even if the initial recruitment target (n = 60) had been achieved any conclusions relating to changes in HRQOL, post-operative LOS or complication rates would have needed to be interpreted with caution. In a recent systematic review, Boereboom, Doleman, Lund, and Williams (2016) suggested to detect a 10% reduction in absolute post-operative complication rates with 0.80 power, a sample of ~400 patients would be required. Equally, in order to detect a one day reduction in hospital LOS with 0.80 power (equating to a potential £6.5 million a year saving based on the latest NBOCAP audit of major resections per year), a sample of 388 patients would be required. Both of these values are far in excess of even the initial recruitment target (n = 60) of this thesis or of any of the previously published colorectal cancer literature (Carli et al., 2010; Li et al., 2013).

Similar to this thesis, Jensen, Laustsen, Jensen, Borre, and Petersen (2016) recently reported two weeks of twice daily strength exercises (10-15 reps of six exercises targeting major muscle groups) plus at least 30 minutes endurance training

(stepping) increased muscle power (+18% W.kg) during the pre-operative period in 107 (PREHAB: n = 50; control: n =57) radical cystectomy patients. This represented a 0.3 W.kg improvement compared to the control group (Jensen et al., 2016), adding to the improved mobilisation (distance walked during first seven days post-operatively; PREHAB: +1900 metres compared to control; $p < 0.001$) and return of ability to perform personal activities of daily living (PREHAB: -1 day compared to control; $p < 0.05$) previously reported (Jensen, Petersen, Jensen, Laustsen, & Borre, 2015). Importantly though, there was no decrease in median LOS (8 days for both groups), number of complications or complication severity between groups (Jensen et al., 2015). Therefore the improvements in physical functioning observed within this thesis are unlikely to be sufficient to justify the funding required to implement the intervention in its current format. However, as the premise of the ERAS approach is based on incorporating multiple components to improve surgical outcomes (Fearon et al., 2005; Gustafsson et al., 2012), the refinement of the intervention to provide a more cost-effective home-based intervention may be a viable alternative.

8.3. Effect of PREHAB on markers of circulatory stress

Having investigated the effect of PREHAB on pre-operative physical functioning, HRQOL and clinical outcomes in chapter 5, this thesis aimed to investigate whether any changes in circulatory markers of cellular stress would occur with a particular focus on Hsp72. Previous research has reported increased basal leukocyte Hsp72 expression following as little as 3-10 days of concurrent heat acclimation and exercise training (Lee et al., 2015; McClung et al., 2008). Furthermore these elevated basal levels are suggested to provide cross-tolerance to any subsequent stresses (Madden et al., 2008). Therefore in chapter 6, it had been hypothesised that participation in PREHAB would upregulate the basal leukocyte Hsp72 and Hsp32 expression and/or increase circulatory glutathione levels prior to surgery. Although the results need to be interpreted with caution given the small sample size, changes in neither marker appeared to be evident following participation in PREHAB compared to the control group. As discussed in chapter 6, whilst a number of factors may explain this lack of change, it was likely that the PREHAB intervention was not of either sufficient duration (mean aerobic exercise duration: 22.5 ± 4.0 minutes), intensity (mean %HRR for aerobic exercise: 53.1 ± 6.7%; minimum and peak HRR achieved during resistance exercise: 30 to 90% HRR) or frequency (mean no. sessions completed: 6.6 ± 2.5) to

trigger an adaptive response. Interestingly, a decrease in plasma Hsp27 was observed in eight out of 10 patients ($p < 0.05$; Figures 6.13 & 6.14) in the PREHAB group at pre-operative reassessment; a finding not replicated in the control group. Whether this difference was a result of the PREHAB intervention, another confounding variable (e.g. prior NACRT, cancer stage) or simply due to chance is impossible to determine however it may warrant further investigation.

As chapter 6 failed to establish any change in basal Hsp72 in monocytes following PREHAB, in chapter 7 it was intended to investigate whether participation in PREHAB changed the inducibility of Hsp72 in this cell type in response to heat shock. In long term trained endurance runners, the induction of leukocyte Hsp72 expression following a subsequent stressor is reported to be increased compared to sedentary controls (Fehrenbach et al., 2000a), whilst the decline in Hsp72 stress response that is reported to be associated with ageing (Njemini et al., 2002) is potentially attenuated by maintaining physical activity levels into later life (Simar et al., 2004). Bautmans et al. (2005) had reported increased inducibility following 6 weeks of progressive resistance training (3 x 10 reps at 50% 1RM progressing to 3 x 10 reps at 70-80% 1RM of leg press, leg abductor, leg adductor, vertical traction, chest press and shoulder press) in elderly individuals (n = 31; mean age: 68.4 ± 5.4 years). Whether a shorter period of functional resistance training such as that used in this thesis could result in similar change is however not known, especially in a clinical population. Unfortunately initial low numbers of patients with both baseline and pre-operative PBMCs (n = 16), were further reduced (Control: n = 5; PREHAB: n = 4) following issues with cell viability during thawing, meaning this could not be investigated.

The issues with cell viability may also have been one of a number of factors to influence the presence of a higher MFR for patient monocyte Hsp72 expression in chapter 6 compared to their corresponding samples following incubation in chapter 7. At the point of comparing the Hsp72 expression in PBMCs in chapter 7 to the values observed in whole blood in chapter 6, the PBMC would have been exposed to the isolation process, freezing, long-term storage in liquid nitrogen and the subsequent thawing as well as a minimum of 90 minutes incubation in a sealed polypropylene tube; all of which may have affected cell viability. In theory, the use of red blood cell lysis in chapter 6 as a means of isolating leukocytes rather than density gradient centrifugation could artificially activate Hsp72 expression as a result of the Fenton reaction. Despite

this, the use of red blood cell lysis prior to the analysis of leukocyte Hsp72 is a well-established and accepted method within the literature (Lee et al., 2015; Sandstrom et al., 2009; Taylor et al., 2012; Vince et al., 2010). It is also worth considering that although flow cytometry represents a semi-quantitative measure of Hsp72 expression in leukocytes (Bachelet et al., 1998), the use of MFR remains an arbitrary unit of measurement. Therefore, differences in the FSC/SCC setting used in chapter 6, where Hsp72 expression in monocytes, lymphocytes and granulocytes was measured compared to just monocyte expression in chapter 7 may account for the higher MFR observed. Furthermore, as the response to a subsequent stressor was not measured in the cells in chapter 7, whether a different stress response existed is unknown.

8.4. Influence of basal Hsp72 on subsequent expression

Whilst strategies such as heat acclimation (McClung et al., 2008), exercise preconditioning (Madden et al., 2008) and nutritional interventions (Singleton & Wischmeyer, 2007; Wischmeyer et al., 2001) have been used to increase basal content of Hsp72 with the intension of increasing cross-tolerance to a secondary more severe stressor (e.g. extreme heat, major surgery, intense exercise); the ability to do so may be in part dependent on the basal content already present (Vince et al., 2010). As a result, the second aim of chapter 7 was to investigate the stress response in cells with an already elevated Hsp72 content. In the cancer cell lines, where basal Hsp72 content is typically elevated, it was only after 180 minutes of heat shock that sufficient stimulus was available to induce further expression. This is likely explained by the repressive effect of Hsp72 on further Hsp72 synthesis (Mosser et al., 1993).

The proportional relationship between basal Hsp72 content and subsequent induction previously observed in the monocytes of healthy young males exposed to 90 minutes of heat shock (Vince et al., 2010) was not seen in cells from colorectal cancer patients (Figures 8.7). As discussed in chapter 7, this may be as a result of the presence of cancer-related elevations in basal Hsp72 content meaning sufficient expression is already present preventing further induction. Furthermore, the older age of the patients (range: 46 to 79 years) in this thesis may have reflected the age-related decline in the Hsp72 stress response (Njemini et al., 2002). Despite this, the presence of a negative relationship between Hsp72 expression after 90 minutes at 37°C and change in

expression at 180 minute at 37°C further supports the association between Hsp72 content and subsequent expression.

10.5. Experimental limitations

Working with a clinical population unfortunately resulted in a number of unavoidable experimental limitations occurring, many of which have been discussed in detail within the specific experimental chapters. The poor recruitment to the main trial, as discussed in chapter 5 and in section 8.1, obviously represents a major limitation to the thesis, impacting on the sample size achieved for chapters 5, 6 and 7, and meaning all results require interpreting with some caution. Despite efforts to increase recruitment through amending the inclusion criteria to make patients with benign disease eligible for the final seven months of recruitment, this only contributed two additional patients. Whilst disappointing, the issues with recruitment has proved to be one of the most pertinent findings of this thesis.

CPET remains the 'gold standard' measure of cardiorespiratory fitness in both clinical and healthy populations alike therefore the absence of baseline and pre-operative reassessment CPET in both groups in chapter 5 is an obvious limitation. Whilst ideally these would have been performed allowing for objective measures of cardiorespiratory fitness such as VO_{2peak}, AT, VE/VO_2 and VE/VCO_2 to be obtained, given the facilities and equipment available to perform the CPET at the research site this was deemed an necessary risk in this population. The minimum workload available on the Daum cycle ergometer used for performing the CPET was 20 W with the option of unloaded cycling not available. Whilst in healthy young populations this would not have been an issue, in a typically ageing clinical population such as the one used in this thesis, this was a sufficient intensity to achieve AT in the first three minutes of the test making the identification of the AT difficult thus limiting the quality of the CPET data attainable. It also meant the protocol used was not as previously recommended within clinical populations (ATS, 2003) therefore the validity of the data beyond exercise prescription would be questionable. As the PREHAB intervention in this thesis placed greater emphasis on functional resistance training with the aim of improving physical functioning rather than predominantly focusing on aerobic fitness, the likelihood of achieving measurable changes in cardiorespiratory fitness were minimal, especially in patients with only two weeks between enrolment and surgery.

It is acknowledged that as a single centre trial, the generalisability beyond the local NHS Trust where the trial was based is limited. Across different NHS trusts and sites there are likely to be differing waiting times for colorectal cancer surgery meaning opportunities to implement PREHAB may be greater in some sites than others. Moreover, different sites are likely to incorporate different elements of the ERAS pathway into their standard pre-, peri- and post-operative care pathways. The heterogeneity of the sample population for the main trial also affects the generalisability of study to a certain extent. Within the 23 patients recruited in chapters 5 and 6, there were a number of confounding variables (colon cancer vs. rectal cancer vs. benign disease; NACRT vs. no NACRT) within the sample all of which potentially could have affected the results, especially in terms of the biochemical analysis in chapter 6.

In chapter 6, changes in plasma volume between baseline and pre-operative blood samples were not measured as access to the equipment required to measure haematocrit and haemoglobin was limited at the hospital site where the samples were obtained. This meant whether a change in plasma volume occurred between baseline and pre-operative reassessment cannot be ruled out although given that patients had been exposed to neither extreme temperatures nor intensive exercise prior to providing their samples a significant change was not expected to have occurred.

10.6. Recommendations for future research

It was accepted at the concept of the PREHAB intervention used in this thesis that in the long term, providing one to one sessions would not be cost-effective within the current NHS structure. The programme was designed to incorporate predominantly simple exercises with minimal equipment requirements which could eventually be adapted and implemented as a home exercise intervention if effective. The apparent improvement in physical functioning observed prior to surgery in the PREHAB group in chapter 6 therefore means further research is warranted into such a home-based programme. As discussed in section 8.2, recent publications by Boereboom et al. (2016b) and Dunne et al. (2016), and the presence of at least two clinical trials currently on going (Clinical trials.gov. 2016a; 2016b) shows there is support for research into the use of HIT as a potential modality of pre-operative training care, at least in patients physically capable of performing such exercise. Regardless of which approach is taken

in the future, correctly powered multicentre RCTs (n = ~400 patients) are required in order to establish whether exercise PREHAB alone can improve clinical outcomes (LOS, complications). An alternative viewpoint however is that the refinement of ERAS protocols is now about achieving marginal gains though incorporating multiple components (e.g. exercise training, nutritional supplementation, psychological interventions, all of which may improve patient recovery) in a 'complex intervention' as specific components may not achieve noticeable differences individually (Durrand, Batterham, & Danjoux, 2014). There is therefore an argument for such interventions to be developed and researched in the future (Durrand et al., 2014).

Based on the results of chapter 6, it appears unlikely a PREHAB intervention such that used in this thesis is of sufficient intensity or duration to elicit a change in leukocyte Hsp72 or Hsp32 expression prior to surgery. Whether a HIT intervention, as previously discussed, given its higher intensity would induce adaptive changes in basal leukocyte Hsp72 or Hsp32 expression and well as plasma Hsp27 expression may be of future interest. In order to do this however, strategies for controlling for external confounding factors such as time of day, prior treatments such as NACRT and age would need to be in place first.

10.7. Conclusions

In conclusion, given the associations that have been observed between objective physical fitness and post-operative outcomes (Older et al., 1999; Wilson et al, 2010), interest will remain for the foreseeable future in the potential use of PREHAB as a means of preparing colorectal cancer patients for major surgery. This thesis however has highlighted a number of important issues (e.g. short time to surgery, low participant uptake, patient incapable of performing prolonged aerobic exercise) that would need to be overcome in order to implement such a programme within the current NHS colorectal cancer care pathway. Despite this, by adopting a joint-by-joint approach to PREHAB, pre-operative improvements in physical functioning (chapter 5) appear to have been observed following as little as five PREHAB sessions. If these improvements could be reproduced by adapting the intervention for home based use, than this approach may represent a cost effective addition to ERAS pathways, especially in patients who are unable to perform more intensive aerobic exercise.

Chapter 11: References

Aaronson, N. K., Ahmedzai, S., Bergman, B., Bullinger, M., Cull, A., Duez, N. J., . . . et al. (1993). The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *Journal of the National Cancer Institute, 85*(5), 365-376.

Aarts, M. A., Okrainec, A., Glicksman, A., Pearsall, E., Victor, J. C., & McLeod, R. S. (2012). Adoption of enhanced recovery after surgery (ERAS) strategies for colorectal surgery at academic teaching hospitals and impact on total length of hospital stay. *Surgical Endoscopy, 26*(2), 442-450. doi: 10.1007/s00464-011-1897-5

Abravaya, K., Myers, M. P., Murphy, S. P., & Morimoto, R. I. (1992). The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. *Genes & Development, 6*(7), 1153-1164.

Acott, A. A., Theus, S. A., Marchant-Miros, K. E., & Mancino, A. T. (2008). Association of tobacco and alcohol use with earlier development of colorectal cancer: should we modify screening guidelines? *American journal of Surgery, 196*(6), 915-918; discussion 918-919. doi: 10.1016/j.amjsurg.2008.07.033

Agnew, N. (2010). Preoperative cardiopulmonary exercise testing. *Continuing Education in Anaesthesia, Critical Care & Pain, 10*(2), 33-37. doi: 10.1093/bjaceaccp/mkq001

Ahlberg, K., Ekman, T., Gaston-Johansson, F., & Mock, V. (2003). Assessment and management of cancer-related fatigue in adults. *Lancet, 362*(9384), 640-650. doi: 10.1016/s0140-6736(03)14186-4

Akasu, T., Takawa, M., Yamamoto, S., Yamaguchi, T., Fujita, S., & Moriya, Y. (2010). Risk factors for anastomotic leakage following intersphincteric resection for very low rectal adenocarcinoma. *Journal of Gastrointestinal Surgery, 14*(1), 104-111. doi: 10.1007/s11605-009-1067-4

Alacacioglu, A., Binicier, O., Gungor, O., Oztop, I., Dirioz, M., & Yilmaz, U. (2010). Quality of life, anxiety, and depression in Turkish colorectal cancer patients. *Supportive Care in Cancer, 18*(4), 417-421. doi: 10.1007/s00520-009-0679-2

Alcock, L., Vanicek, N., & O'Brien, T. D. (2013). Alterations in gait speed and age do not fully explain the changes in gait mechanics associated with healthy older women. *Gait & Posture, 37*(4), 586-592. doi: 10.1016/j.gaitpost.2012.09.023

Alfonso-Rosa, R. M., Del Pozo-Cruz, B., Del Pozo-Cruz, J., Sanudo, B., & Rogers, M. E. (2014). Test-retest reliability and minimal detectable change scores for fitness assessment in older adults with type 2 diabetes. *Rehabilitation Nursing, 39*(5), 260-268. doi: 10.1002/rnj.111

American College of Sports Medicine. (2013). *ACSM's Guidelines for Exercise Testing and Prescription*: Wolters Kluwer/Lippincott Williams & Wilkins Health

American Thoracic Society. (2002). ATS statement: guidelines for the six-minute walk test. *American Journal of Respiratory and Critical Care Medicine, 166*(1), 111-117. doi: 10.1164/ajrccm.166.1.at1102

American Thorcic Society. (2003). ATS/ACCP Statement on cardiopulmonary exercise testing. *American Journal of Respiratory and Critical Care Medicine,, 167*(2), 211-277. doi: 10.1164/rccm.167.2.211

Anderson, A. D., McNaught, C. E., MacFie, J., Tring, I., Barker, P., & Mitchell, C. J. (2003). Randomized clinical trial of multimodal optimization and standard perioperative surgical care. *British Journal of Surgery, 90*(12), 1497-1504. doi: 10.1002/bjs.4371

Anup, R., Aparna, V., Pulimood, A., & Balasubramanian, K. A. (1999). Surgical stress and the small intestine: role of oxygen free radicals. *Surgery, 125*(5), 560-569.

Anup, R., & Balasubramanian, K. A. (2000). Surgical stress and the gastrointestinal tract. *Journal of Surgical Research, 92*(2), 291-300. doi: 10.1006/jsre.2000.5874

Anup, R., Susama, P., & Balasubramanian, K. A. (2000). Role of xanthine oxidase in small bowel mucosal dysfunction after surgical stress. *British Journal of Surgery, 87*(8), 1094-1101. doi: 10.1046/j.1365-2168.2000.01469.x

Arora, V., Velanovich, V., & Alarcon, W. (2011). Preoperative assessment of cardiac risk and perioperative cardiac management in noncardiac surgery. *International Journal of Surgery, 9*(1), 23-28. doi: 10.1016/j.ijsu.2010.09.010

Arthur, H. M., Daniels, C., McKelvie, R., Hirsh, J., & Rush, B. (2000). Effect of a preoperative intervention on preoperative and postoperative outcomes in low-risk patients awaiting

elective coronary artery bypass graft surgery. A randomized, controlled trial. *Annals of Internal Medicine, 133*(4), 253-262.
Asoh, T., & Tsuji, H. (1981). Preoperative physical training for cardiac patients requiring non-cardiac surgery. *Japanese Journal of Surgery, 11*(4), 251-255.
Atkinson, G., & Nevill, A. M. (1998). Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Medicine, 26*(4), 217-238.
Atkinson, G., & Reilly, T. (1996). Circadian Variation in Sports Performance. *Sports Medicine,* 21 (4), 292-312
Aune, D., Chan, D. S., Lau, R., Vieira, R., Greenwood, D. C., Kampman, E., & Norat, T. (2011). Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ, 343*, d6617. doi: 10.1136/bmj.d6617
Babior, B. M. (2004). NADPH oxidase. *Current opinion in immunology. 16* (1), 42-47.
Bachelet, M., Mariethoz, E., Banzet, N., Souil, E., Pinot, F., Polla, C. Z., . . . Polla, B. S. (1998). Flow cytometry is a rapid and reliable method for evaluating heat shock protein 70 expression in human monocytes. *Cell Stress & Chaperones, 3*(3), 168-176.
Baker, C., & Nakatudde, N. (2015). *Cancer: Waiting Times for Diagnosis and Treatment*. Retrieved from http://www.parliament.uk/business/publications/research/briefing-papers/SN07043/cancer-waiting-times-for-diagnosis-and-treatment.
Barakat, H. M., Shahin, Y., Barnes, R., Gohil, R., Souroullas, P., Khan, J., . . . Chetter, I. C. (2014). Supervised exercise program improves aerobic fitness in patients awaiting abdominal aortic aneurysm repair. *Annals of Vascular Surgery, 28*(1), 74-79. doi: 10.1016/j.avsg.2013.09.001
Bauer, K., Nitsche, U., Slotta-Huspenina, J., Drecoll, E., von Weyhern, C. H., Rosenberg, R., . . . Langer, R. (2012). High HSP27 and HSP70 expression levels are independent adverse prognostic factors in primary resected colon cancer. *Cellular Oncology, 35*(3), 197-205. doi: 10.1007/s13402-012-0079-3
Bautmans, I., Njemini, R., De Backer, J., De Waele, E., & Mets, T. (2010). Surgery-induced inflammation in relation to age, muscle endurance, and self-perceived fatigue. *Journal of Gerontology: Biological Sciences & Medical Sciences, 65*(3), 266-273. doi: 10.1093/gerona/glp145
Bautmans, I., Njemini, R., Vasseur, S., Chabert, H., Moens, L., Demanet, C., & Mets, T. (2005). Biochemical changes in response to intensive resistance exercise training in the elderly. *Gerontology, 51*(4), 253-265. doi: 10.1159/000085122
Beckham, S. G., & Harper, M. (2010). Functional Training: Fad or Here to Stay? *ACSM's Health & Fitness Journal, 14*(6), 24-30. doi: 10.1249/FIT.0b013e3181f8b3b7
Beckman, K. B., & Ames, B. N. (1998). The free radical theory of aging matures. *Physiological Reviews, 78*(2), 547-581.
Beckmann, R. P., Mizzen, L. E., & Welch, W. J. (1990). Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. *Science, 248*(4957), 850-854.
Beere, H. M., Wolf, B. B., Cain, K., Mosser, D. D., Mahboubi, A., Kuwana, T., . . . Green, D. R. (2000). Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nature Cell Biology, 2*(8), 469-475. doi: 10.1038/35019501
Behrens, B. C., Hamilton, T. C., Masuda, H., Grotzinger, K. R., Whang-Peng, J., Louie, K. G., . . . Ozols, R. F. (1987). Characterization of a cis-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Research, 47*(2), 414-418.
Bellet, R. N., Adams, L., & Morris, N. R. (2012). The 6-minute walk test in outpatient cardiac rehabilitation: validity, reliability and responsiveness--a systematic review. *Physiotherapy, 98*(4), 277-286. doi: 10.1016/j.physio.2011.11.003
Beltran Valls, M. R., Dimauro, I., Brunelli, A., Tranchita, E., Ciminelli, E., Caserotti, P., . . . Caporossi, D. (2014). Explosive type of moderate-resistance training induces functional, cardiovascular, and molecular adaptations in the elderly. *Age, 36*(2), 759-772. doi: 10.1007/s11357-013-9584-1

Benjamin, I. J., & McMillan, D. R. (1998). Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circulation Research, 83*(2), 117-132.

Bennell, K., Dobson, F., & Hinman, R. (2011). Measures of physical performance assessments: Self-Paced Walk Test (SPWT), Stair Climb Test (SCT), Six-Minute Walk Test (6MWT), Chair Stand Test (CST), Timed Up & Go (TUG), Sock Test, Lift and Carry Test (LCT), and Car Task. *Arthritis Care & Research, 63 Suppl 11*, S350-370. doi: 10.1002/acr.20538

Benzo, R., Wigle, D., Novotny, P., Wetzstein, M., Nichols, F., Shen, R. K., . . . Deschamps, C. (2011). Preoperative pulmonary rehabilitation before lung cancer resection: results from two randomized studies. *Lung Cancer, 74*(3), 441-445. doi: 10.1016/j.lungcan.2011.05.011

Bischoff, H. A., Stahelin, H. B., Monsch, A. U., Iversen, M. D., Weyh, A., von Dechend, M., . . . Theiler, R. (2003). Identifying a cut-off point for normal mobility: a comparison of the timed 'up and go' test in community-dwelling and institutionalised elderly women. *Age & Ageing, 32*(3), 315-320.

Blanco, R. A., Zeigler, T. R., Carlson, B. A., Cheng, P. Y, Park, Y., Cotsonis, G. A., Accardi, C. J., Jones, D. P. (2007). Diurnal variation in glutathione and cycteine redox states in human plasma. American Journal of Clinical Nutrition, 86 (4), 1016-1023.

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet, 1*(8476), 307-310.

Blumetti, J., Luu, M., Sarosi, G., Hartless, K., McFarlin, J., Parker, B., . . . Anthony, T. (2007). Surgical site infections after colorectal surgery: do risk factors vary depending on the type of infection considered? *Surgery, 142*(5), 704-711. doi: 10.1016/j.surg.2007.05.012

Bobbio, A., Chetta, A., Ampollini, L., Primomo, G. L., Internullo, E., Carbognani, P., . . . Olivieri, D. (2008). Preoperative pulmonary rehabilitation in patients undergoing lung resection for non-small cell lung cancer. *European Journal of Cardiothoracic Surgery, 33*(1), 95-98. doi: 10.1016/j.ejcts.2007.10.003

Boereboom, C., Doleman, B., Lund, J. N., & Williams, J. P. (2016a). Systematic review of pre-operative exercise in colorectal cancer patients. *Techniques in Coloproctology, 20*(2), 81-89. doi: 10.1007/s10151-015-1407-1

Boereboom, C. L., Phillips, B. E., Williams, J. P., & Lund, J. N. (2016b). A 31-day time to surgery compliant exercise training programme improves aerobic health in the elderly. *Techniques in Coloproctology, 20*(6), 375-382. doi: 10.1007/s10151-016-1455-1

Boereboom, C. L., Williams, J. P., Leighton, P., & Lund, J. N. (2015). Forming a consensus opinion on exercise prehabilitation in elderly colorectal cancer patients: a Delphi study. *Techniques in Coloproctology, 19*(6), 347-354. doi: 10.1007/s10151-015-1317-2

Bohannon, R. W. (2008). Hand-grip dynamometry predicts future outcomes in aging adults. *Journal of Geriatric Physical Therapy, 31*(1), 3-10.

Bohannon, R. W. (2011). Test-retest reliability of the five-repetition sit-to-stand test: a systematic review of the literature involving adults. *Journal of Strength & Conditioning Research, 25*(11), 3205-3207. doi: 10.1519/JSC.0b013e318234e59f

Bohannon, R. W., Bubela, D. J., Magasi, S. R., & Gershon, R. C. (2011). Relative reliability of three objective tests of limb muscle strength. *Isokinetics & Exercise Science, 19*(2). doi: 10.3233/IES-2011-0400

Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., . . . Sibbald, W. J. (1992). American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Critical Care Medicine, 20*(6), 864-874.

Boni, L., Benevento, A., Rovera, F., Dionigi, G., Di Giuseppe, M., Bertoglio, C., & Dionigi, R. (2006). Infective complications in laparoscopic surgery. *Surgical Infections, 7 Suppl 2*, S109-111. doi: 10.1089/sur.2006.7.s2-109

Bordes, J., Cardinal, M., & Kaiser, E. (2015). Prehabilitation versus Rehabilitation. *Anesthesiology, 122*(6), 1438. doi: 10.1097/aln.0000000000000661

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise, 14*(5), 377-381.

Botteri, E., Iodice, S., Bagnardi, V., Raimondi, S., Lowenfels, A. B., & Maisonneuve, P. (2008). Smoking and colorectal cancer: a meta-analysis. *JAMA, 300*(23), 2765-2778. doi: 10.1001/jama.2008.839

Botteri, E., Iodice, S., Raimondi, S., Maisonneuve, P., & Lowenfels, A. B. (2008). Cigarette smoking and adenomatous polyps: a meta-analysis. *Gastroenterology, 134*(2), 388-395. doi: 10.1053/j.gastro.2007.11.007

Boyle, M. (2010). *Advances in Functional Training: Training Techniques for Coaches, Personal Trainers and Athletes*. Aptos, CA: On Target Publications, LLC.

Boyle, T., Keegel, T., Bull, F., Heyworth, J., & Fritschi, L. (2012). Physical activity and risks of proximal and distal colon cancers: a systematic review and meta-analysis. *Journal of the National Cancer Institute, 104*(20), 1548-1561. doi: 10.1093/jnci/djs354

Boynton, P. M., & Greenhalgh, T. (2004). Selecting, designing, and developing your questionnaire. *BMJ, 328*(7451), 1312-1315. doi: 10.1136/bmj.328.7451.1312

Brerro-Saby, C., Delliaux, S., Steinberg, J. G., Boussuges, A., Gole, Y., & Jammes, Y. (2010). Combination of two oxidant stressors suppresses the oxidative stress and enhances the heat shock protein 27 response in healthy humans. *Metabolism, 59*(6), 879-886. doi: 10.1016/j.metabol.2009.10.006

British Heart Foundation. (2014). *The National Audit of Cardiac Rehabilitation Annual Statistical Report 2014*. Retrieved from http://www.cardiacrehabilitation.org.uk/docs/2014.pdf

Brown, S. R., & Tiernan, J. (2005). Transverse verses midline incisions for abdominal surgery. *Cochrane Database of Systematic Reviews*, 4, CD005199. doi: 10.1002/14651858.CD005199.pub2

Buchs, N. C., Gervaz, P., Secic, M., Bucher, P., Mugnier-Konrad, B., & Morel, P. (2008). Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. *International Journal of Colorectal Disease, 23*(3), 265-270. doi: 10.1007/s00384-007-0399-3

Burke, S. M., Brunet, J., Sabiston, C. M., Jack, S., Grocott, M. P., & West, M. A. (2013). Patients' perceptions of quality of life during active treatment for locally advanced rectal cancer: the importance of preoperative exercise. *Supportive Care in Cancer, 21*(12), 3345-3353. doi: 10.1007/s00520-013-1908-2

Burke, S. M., West, M. A., Grocott, M. P. W., Brunet, J., & Jack, S. (2015). Exploring the experience of adhering to a prescribed pre-surgical exercise program for patients with advanced rectal cancer: A phenomenological study. *Psychology of Sport and Exercise, 16, Part 3*, 88-95. doi: 10.1016/j.psychsport.2014.09.005

Calderwood, S. K., Khaleque, M. A., Sawyer, D. B., & Ciocca, D. R. (2006). Heat shock proteins in cancer: chaperones of tumorigenesis. *Trends in Biochemical Sciences, 31*(3), 164-172. doi: 10.1016/j.tibs.2006.01.006

Campbell, D. (2016). Hospitals told to cut staff amid spiralling NHS cash crisis, *The Guardian*. Retrieved from http://www.theguardian.com/society/2016/jan/29/hospitals-told-cut-staff-nhs-cash-crisis

Cancer Research UK. (2016a). *Bowel cancer incidence statistics.* Retrieved 3rd June, 2016, from http://www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/incidence/#By2

Cancer Research UK. (2016b). *Bowel cancer survival statistics.* Retrieved 5th June, 2016, from http://www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/survival/#stage

Carli, F. (2015). Physiologic considerations of Enhanced Recovery After Surgery (ERAS) programs: implications of the stress response. *Canadian Journal of Anaesthesia, 62*(2), 110-119. doi: 10.1007/s12630-014-0264-0

Carli, F., Charlebois, P., Stein, B., Feldman, L., Zavorsky, G., Kim, D. J., . . . Mayo, N. E. (2010). Randomized clinical trial of prehabilitation in colorectal surgery. *British Journal of Surgery, 97*(8), 1187-1197. doi: 10.1002/bjs.7102

Carli, F., & Zavorsky, G. S. (2005). Optimizing functional exercise capacity in the elderly surgical population. *Current Opinion in Clinical Nutrition & Metabolic Care , 8*(1), 23-32.

Carlsson, E., Berndtsson, I., Hallen, A. M., Lindholm, E., & Persson, E. (2010). Concerns and quality of life before surgery and during the recovery period in patients with rectal

cancer and an ostomy. *Journal of Wound Ostomy & Continence Nursing, 37*(6), 654-661. doi: 10.1097/WON.0b013e3181f90f0c

Cerantola, Y., Valerio, M., Persson, B., Jichlinski, P., Ljungqvist, O., Hubner, M., . . . Patel, H. R. (2013). Guidelines for perioperative care after radical cystectomy for bladder cancer: Enhanced Recovery After Surgery (ERAS((R))) society recommendations. *Clinical Nutrtion , 32*(6), 879-887. doi: 10.1016/j.clnu.2013.09.014

Cesario, A., Ferri, L., Galetta, D., Cardaci, V., Biscione, G., Pasqua, F., . . . Granone, P. (2007). Pre-operative pulmonary rehabilitation and surgery for lung cancer. *Lung Cancer, 57*(1), 118-119. doi: 10.1016/j.lungcan.2007.03.022

Chan, D. S., Lau, R., Aune, D., Vieira, R., Greenwood, D. C., Kampman, E., & Norat, T. (2011). Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PLoS One, 6*(6), e20456. doi: 10.1371/journal.pone.0020456

Cho, E., Smith-Warner, S. A., Ritz, J., van den Brandt, P. A., Colditz, G. A., Folsom, A. R., . . . Hunter, D. J. (2004). Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Annals of Internal Medicine, 140*(8), 603-613.

Ciocca, D. R., Arrigo, A. P., & Calderwood, S. K. (2013). Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. *Archives of Toxicology, 87*(1), 19-48. doi: 10.1007/s00204-012-0918-z

Ciocca, D. R., Clark, G. M., Tandon, A. K., Fuqua, S. A., Welch, W. J., & McGuire, W. L. (1993). Heat shock protein hsp70 in patients with axillary lymph node-negative breast cancer: prognostic implications. *Journal of the National Cancer Institute, 85*(7), 570-574.

Clark, A. M., King-Shier, K. M., Thompson, D. R., Spaling, M. A., Duncan, A. S., Stone, J. A., . . . Angus, J. E. (2012). A qualitative systematic review of influences on attendance at cardiac rehabilitation programs after referral. *American Heart Journal, 164*(6), 835-845 e832. doi: 10.1016/j.ahj.2012.08.020

ClinicalTrials.gov (2016a). *Assessing the Effectiveness of a Preoperative High Intensity Interval Training Programme in Older Colorectal Cancer Patients (NCT 02188342)*. Retrieved from https://clinicaltrials.gov/ct2/show/NCT02188342?term=NCT+02188342&rank=1

ClinicalTrials.gov (2016b). *Preop CPET Testing and Exercise Training in Colorectal Patients (NCT 01924897).* Retrieved from https://clinicaltrials.gov/ct2/show/NCT01924897?term=01924897&rank=1

Coats, V., Maltais, F., Simard, S., Frechette, E., Tremblay, L., Ribeiro, F., & Saey, D. (2013). Feasibility and effectiveness of a home-based exercise training program before lung resection surgery. *Canadian Respiratory Journal, 20*(2), e10-16.

Cook, G., & Burton, L. (2010). *Movement: Functional Movement Systems : Screening, Assessment, and Corrective Strategies*. Aptos, CA.: On Target Publications.

Costa-Santos, C., Bernardes, J., Ayres-de-Campos, D., Costa, A., & Amorim-Costa, C. (2011). The limits of agreement and the intraclass correlation coefficient may be inconsistent in the interpretation of agreement. *Journal of Clinical Epidemiology, 64*(3), 264-269. doi: 10.1016/j.jclinepi.2009.11.010

Cramp, F., & Byron-Daniel, J. (2012). Exercise for the management of cancer-related fatigue in adults. *Cochrane Database of Systematic Reviews, 11*, CD006145. doi: 10.1002/14651858.CD006145.pub3

Daams, F., Luyer, M., & Lange, J. F. (2013). Colorectal anastomotic leakage: aspects of prevention, detection and treatment. *World Journal of Gastroenterology, 19*(15), 2293-2297. doi: 10.3748/wjg.v19.i15.2293

Daley, M. J., & Spinks, W. L. (2000). Exercise, mobility and aging. *Sports Medicine, 29*(1), 1-12.

Daly, J., Sindone, A. P., Thompson, D. R., Hancock, K., Chang, E., & Davidson, P. (2002). Barriers to participation in and adherence to cardiac rehabilitation programs: a critical literature review. *Progress in Cardiovascular Nursing, 17*(1), 8-17.

Damrauer, S. M., Bordeianou, L., & Berger, D. (2009). Contained anastomotic leaks after colorectal surgery: are we too slow to act? *Archives of Surgery, 144*(4), 333-338; discussion 338. doi: 10.1001/archsurg.2008.589

Daugaard, M., Rohde, M., & Jaattela, M. (2007). The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Letters, 581*(19), 3702-3710. doi: 10.1016/j.febslet.2007.05.039

De Vos, C., Li, X., Van Vlaenderen, I., Saka, O., Dendale, P., Eyssen, M., & Paulus, D. (2013). Participating or not in a cardiac rehabilitation programme: factors influencing a patient's decision. *European Journal of Preventive Cardiology, 20*(2), 341-348. doi: 10.1177/2047487312437057

Demers, C., McKelvie, R. S., Negassa, A., & Yusuf, S. (2001). Reliability, validity, and responsiveness of the six-minute walk test in patients with heart failure. *American Heart Journal, 142*(4), 698-703. doi: 10.1067/mhj.2001.118468

Demirel, H. A., Powers, S. K., Zergeroglu, M. A., Shanely, R. A., Hamilton, K., Coombes, J., & Naito, H. (2001). Short-term exercise improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. *Journal of Applied Physiology , 91*(5), 2205-2212.

Dempsey, N. C., Leoni, F., Ireland, H. E., Hoyle, C., & Williams, J. H. (2010). Differential heat shock protein localization in chronic lymphocytic leukemia. *Journal of Leukocyte Biology, 87*(3), 467-476. doi: 10.1189/jlb.0709502

Desborough, J. P. (2000). The stress response to trauma and surgery. *British Journal of Anaesthesia, 85*(1), 109-117.

Desmeules, F., Hall, J., & Woodhouse, L. J. (2013). Prehabilitation improves physical function of individuals with severe disability from hip or knee osteoarthritis. *Physiotherapy Canada, 65*(2), 116-124. doi: 10.3138/ptc.2011-60

Dhalla, N. S., Temsah, R. M., & Netticadan, T. (2000). Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension, 18*(6), 655-673.

Dickinson, D. A., & Forman, H. J. (2002). Glutathione in defense and signaling: lessons from a small thiol. *Annals of the New York Academy of Science, 973*, 488-504.

Dodson, J. A., Arnold, S. V., Reid, K. J., Gill, T. M., Rich, M. W., Masoudi, F. A., . . . Alexander, K. P. (2012). Physical function and independence 1 year after myocardial infarction: observations from the Translational Research Investigating Underlying disparities in recovery from acute Myocardial infarction: Patients' Health status registry. *American Heart Journal, 163*(5), 790-796. doi: 10.1016/j.ahj.2012.02.024

Department of Health. (2015). *NHS Reference Costs 2014/2015*. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/477919/2014-15_Reference_costs_publication.pdf.

Douris, P. C., Elokda, A. S., Handrakis, J. P., Principal, S., Rondo, E., Bovell, J., . . . Zimmerman, T. (2009). Martial art training enhances the glutathione antioxidant system in middle-aged adults. *Journal of Strength & Conditioning Research, 23*(5), 1518-1523. doi: 10.1519/JSC.0b013e3181b339ac

Dronkers, J. J., Chorus, A. M., van Meeteren, N. L., & Hopman-Rock, M. (2013). The association of pre-operative physical fitness and physical activity with outcome after scheduled major abdominal surgery. *Anaesthesia, 68*(1), 67-73. doi: 10.1111/anae.12066

Dronkers, J. J., Lamberts, H., Reutelingsperger, I. M., Naber, R. H., Dronkers-Landman, C. M., Veldman, A., & van Meeteren, N. L. (2010). Preoperative therapeutic programme for elderly patients scheduled for elective abdominal oncological surgery: a randomized controlled pilot study. *Clinical Rehabilitation, 24*(7), 614-622. doi: 10.1177/0269215509358941

Dunlay, S. M., Witt, B. J., Allison, T. G., Hayes, S. N., Weston, S. A., Koepsell, E., & Roger, V. L. (2009). Barriers to participation in cardiac rehabilitation. *American Heart Journal, 158*(5), 852-859. doi: 10.1016/j.ahj.2009.08.010

Dunne, D. F., Jack, S., Jones, R. P., Jones, L., Lythgoe, D. T., Malik, H. Z., . . . Fenwick, S. W. (2016). Randomized clinical trial of prehabilitation before planned liver resection. *British Journal of Surgery, 103*(5), 504-512. doi: 10.1002/bjs.10096

Durrand, J. W., Batterham, A. M., & Danjoux, G. R. (2014). Pre-habilitation. I: aggregation of marginal gains. *Anaesthesia, 69*(5), 403-406. doi: 10.1111/anae.12666

Edge, S.B., Byrd, D.R., Compton, C.C., Fritz, A.G., Greene, F.L., & Trotti, A. (2010). *AJCC cancer staging manual, 7th edition.* France: Springer.

Elokda, A. S., & Nielsen, D. H. (2007). Effects of exercise training on the glutathione antioxidant system. *European Journal of Cardiovascular Prevention & Rehabilitation, 14*(5), 630-637. doi: 10.1097/HJR.0b013e32828622d7

Erden-Inal, M., Sunal, E., & Kanbak, G. (2002). Age-related changes in the glutathione redox system. *Cell Biochemistry & Function, 20*(1), 61-66. doi: 10.1002/cbf.937

Fearon, K. C., Ljungqvist, O., Von Meyenfeldt, M., Revhaug, A., Dejong, C. H., Lassen, K., . . . Kehlet, H. (2005). Enhanced recovery after surgery: a consensus review of clinical care for patients undergoing colonic resection. *Clinical Nutrtion , 24*(3), 466-477. doi: 10.1016/j.clnu.2005.02.002

Febbraio, M. A., Mesa, J. L., Chung, J., Steensberg, A., Keller, C., Nielsen, H. B., . . . Pedersen, B. K. (2004). Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. *Cell Stress & Chaperones, 9*(4), 390-396.

Fehrenbach, E., Niess, A. M., Passek, F., Sorichter, S., Schwirtz, A., Berg, A., . . . Northoff, H. (2003). Influence of different types of exercise on the expression of haem oxygenase-1 in leukocytes. *Journal of Sports Sciences, 21*(5), 383-389. doi: 10.1080/0264041031000071164

Fehrenbach, E., Niess, A. M., Schlotz, E., Passek, F., Dickhuth, H. H., & Northoff, H. (2000a). Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *Journal of Applied Physiology , 89*(2), 704-710.

Fehrenbach, E., Passek, F., Niess, A. M., Pohla, H., Weinstock, C., Dickhuth, H. H., & Northoff, H. (2000b). HSP expression in human leukocytes is modulated by endurance exercise. *Medicine & Science in Sports & Exercise, 32*(3), 592-600.

Ferrell, B. R., Dow, K. H., & Grant, M. (1995). Measurement of the quality of life in cancer survivors. *Quality of Life Research, 4*(6), 523-531.

Fleshner, M., Campisi, J., Amiri, L., & Diamond, D. M. (2004). Cat exposure induces both intra- and extracellular Hsp72: the role of adrenal hormones. *Psychoneuroendocrinology, 29*(9), 1142-1152. doi: 10.1016/j.psyneuen.2004.01.007

Fransen, M., Nairn, L., Winstanley, J., Lam, P., & Edmonds, J. (2007). Physical activity for osteoarthritis management: a randomized controlled clinical trial evaluating hydrotherapy or Tai Chi classes. *Arthritis & Rheumatology, 57*(3), 407-414. doi: 10.1002/art.22621

Fujimori, S., Otaka, M., Otani, S., Jin, M., Okuyama, A., Itoh, S., . . . Masamune, O. (1997). Induction of a 72-kDa heat shock protein and cytoprotection against thioacetamide-induced liver injury in rats. *Digestive Disease & Sciences, 42*(9), 1987-1994.

Fujita, F., Torashima, Y., Kuroki, T., & Eguchi, S. (2014). The risk factors and predictive factors for anastomotic leakage after resection for colorectal cancer: reappraisal of the literature. *Surgery Today, 44*(9), 1595-1602. doi: 10.1007/s00595-013-0685-3

Gabai, V. L., Budagova, K. R., & Sherman, M. Y. (2005). Increased expression of the major heat shock protein Hsp72 in human prostate carcinoma cells is dispensable for their viability but confers resistance to a variety of anticancer agents. *Oncogene, 24*(20), 3328-3338. doi: 10.1038/sj.onc.1208495

Gabai, V. L., Yaglom, J. A., Waldman, T., & Sherman, M. Y. (2009). Heat shock protein Hsp72 controls oncogene-induced senescence pathways in cancer cells. *Molecular & Cellular Biology, 29*(2), 559-569. doi: 10.1128/mcb.01041-08

Garrido, C., Brunet, M., Didelot, C., Zermati, Y., Schmitt, E., & Kroemer, G. (2006). Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. *Cell Cycle, 5*(22), 2592-2601.

Giannoudis, P. V., Dinopoulos, H., Chalidis, B., & Hall, G. M. (2006). Surgical stress response. *Injury, 37 Suppl 5*, S3-9. doi: 10.1016/S0020-1383(07)70005-0

Gil, L., Siems, W., Mazurek, B., Gross, J., Schroeder, P., Voss, P., & Grune, T. (2006). Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radical Research, 40*(5), 495-505. doi: 10.1080/10715760600592962

Gillis, C., Li, C., Lee, L., Awasthi, R., Augustin, B., Gamsa, A., . . . Carli, F. (2014). Prehabilitation versus Rehabilitation: A Randomized Control Trial in Patients Undergoing Colorectal Resection for Cancer. *Anesthesiology, 121*(5), 937-947. doi: 10.1097/ALN.0000000000000393

Gjovaag, T. F., & Dahl, H. A. (2006). Effect of training and detraining on the expression of heat shock proteins in m. triceps brachii of untrained males and females. *European Journal of Applied Physiology, 98*(3), 310-322. doi: 10.1007/s00421-006-0281-y

Goldberg, A., Chavis, M., Watkins, J., & Wilson, T. (2012). The five-times-sit-to-stand test: validity, reliability and detectable change in older females. *Aging Clinical & Experimental Research, 24*(4), 339-344.

Gomes, E. C., Silva, A. N., & de Oliveira, M. R. (2012). Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative Medicine & Cell Longevity*, 756132. doi: 10.1155/2012/756132

Gozzelino, R., Jeney, V., & Soares, M. P. (2010). Mechanisms of cell protection by heme oxygenase-1. *Annual Review of Pharmacology & Toxicology, 50*, 323-354. doi: 10.1146/annurev.pharmtox.010909.105600

Grace, S. L., Gravely-Witte, S., Brual, J., Monette, G., Suskin, N., Higginson, L., . . . Stewart, D. E. (2008). Contribution of patient and physician factors to cardiac rehabilitation enrollment: a prospective multilevel study. *European Journal of Cardiovascular Prevention & Rehabilitation, 15*(5), 548-556. doi: 10.1097/HJR.0b013e328305df05

Grace, S. L., Shanmugasegaram, S., Gravely-Witte, S., Brual, J., Suskin, N., & Stewart, D. E. (2009). Barriers to cardiac rehabilitation: Does age make a difference? *Journal of Cardiopulmonary Rehabilitation & Prevention, 29*(3), 183-187. doi: 10.1097/HCR.0b013e3181a3333c

Gravely-Witte, S., De Gucht, V., Heiser, W., Grace, S. L., & Van Elderen, T. (2007). The impact of angina and cardiac history on health-related quality of life and depression in coronary heart disease patients. *Chronic Illness, 3*(1), 66-76. doi: 10.1177/1742395307079192

Gray, N. M., Hall, S. J., Browne, S., Johnston, M., Lee, A. J., Macleod, U., . . . Campbell, N. C. (2014). Predictors of anxiety and depression in people with colorectal cancer. *Supportive Care in Cancer, 22*(2), 307-314. doi: 10.1007/s00520-013-1963-8

Gremeaux, V., Troisgros, O., Benaim, S., Hannequin, A., Laurent, Y., Casillas, J. M., & Benaim, C. (2011). Determining the minimal clinically important difference for the six-minute walk test and the 200-meter fast-walk test during cardiac rehabilitation program in coronary artery disease patients after acute coronary syndrome. *Archives of Physical Medicine & Rehabilitation, 92*(4), 611-619. doi: 10.1016/j.apmr.2010.11.023

Grivicich, I., Regner, A., Zanoni, C., Correa, L. P., Jotz, G. P., Henriques, J. A., . . . da Rocha, A. B. (2007). Hsp70 response to 5-fluorouracil treatment in human colon cancer cell lines. *International Journal of Colorectal Disease, 22*(10), 1201-1208. doi: 10.1007/s00384-007-0307-x

Guo, X., Shin, V. Y., & Cho, C. H. (2001). Modulation of heme oxygenase in tissue injury and its implication in protection against gastrointestinal diseases. *Life Sciences, 69*(25-26), 3113-3119.

Gustafsson, U. O., Scott, M. J., Schwenk, W., Demartines, N., Roulin, D., Francis, N., . . . Ljungqvist, O. (2012). Guidelines for perioperative care in elective colonic surgery: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Clinical Nutrtion , 31*(6), 783-800. doi: 10.1016/j.clnu.2012.08.013

Haas, S. L., Ye, W., & Lohr, J. M. (2012). Alcohol consumption and digestive tract cancer. *Current Opinion in Clinical Nutrition & Metabolic Care , 15*(5), 457-467. doi: 10.1097/MCO.0b013e3283566699

Hakola, L., Komulainen, P., Hassinen, M., Savonen, K., Litmanen, H., Lakka, T. A., & Rauramaa, R. (2011). Cardiorespiratory fitness in aging men and women: the DR's EXTRA study. *Scandinavian Journal of Medicine & Science in Sports, 21*(5), 679-687. doi: 10.1111/j.1600-0838.2010.01127.x

Haley, S. M., & Fragala-Pinkham, M. A. (2006). Interpreting change scores of tests and measures used in physical therapy. *Physical Therapy, 86*(5), 735-743.

Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs & Aging, 18*(9), 685-716.

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine* (4th ed.). Oxford: Oxford University Press.

Hamilton, D. M., & Haennel, R. G. (2000). Validity and reliability of the 6-minute walk test in a cardiac rehabilitation population. *Journal of Cardiopulmonary Rehabilitation, 20*(3), 156-164.

Hanson, L. C., McBurney, H., & Taylor, N. F. (2012). The retest reliability of the six-minute walk test in patients referred to a cardiac rehabilitation programme. *Physiotherapy Research International, 17*(1), 55-61. doi: 10.1002/pri.513

Harada, N. D., Chiu, V., & Stewart, A. L. (1999). Mobility-related function in older adults: assessment with a 6-minute walk test. *Archives of Physical Medicine & Rehabilitation, 80*(7), 837-841.

Hardy, R. G., Meltzer, S. J., & Jankowski, J. A. (2000). ABC of colorectal cancer. Molecular basis for risk factors. *BMJ, 321*(7265), 886-889.

Harriss, D. J., Atkinson, G., Batterham, A., George, K., Cable, N. T., Reilly, T., . . . Renehan, A. G. (2009). Lifestyle factors and colorectal cancer risk (2): a systematic review and meta-analysis of associations with leisure-time physical activity. *Colorectal Disease, 11*(7), 689-701. doi: 10.1111/j.1463-1318.2009.01767.x

Health & Social Care Information Centre. (2015). *National Bowel Cancer Audit Report 2015.* Retrieved from http://www.acpgbi.org.uk/content/uploads/2016/03/nati-clin-audi-supp-prog-bowe-canc-2015.pdf

Heran, B. S., Chen, J. M., Ebrahim, S., Moxham, T., Oldridge, N., Rees, K., . . . Taylor, R. S. (2011). Exercise-based cardiac rehabilitation for coronary heart disease. *Cochrane Database of Systematic Reviews*, 7, CD001800. doi: 10.1002/14651858.CD001800.pub2

Hillman, A. R., Vince, R. V., Taylor, L., McNaughton, L., Mitchell, N., & Siegler, J. (2011). Exercise-induced dehydration with and without environmental heat stress results in increased oxidative stress. *Applied Physiology, Nutrition & Metabolism, 36*(5), 698-706. doi: 10.1139/h11-080

Hogan , B. V., Peter, M. B., Shenoy, H. G., Horgan, K.., & Hughes, T. A. (2011). Surgery induced immunosuppression. The Surgeon, 9 (1), 38-43. doi: 10.1016/j.surge.2010.07.011.

Holte, K., & Kehlet, H. (2000). Postoperative ileus: a preventable event. *British Journal of Surgery, 87*(11), 1480-1493. doi: 10.1046/j.1365-2168.2000.01595.x

Holte, K., Nielsen, K. G., Madsen, J. L., & Kehlet, H. (2004). Physiologic effects of bowel preparation. *Diseases of the Colon & Rectum, 47*(8), 1397-1402.

Horowitz, M., Maloyan, A., & Shlaier, J. (1997). HSP 70 kDa dynamics in animals undergoing heat stress superimposed on heat acclimation. *Annals of the New York Academy of Science, 813*, 617-619.

Hulzebos, E. H., Helders, P. J., Favie, N. J., De Bie, R. A., Brutel de la Riviere, A., & Van Meeteren, N. L. (2006). Preoperative intensive inspiratory muscle training to prevent postoperative pulmonary complications in high-risk patients undergoing CABG surgery: a randomized clinical trial. *JAMA, 296*(15), 1851-1857. doi: 10.1001/jama.296.15.1851

Huxley, R. R., Ansary-Moghaddam, A., Clifton, P., Czernichow, S., Parr, C. L., & Woodward, M. (2009). The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *International Journal of Cancer, 125*(1), 171-180. doi: 10.1002/ijc.24343

Hwang, T. S., Han, H. S., Choi, H. K., Lee, Y. J., Kim, Y. J., Han, M. Y., & Park, Y. M. (2003). Differential, stage-dependent expression of Hsp70, Hsp110 and Bcl-2 in colorectal cancer. *Journal of Gastroenterology & Hepatology, 18*(6), 690-700.

Ingle, L., Cleland, J. G., & Clark, A. L. (2014a). The long-term prognostic significance of 6-minute walk test distance in patients with chronic heart failure. *Biomed Research International, 2014*, 505969. doi: 10.1155/2014/505969

Ingle, L., Cleland, J. G., & Clark, A. L. (2014b). The relation between repeated 6-minute walk test performance and outcome in patients with chronic heart failure. *Annals of Physical & Rehabilitation Medicine, 57*(4), 244-253. doi: 10.1016/j.rehab.2014.03.004

Izumi, Y., Otaka, M., Takahashi, T., Takada, M., Shimada, Y., Asaoka, D., . . . Watanabe, S. (2009). Specific induction of a 72-kDa heat shock protein protects esophageal mucosa

from reflux esophagitis. *Life Sciences, 84*(15-16), 517-522. doi: 10.1016/j.lfs.2009.01.016

Jaattela, M., Wissing, D., Kokholm, K., Kallunki, T., & Egeblad, M. (1998). Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. *EMBO Journal, 17*(21), 6124-6134. doi: 10.1093/emboj/17.21.6124

Jack, S., West, M. A., Raw, D., Marwood, S., Ambler, G., Cope, T. M., . . . Grocott, M. P. (2014). The effect of neoadjuvant chemotherapy on physical fitness and survival in patients undergoing oesophagogastric cancer surgery. *European Journal of Surgical Oncology, 40*(10), 1313-1320. doi: 10.1016/j.ejso.2014.03.010

Jammes, Y., Steinberg, J. G., & Delliaux, S. (2012). Chronic fatigue syndrome: acute infection and history of physical activity affect resting levels and response to exercise of plasma oxidant/antioxidant status and heat shock proteins. *Journal of Internal Medicine, 272*(1), 74-84. doi: 10.1111/j.1365-2796.2011.02488.x

Jenkins, S. C. (2007). 6-Minute walk test in patients with COPD: clinical applications in pulmonary rehabilitation. *Physiotherapy, 93*(3), 175-182. doi: 10.1016/j.physio.2007.02.001

Jensen, B. T., Laustsen, S., Jensen, J. B., Borre, M., & Petersen, A. K. (2016). Exercise-based pre-habilitation is feasible and effective in radical cystectomy pathways-secondary results from a randomized controlled trial. *Supportive Care in Cancer*. doi: 10.1007/s00520-016-3140-3

Jensen, B. T., Petersen, A. K., Jensen, J. B., Laustsen, S., & Borre, M. (2015). Efficacy of a multiprofessional rehabilitation programme in radical cystectomy pathways: a prospective randomized controlled trial. *Scandinavian Journal of Urology, 49*(2), 133-141. doi: 10.3109/21681805.2014.967810

Jette, A. M., Jette, D. U., Ng, J., Plotkin, D. J., & Bach, M. A. (1999). Are performance-based measures sufficiently reliable for use in multicenter trials? Musculoskeletal Impairment (MSI) Study Group. *Journal of Gerontology: Biological Sciences & Medical Sciences, 54*(1), M3-6.

Jiang, Y., Ben, Q., Shen, H., Lu, W., Zhang, Y., & Zhu, J. (2011). Diabetes mellitus and incidence and mortality of colorectal cancer: a systematic review and meta-analysis of cohort studies. *European Journal of Epidemiology, 26*(11), 863-876. doi: 10.1007/s10654-011-9617-y

Jin, X., Wang, R., Xiao, C., Cheng, L., Wang, F., Yang, L., . . . Wu, T. (2004). Serum and lymphocyte levels of heat shock protein 70 in aging: a study in the normal Chinese population. *Cell Stress & Chaperones, 9*(1), 69-75.

Jones, D. P., Mody, V. C., Jr., Carlson, J. L., Lynn, M. J., & Sternberg, P., Jr. (2002). Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. *Free Radical Biology & Medicine, 33*(9), 1290-1300.

Jones, J. M., Olson, K., Catton, P., Catton, C. N., Fleshner, N. E., Krzyzanowska, M. K., . . . Howell, D. (2016). Cancer-related fatigue and associated disability in post-treatment cancer survivors. *Journal of Cancer Survivorship, 10*(1), 51-61. doi: 10.1007/s11764-015-0450-2

Jones, L. W., Eves, N. D., Peddle, C. J., Courneya, K. S., Haykowsky, M., Kumar, V., . . . Reiman, T. (2009). Effects of presurgical exercise training on systemic inflammatory markers among patients with malignant lung lesions. *Applied Physiology, Nutrition & Metabolism, 34*(2), 197-202. doi: 10.1139/H08-104

Jones, L. W., Peddle, C. J., Eves, N. D., Haykowsky, M. J., Courneya, K. S., Mackey, J. R., . . . Reiman, T. (2007). Effects of presurgical exercise training on cardiorespiratory fitness among patients undergoing thoracic surgery for malignant lung lesions. *Cancer, 110*(3), 590-598. doi: 10.1002/cncr.22830

Jung, B., Lannerstad, O., Pahlman, L., Arodell, M., Unosson, M., & Nilsson, E. (2007). Preoperative mechanical preparation of the colon: the patient's experience. *BMC Surgery, 7*, 5. doi: 10.1186/1471-2482-7-5

Kahraman, A., Cakar, H., & Koken, T. (2012). The protective effect of quercetin on long-term alcohol consumption-induced oxidative stress. *Molecular Biology Reports, 39*(3), 2789-2794. doi: 10.1007/s11033-011-1037-2

Kamper, A. M., Stott, D. J., Hyland, M., Murray, H. M., & Ford, I. (2005). Predictors of functional decline in elderly people with vascular risk factors or disease. *Age & Ageing, 34*(5), 450-455. doi: 10.1093/ageing/afi137

Kanazawa, Y., Isomoto, H., Oka, M., Yano, Y., Soda, H., Shikuwa, S., . . . Kohno, S. (2003). Expression of heat shock protein (Hsp) 70 and Hsp 40 in colorectal cancer. *Medical Oncology, 20*(2), 157-164. doi: 10.1385/mo:20:2:157

Karolkiewicz, J., Michalak, E., Pospieszna, B., Deskur-Smielecka, E., Nowak, A., & Pilaczynska-Szczesniak, L. (2009). Response of oxidative stress markers and antioxidant parameters to an 8-week aerobic physical activity program in healthy, postmenopausal women. *Archives of Gerontology & Geriatrics, 49*(1), e67-71. doi: 10.1016/j.archger.2008.09.001

Karolkiewicz, J., Szczesniak, L., Deskur-Smielecka, E., Nowak, A., Stemplewski, R., & Szeklicki, R. (2003). Oxidative stress and antioxidant defense system in healthy, elderly men: relationship to physical activity. *The Aging Male, 6*(2), 100-105.

Kehlet, H. (1997). Multimodal approach to control postoperative pathophysiology and rehabilitation. *British Journal of Anaesthesia, 78*(5), 606-617.

Kehlet, H., & Wilmore, D. W. (2002). Multimodal strategies to improve surgical outcome. *American Journal of Surgery, 183*(6), 630-641.

Kehlet, H., & Wilmore, D. W. (2005). Fast-track surgery. *British Journal of Surgery, 92*(1), 3-4. doi: 10.1002/bjs.4841

Kennedy, D., Jager, R., Mosser, D. D., & Samali, A. (2014). Regulation of apoptosis by heat shock proteins. *IUBMB Life, 66*(5), 327-338. doi: 10.1002/iub.1274

Kerrigan, D. C., Lee, L. W., Collins, J. J., Riley, P. O., & Lipsitz, L. A. (2001). Reduced hip extension during walking: healthy elderly and fallers versus young adults. *Archives of Physical Medicine & Rehabilitation, 82*(1), 26-30. doi: 10.1053/apmr.2001.18584

Kervio, G., Carre, F., & Ville, N. S. (2003). Reliability and intensity of the six-minute walk test in healthy elderly subjects. *Medicine & Science in Sports & Exercise, 35*(1), 169-174. doi: 10.1249/01.MSS.0000043545.02712.A7

Kervio, G., Ville, N. S., Leclercq, C., Daubert, J. C., & Carre, F. (2004). Intensity and daily reliability of the six-minute walk test in moderate chronic heart failure patients. *Archives of Physical Medicine & Rehabilitation, 85*(9), 1513-1518.

Khalil, A. A., Kabapy, N. F., Deraz, S. F., & Smith, C. (2011). Heat shock proteins in oncology: diagnostic biomarkers or therapeutic targets? *Biochimica et Biophysica Acta, 1816*(2), 89-104. doi: 10.1016/j.bbcan.2011.05.001

Khan, A. A., Wheeler, J. M., Cunningham, C., George, B., Kettlewell, M., & Mortensen, N. J. (2008). The management and outcome of anastomotic leaks in colorectal surgery. *Colorectal Disease, 10*(6), 587-592. doi: 10.1111/j.1463-1318.2007.01417.x

Khan, S., Gatt, M., Horgan, A., Anderson, I., & MacFie, J. (2009). *Guidelines for implementation of enhanced recovery protocols*. Association of Surgeons of Great Britain and Ireland, London.

Khassaf, M., McArdle, A., Esanu, C., Vasilaki, A., McArdle, F., Griffiths, R. D., . . . Jackson, M. J. (2003). Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *Journal of Physiology, 549*(Pt 2), 645-652. doi: 10.1113/jphysiol.2003.040303

Kiang, J. G., & Tsokos, G. C. (1998). Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacology & Therapeutics, 80*(2), 183-201.

Kiecolt-Glaser, J. K., Page, G. G., Marucha, P. T., MacCallum, R. C., & Glaser, R. (1998). Psychological influences on surgical recovery. Perspectives from psychoneuroimmunology. *American Psychologist, 53*(11), 1209-1218.

Kim do, J., Mayo, N. E., Carli, F., Montgomery, D. L., & Zavorsky, G. S. (2009). Responsive measures to prehabilitation in patients undergoing bowel resection surgery. *Tohoku Journal of Experimental Medicine, 217*(2), 109-115.

Kirchhoff, P., Clavien, P. A., & Hahnloser, D. (2010). Complications in colorectal surgery: risk factors and preventive strategies. *Patient Safety in Surgery, 4*(1), 5. doi: 10.1186/1754-9493-4-5

Kocsis, J., Madaras, B., Toth, E. K., Fust, G., & Prohaszka, Z. (2010). Serum level of soluble 70-kD heat shock protein is associated with high mortality in patients with colorectal

cancer without distant metastasis. *Cell Stress & Chaperones, 15*(2), 143-151. doi: 10.1007/s12192-009-0128-7

Kothmann, E., Batterham, A. M., Owen, S. J., Turley, A. J., Cheesman, M., Parry, A., & Danjoux, G. (2009). Effect of short-term exercise training on aerobic fitness in patients with abdominal aortic aneurysms: a pilot study. *British Journal of Anaesthesia, 103*(4), 505-510. doi: 10.1093/bja/aep205

Kregel, K. C. (2002). Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physiology (1985), 92*(5), 2177-2186. doi: 10.1152/japplphysiol.01267.2001

Kristjánsdóttir, Á., Ragnarsdóttir, M., Einarsson, M. B., & Torfason, B. (2004). A Comparison of the 6-Minute Walk Test and Symptom Limited Graded Exercise Test for Phase II Cardiac Rehabilitation of Older Adults. *Journal of Geriatric Physical Therapy, 27*(2), 65-68.

Kurz, A., Fleischmann, E., Sessler, D. I., Buggy, D. J., Apfel, C., & Akca, O. (2015). Effects of supplemental oxygen and dexamethasone on surgical site infection: a factorial randomized trialdouble dagger. *British Journal of Anaesthesia, 115*(3), 434-443. doi: 10.1093/bja/aev062

Kvam, S., Kleppe, C. L., Nordhus, I. H., & Hovland, A. (2016). Exercise as a treatment for depression: A meta-analysis. *Journal of Affective Disorders, 202*, 67-86. doi: 10.1016/j.jad.2016.03.063

Lancaster, G. (2015). Pilot and feasibility studies come of age! *Pilot and Feasibility Studies, 1*(1), 1-4. doi: 10.1186/2055-5784-1-1

Lancaster, G. A., Dodd, S., & Williamson, P. R. (2004). Design and analysis of pilot studies: recommendations for good practice. *Journal of Evaluation in Clinical Practice, 10*(2), 307-312. doi: 10.1111/j.2002.384.doc.x

Lane, D., Carroll, D., Ring, C., Beevers, D. G., & Lip, G. Y. (2002). The prevalence and persistence of depression and anxiety following myocardial infarction. *British Journal of Health Psychology, 7*(Pt 1), 11-21. doi: 10.1348/135910702169321

Larsson, S. C., & Wolk, A. (2006). Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. *International Journal of Cancer, 119*(11), 2657-2664. doi: 10.1002/ijc.22170

Lassen, K., Coolsen, M. M., Slim, K., Carli, F., de Aguilar-Nascimento, J. E., Schafer, M., . . . Dejong, C. H. (2012). Guidelines for perioperative care for pancreaticoduodenectomy: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Clinical Nutrtion , 31*(6), 817-830. doi: 10.1016/j.clnu.2012.08.011

Lassen, K., Soop, M., Nygren, J., Cox, P. B., Hendry, P. O., Spies, C., . . . Dejong, C. H. (2009). Consensus review of optimal perioperative care in colorectal surgery: Enhanced Recovery After Surgery (ERAS) Group recommendations. *Archives of Surgery, 144*(10), 961-969. doi: 10.1001/archsurg.2009.170

Lee, B. J., Emery-Sinclair, E. L., Mackenzie, R. W., Hussain, A., Taylor, L., James, R. S., & Thake, C. D. (2014). The impact of submaximal exercise during heat and/or hypoxia on the cardiovascular and monocyte HSP72 responses to subsequent (post 24 h) exercise in hypoxia. *Extreme Physiology & Medicine, 3*, 15. doi: 10.1186/2046-7648-3-15

Lee, B. J., Mackenzie, R. W., Cox, V., James, R. S., & Thake, C. D. (2015). Human monocyte heat shock protein 72 responses to acute hypoxic exercise after 3 days of exercise heat acclimation. *Biomed Research International, 2015*, 849809. doi: 10.1155/2015/849809

Lee, L., Schwartzman, K., Carli, F., Zavorsky, G. S., Li, C., Charlebois, P., . . . Feldman, L. S. (2013). The association of the distance walked in 6 min with pre-operative peak oxygen consumption and complications 1 month after colorectal resection. *Anaesthesia, 68*(8), 811-816. doi: 10.1111/anae.12329

Lee, R., Margaritis, M., Channon, K. M., & Antoniades, C. (2012). Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. *Current Medicinal Chemistry, 19*(16), 2504-2520.

Lemanu, D. P., Singh, P. P., MacCormick, A. D., Arroll, B., & Hill, A. G. (2013). Effect of preoperative exercise on cardiorespiratory function and recovery after surgery: a systematic review. *World Journal of Surgery, 37*(4), 711-720. doi: 10.1007/s00268-012-1886-4

Lemmink, K., Han, K., de Greef, M. H. G., Rispens, P., & Stevens, M. (2001). Reliability of the Groningen Fitness Test for the Elderly. *Journal of Aging and Physical Activity, 9*(2), 194-212.

Lennon, S. L., Quindry, J. C., French, J. P., Kim, S., Mehta, J. L., & Powers, S. K. (2004). Exercise and myocardial tolerance to ischaemia-reperfusion. *Acta Physiologica Scandinavica, 182*(2), 161-169. doi: 10.1111/j.1365-201X.2004.01346.x

Levett, D. Z., & Grocott, M. P. (2015). Cardiopulmonary exercise testing, prehabilitation, and Enhanced Recovery After Surgery (ERAS). *Canadian Journal of Anaesthesia, 62*(2), 131-142. doi: 10.1007/s12630-014-0307-6

Levy, M. M., Fink, M. P., Marshall, J. C., Abraham, E., Angus, D., Cook, D., . . . Ramsay, G. (2003). 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Medicine, 29*(4), 530-538. doi: 10.1007/s00134-003-1662-x

Lewis, S. J., Egger, M., Sylvester, P. A., & Thomas, S. (2001). Early enteral feeding versus "nil by mouth" after gastrointestinal surgery: systematic review and meta-analysis of controlled trials. *BMJ, 323*(7316), 773-776.

Li, C., Carli, F., Lee, L., Charlebois, P., Stein, B., Liberman, A. S., . . . Feldman, L. S. (2013). Impact of a trimodal prehabilitation program on functional recovery after colorectal cancer surgery: a pilot study. *Surgical Endoscopy, 27*(4), 1072-1082. doi: 10.1007/s00464-012-2560-5

Li, C. Y., Lee, J. S., Ko, Y. G., Kim, J. I., & Seo, J. S. (2000). Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase-3 activation. *Journal of Biological Chemistry, 275*(33), 25665-25671. doi: 10.1074/jbc.M906383199

Liang, P. S., Chen, T. Y., & Giovannucci, E. (2009). Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. *International Journal of Cancer, 124*(10), 2406-2415. doi: 10.1002/ijc.24191

Liao, Y. F., Zhu, W., Li, D. P., & Zhu, X. (2013). Heme oxygenase-1 and gut ischemia/reperfusion injury: A short review. *World Journal of Gastroenterology, 19*(23), 3555-3561. doi: 10.3748/wjg.v19.i23.3555

Lin, Y. C., Davey, R. C., & Cochrane, T. (2001). Tests for physical function of the elderly with knee and hip osteoarthritis. *Scandinavian Journal of Medicine & Science in Sports, 11*(5), 280-286.

Linden, W., Vodermaier, A., Mackenzie, R., & Greig, D. (2012). Anxiety and depression after cancer diagnosis: prevalence rates by cancer type, gender, and age. *Journal of Affective Disorders, 141*(2-3), 343-351. doi: 10.1016/j.jad.2012.03.025

Liu, R. M., & Dickinson, D. A. (2003). Decreased synthetic capacity underlies the age-associated decline in glutathione content in Fisher 344 rats. *Antioxidant & Redox Signaling, 5*(5), 529-536. doi: 10.1089/152308603770310176

Ljungqvist, O., & Soreide, E. (2003). Preoperative fasting. *British Journal of Surgery, 90*(4), 400-406. doi: 10.1002/bjs.4066

Locke, M., Noble, E. G., & Atkinson, B. G. (1991). Inducible isoform of HSP70 is constitutively expressed in a muscle fiber type specific pattern. *American Journal of Physiology, 261*(5 Pt 1), C774-779.

Lovell, R., Madden, L., Carroll, S., & McNaughton, L. (2007). The time-profile of the PBMC HSP70 response to in vitro heat shock appears temperature-dependent. *Amino Acids, 33*(1), 137-144. doi: 10.1007/s00726-006-0400-4

Luo, W., Cao, Y., Liao, C., & Gao, F. (2012). Diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. *Colorectal Disease, 14*(11), 1307-1312. doi: 10.1111/j.1463-1318.2012.02875.x

Lutgens, M. W., van Oijen, M. G., van der Heijden, G. J., Vleggaar, F. P., Siersema, P. D., & Oldenburg, B. (2013). Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflammatory Bowel Disease, 19*(4), 789-799. doi: 10.1097/MIB.0b013e31828029c0

Lv, L., Shao, Y. F., & Zhou, Y. B. (2012). The enhanced recovery after surgery (ERAS) pathway for patients undergoing colorectal surgery: an update of meta-analysis of randomized controlled trials. *International Journal of Colorectal Disease, 27*(12), 1549-1554. doi: 10.1007/s00384-012-1577-5

Madden, L. A., Hayman, Y. A., Underwood, C., Vince, R. V., Greenman, J., Allsup, D., & Ali, S. (2012). Increased inducible heat shock protein 72 expression associated with PBMC isolated from patients with haematological tumours. *Scandinavian Journal of Clinical & Laboratory Investigation, 72*(5), 380-386. doi: 10.3109/00365513.2012.681683

Madden, L. A., Sandstrom, M. E., Lovell, R. J., & McNaughton, L. (2008). Inducible heat shock protein 70 and its role in preconditioning and exercise. *Amino Acids, 34*(4), 511-516. doi: 10.1007/s00726-007-0004-7

Magalhaes Fde, C., Amorim, F. T., Passos, R. L., Fonseca, M. A., Oliveira, K. P., Lima, M. R., . . . Rodrigues, L. O. (2010). Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. *Cell Stress & Chaperones, 15*(6), 885-895. doi: 10.1007/s12192-010-0197-7

Maher, P. (2005). The effects of stress and aging on glutathione metabolism. *Ageing Research Reviews, 4*(2), 288-314. doi: 10.1016/j.arr.2005.02.005

Maloyan, A., Palmon, A., & Horowitz, M. (1999). Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. *American Journal of Physiology, 276*(5 Pt 2), R1506-1515.

Markovitch, D., Tyrrell, R. M., & Thompson, D. (2008). Acute moderate-intensity exercise in middle-aged men has neither an anti- nor proinflammatory effect. *Journal of Applied Physiology (1985), 105*(1), 260-265. doi: 10.1152/japplphysiol.00096.2008

Mathias, S., Nayak, U. S., & Isaacs, B. (1986). Balance in elderly patients: the "get-up and go" test. *Archives of Physical Medicine & Rehabilitation, 67*(6), 387-389.

McCall, M. C., Ward, A., & Heneghan, C. (2015). Yoga in adult cancer: a pilot survey of attitudes and beliefs among oncologists. *Curr Oncol, 22*(1), 13-19. doi: 10.3747/co.22.2129

McClung, J. P., Hasday, J. D., He, J. R., Montain, S. J., Cheuvront, S. N., Sawka, M. N., & Singh, I. S. (2008). Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. *American Journal of Physiology: Regulatory, Integrative & Comparative Physiology, 294*(1), R185-191. doi: 10.1152/ajpregu.00532.2007

Meneses-Echavez, J. F., Gonzalez-Jimenez, E., & Ramirez-Velez, R. (2015). Supervised exercise reduces cancer-related fatigue: a systematic review. *Journal of Physiotherapy, 61*(1), 3-9. doi: 10.1016/j.jphys.2014.08.019

Menz, H. B., Morris, M. E., & Lord, S. R. (2005). Foot and ankle characteristics associated with impaired balance and functional ability in older people. *Journal of Gerontology: Biological Sciences & Medical Sciences, 60*(12), 1546-1552.

Menz, H. B., Morris, M. E., & Lord, S. R. (2006). Foot and ankle risk factors for falls in older people: a prospective study. *Journal of Gerontology: Biological Sciences & Medical Sciences, 61*(8), 866-870.

Mesquita, R., Janssen, D. J., Wouters, E. F., Schols, J. M., Pitta, F., & Spruit, M. A. (2013). Within-day test-retest reliability of the Timed Up & Go test in patients with advanced chronic organ failure. *Archives of Physical Medicine & Rehabilitation, 94*(11), 2131-2138. doi: 10.1016/j.apmr.2013.03.024

Milicevic, Z. T., Petkovic, M. Z., Drndarevic, N. C., Pavlovic, M. D., & Todorovic, V. N. (2007). Expression of heat shock protein 70 (HSP70) in patients with colorectal adenocarcinoma--immunohistochemistry and Western blot analysis. *Neoplasma, 54*(1), 37-45.

Miyazaki, H., Oh-ishi, S., Ookawara, T., Kizaki, T., Toshinai, K., Ha, S., . . . Ohno, H. (2001). Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *European Journal of Applied Physiology, 84*(1-2), 1-6. doi: 10.1007/s004210000342

Mock, V. (2001). Fatigue management: evidence and guidelines for practice. *Cancer, 92*(6 Suppl), 1699-1707.

Monkhouse, D. (2006). Postoperative sepsis. *Current Anaesthesia & Critical Care, 17*(1), 65-70.

Mons, U., Muscat, J. E., Modesto, J., Richie Jr, J. P., & Brenner, H. (2016). Effect of smoking reduction and cessation on the plasma levels of the oxidative stress biomarker

glutathione – Post-hoc analysis of data from a smoking cessation trial. *Free Radical Biology and Medicine, 91*, 172-177. doi: 10.1016/j.freeradbiomed.2015.12.018
Montgomery, P. S., & Gardner, A. W. (1998). The clinical utility of a six-minute walk test in peripheral arterial occlusive disease patients. *Journal of the American Geriatrics Society, 46*(6), 706-711.
Moore, L. J., Moore, F. A., Jones, S. L., Xu, J., & Bass, B. L. (2009). Sepsis in general surgery: a deadly complication. *American Journal of Surgery, 198*(6), 868-874. doi: 10.1016/j.amjsurg.2009.05.025
Moriello, C., Mayo, N. E., Feldman, L., & Carli, F. (2008). Validating the six-minute walk test as a measure of recovery after elective colon resection surgery. *Archives of Physical Medicine & Rehabilitation, 89*(6), 1083-1089. doi: 10.1016/j.apmr.2007.11.031
Morimoto, R. I. (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes & Development, 12*(24), 3788-3796.
Morrow, G. R., Andrews, P. L., Hickok, J. T., Roscoe, J. A., & Matteson, S. (2002). Fatigue associated with cancer and its treatment. *Supportive Care in Cancer, 10*(5), 389-398. doi: 10.1007/s005200100293
Mortensen, K., Nilsson, M., Slim, K., Schafer, M., Mariette, C., Braga, M., . . . Lassen, K. (2014). Consensus guidelines for enhanced recovery after gastrectomy: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *British Journal of Surgery, 101*(10), 1209-1229. doi: 10.1002/bjs.9582
Morton, J. P., MacLaren, D. P., Cable, N. T., Bongers, T., Griffiths, R. D., Campbell, I. T., . . . Drust, B. (2006). Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. *Journal of Applied Physiology (1985), 101*(1), 176-182. doi: 10.1152/japplphysiol.00046.2006
Morton, J. P., Maclaren, D. P., Cable, N. T., Campbell, I. T., Evans, L., Kayani, A. C., . . . Drust, B. (2008). Trained men display increased basal heat shock protein content of skeletal muscle. *Medicine & Science in Sports & Exercise, 40*(7), 1255-1262. doi: 10.1249/MSS.0b013e31816a7171
Mosoni, L., Breuille, D., Buffiere, C., Obled, C., & Mirand, P. P. (2004). Age-related changes in glutathione availability and skeletal muscle carbonyl content in healthy rats. *Experimental Gerontology, 39*(2), 203-210. doi: 10.1016/j.exger.2003.10.014
Mosser, D. D., Caron, A. W., Bourget, L., Denis-Larose, C., & Massie, B. (1997). Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Molecular & Cellular Biology, 17*(9), 5317-5327.
Mosser, D. D., Duchaine, J., & Massie, B. (1993). The DNA-binding activity of the human heat shock transcription factor is regulated in vivo by hsp70. *Molecular & Cellular Biology, 13*(9), 5427-5438.
Mosser, D. D., & Morimoto, R. I. (2004). Molecular chaperones and the stress of oncogenesis. *Oncogene, 23*(16), 2907-2918. doi: 10.1038/sj.onc.1207529
Muller, F. L., Liu, Y., & Van Remmen, H. (2004). Complex III releases superoxide to both sides of the inner mitochondrial membrane. *Journal of Biological Chemistry, 249* (47), 49064-49073.
Murphy, M. E. (2013). The HSP70 family and cancer. *Carcinogenesis, 34*(6), 1181-1188. doi: 10.1093/carcin/bgt111
Mymrikov, E. V., Seit-Nebi, A. S., & Gusev, N. B. (2011). Large potentials of small heat shock proteins. *Physiological Reviews, 91*(4), 1123-1159. doi: 10.1152/physrev.00023.2010
Nadin, S. B., Vargas-Roig, L. M., Drago, G., Ibarra, J., & Ciocca, D. R. (2007). Hsp27, Hsp70 and mismatch repair proteins hMLH1 and hMSH2 expression in peripheral blood lymphocytes from healthy subjects and cancer patients. *Cancer Letters, 252*(1), 131-146. doi: 10.1016/j.canlet.2006.12.028
Newsholme, P., Krause, M., Newsholme, E. A., Stear, S. J., Burke, L. M., & Castell, L. M. (2011). BJSM reviews: A to Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance--part 18. *British Journal of Sports Medicine, 45*(3), 230-232. doi: 10.1136/bjsm.2010.080978

NHS. (2014). *Bowel cancer - Treatment.* Retrieved 5th June, 2016, from http://www.nhs.uk/conditions/cancer-of-the-colon-rectum-or-bowel/Pages/treatment.aspx

National Institute for Health and Care Excellence. (2011). *Colorectal cancer: the diagnosis and management of colorectal cancer*. Retrieved from https://www.nice.org.uk/guidance/cg131

Nici, L., Donner, C., Wouters, E., Zuwallack, R., Ambrosino, N., Bourbeau, J., . . . Troosters, T. (2006). American Thoracic Society/European Respiratory Society statement on pulmonary rehabilitation. *American Journal of Respiratory & Critical Care Medicine, 173*(12), 1390-1413. doi: 10.1164/rccm.200508-1211ST

Niess, A. M., Passek, F., Lorenz, I., Schneider, E. M., Dickhuth, H. H., Northoff, H., & Fehrenbach, E. (1999). Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes. *Free Radical Biology & Medicine, 26*(1-2), 184-192.

Njemini, R., Abeele, M. V., Demanet, C., Lambert, M., Vandebosch, S., & Mets, T. (2002). Age-related decrease in the inducibility of heat-shock protein 70 in human peripheral blood mononuclear cells. *Journal of Clinical Immunology, 22*(4), 195-205.

Njemini, R., Lambert, M., Demanet, C., Kooijman, R., & Mets, T. (2007). Basal and infection-induced levels of heat shock proteins in human aging. *Biogerontology, 8*(3), 353-364. doi: 10.1007/s10522-006-9078-y

Njemini, R., Lambert, M., Demanet, C., & Mets, T. (2005). Heat shock protein 32 in human peripheral blood mononuclear cells: effect of aging and inflammation. *Journal of Clinical Immunology, 25*(5), 405-417. doi: 10.1007/s10875-005-5361-y

Nocera, J. R., Stegemoller, E. L., Malaty, I. A., Okun, M. S., Marsiske, M., & Hass, C. J. (2013). Using the Timed Up & Go test in a clinical setting to predict falling in Parkinson's disease. *Archives of Physical Medicine & Rehabilitation, 94*(7), 1300-1305. doi: 10.1016/j.apmr.2013.02.020

O'Connell, S. (2014). Barriers to attending cardiac rehabilitation. *Nursing Times, 110*(19), 15-17.

O'Doherty, A. F., West, M., Jack, S., & Grocott, M. P. (2013). Preoperative aerobic exercise training in elective intra-cavity surgery: a systematic review. *British Journal of Anaesthesia, 110*(5), 679-689. doi: 10.1093/bja/aes514

Odashima, M., Otaka, M., Jin, M., Wada, I., Horikawa, Y., Matsuhashi, T., . . . Watanabe, S. (2006). Zinc L-carnosine protects colonic mucosal injury through induction of heat shock protein 72 and suppression of NF-kappaB activation. *Life Sciences, 79*(24), 2245-2250. doi: 10.1016/j.lfs.2006.07.032

Older, P., Hall, A., & Hader, R. (1999). Cardiopulmonary exercise testing as a screening test for perioperative management of major surgery in the elderly. *Chest, 116*(2), 355-362.

Otaka, M., Odashima, M., & Watanabe, S. (2006). Role of heat shock proteins (molecular chaperones) in intestinal mucosal protection. *Biochemical & Biophysical Research Communications, 348*(1), 1-5. doi: 10.1016/j.bbrc.2006.07.028

Otani, S., Otaka, M., Jin, M., Okuyama, A., Itoh, S., Iwabuchi, A., . . . Masamune, O. (1997). Effect of preinduction of heat shock proteins on acetic acid-induced colitis in rats. *Digestive Disease & Sciences, 42*(4), 833-846.

Ozdemirler Erata, G., Kanbagli, O., Durlanik, O., Bulut, T., Toker, G., & Uysal, M. (2005). Induced oxidative stress and decreased expression of inducible heat shock protein 70 (ihsp 70) in patients with colorectal adenocarcinomas. *Japanese Journal of Clinical Oncology, 35*(2), 74-78. doi: 10.1093/jjco/hyi022

Pappas-Gogos, G., Tellis, C., Lasithiotakis, K., Tselepis, A. D., Tsimogiannis, K., Tsimoyiannis, E., . . . Chrysos, E. (2013). Oxidative stress markers in laparoscopic versus open colectomy for cancer: a double-blind randomized study. *Surgical Endoscopy, 27*(7), 2357-2365. doi: 10.1007/s00464-013-2788-8

Paulsen, G., Vissing, K., Kalhovde, J. M., Ugelstad, I., Bayer, M. L., Kadi, F., . . . Raastad, T. (2007). Maximal eccentric exercise induces a rapid accumulation of small heat shock proteins on myofibrils and a delayed HSP70 response in humans. *American Journal of Physiology: Regulatory, Integrative & Comparative Physiology, 293*(2), R844-853. doi: 10.1152/ajpregu.00677.2006

Peart, D. J., Kirk, R. J., Hillman, A. R., Madden, L. A., Siegler, J. C., & Vince, R. V. (2012). The physiological stress response to high-intensity sprint exercise following the ingestion of sodium bicarbonate. *European Journal of Applied Physiology*. doi: 10.1007/s00421-012-2419-4

Peart, D. J., Kirk, R. J., Madden, L. A., Siegler, J. C., & Vince, R. V. (2013). The influence of exogenous carbohydrate provision and pre-exercise alkalosis on the heat shock protein response to prolonged interval cycling. *Amino Acids, 44*(3), 903-910. doi: 10.1007/s00726-012-1419-3

Peart, D. J., McNaughton, L. R., Midgley, A. W., Taylor, L., Towlson, C., Madden, L. A., & Vince, R. V. (2011). Pre-exercise alkalosis attenuates the heat shock protein 72 response to a single-bout of anaerobic exercise. *Journal of Science & Medicine in Sport, 14*(5), 435-440. doi: 10.1016/j.jsams.2011.03.006

Peddle, C. J., Jones, L. W., Eves, N. D., Reiman, T., Sellar, C. M., Winton, T., & Courneya, K. S. (2009). Effects of presurgical exercise training on quality of life in patients undergoing lung resection for suspected malignancy: a pilot study. *Cancer Nursing, 32*(2), 158-165. doi: 10.1097/NCC.0b013e3181982ca1

Perera, S., Mody, S. H., Woodman, R. C., & Studenski, S. A. (2006). Meaningful change and responsiveness in common physical performance measures in older adults. *Journal of the American Geriatric Society, 54*(5), 743-749. doi: 10.1111/j.1532-5415.2006.00701.x

Piccinelli, M., & Wilkinson, G. (2000). Gender differences in depression. Critical review. *British Journal of Psychiatry, 177*, 486-492.

Podsiadlo, D., & Richardson, S. (1991). The timed "Up & Go": a test of basic functional mobility for frail elderly persons. *Journal of the American Geriatric Society, 39*(2), 142-148.

Polkey, M. I., Spruit, M. A., Edwards, L. D., Watkins, M. L., Pinto-Plata, V., Vestbo, J., . . . Celli, B. (2013). Six-minute-walk test in chronic obstructive pulmonary disease: minimal clinically important difference for death or hospitalization. *American Journal of Respiratory & Critical Care Medicine, 187*(4), 382-386. doi: 10.1164/rccm.201209-1596OC

Pommergaard, H. C., Gessler, B., Burcharth, J., Angenete, E., Haglind, E., & Rosenberg, J. (2014). Preoperative risk factors for anastomotic leakage after resection for colorectal cancer: a systematic review and meta-analysis. *Colorectal Disease, 16*(9), 662-671. doi: 10.1111/codi.12618

Portney, L., & Watkins, M. (2008). Foundations of clinical research: applications to practice. *Prentice Hall, Upper Saddle River*.

Pouwels, S., Stokmans, R. A., Willigendael, E. M., Nienhuijs, S. W., Rosman, C., van Ramshorst, B., & Teijink, J. A. (2014). Preoperative exercise therapy for elective major abdominal surgery: a systematic review. *International Journal of Surgery, 12*(2), 134-140. doi: 10.1016/j.ijsu.2013.11.018

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews, 88*(4), 1243-1276. doi: 10.1152/physrev.00031.2007

Powers, S. K., Locke, & Demirel, H. A. (2001). Exercise, heat shock proteins, and myocardial protection from I-R injury. *Medicine & Science in Sports & Exercise, 33*(3), 386-392.

Powers, S. K., Quindry, J. C., & Kavazis, A. N. (2008). Exercise-induced cardioprotection against myocardial ischemia-reperfusion injury. *Free Radical Biology & Medicine, 44*(2), 193-201. doi: 10.1016/j.freeradbiomed.2007.02.006

Preville, X., Salvemini, F., Giraud, S., Chaufour, S., Paul, C., Stepien, G., . . . Arrigo, A. P. (1999). Mammalian small stress proteins protect against oxidative stress through their ability to increase glucose-6-phosphate dehydrogenase activity and by maintaining optimal cellular detoxifying machinery. *Experimental Cell Research, 247*(1), 61-78. doi: 10.1006/excr.1998.4347

Quindry, J. C., Hamilton, K. L., French, J. P., Lee, Y., Murlasits, Z., Tumer, N., & Powers, S. K. (2007). Exercise-induced HSP-72 elevation and cardioprotection against infarct and apoptosis. *Journal of Applied Physiology , 103*(3), 1056-1062. doi: 10.1152/japplphysiol.00263.2007

Quinn, L. A., Moore, G. E., Morgan, R. T., & Woods, L. K. (1979). Cell lines from human colon carcinoma with unusual cell products, double minutes, and homogeneously staining regions. *Cancer Research, 39*(12), 4914-4924.

Ramachandran, A., Patra, S., & Balasubramanian, K. A. (2001). Intestinal mitochondrial dysfunction in surgical stress. *Journal of Surgical Research, 99*(1), 120-128. doi: 10.1006/jsre.2001.6104

Rashmi, R., Kumar, S., & Karunagaran, D. (2004). Ectopic expression of Hsp70 confers resistance and silencing its expression sensitizes human colon cancer cells to curcumin-induced apoptosis. *Carcinogenesis, 25*(2), 179-187. doi: 10.1093/carcin/bgh001

Rebar, A. L., Stanton, R., Geard, D., Short, C., Duncan, M. J., & Vandelanotte, C. (2015). A meta-meta-analysis of the effect of physical activity on depression and anxiety in non-clinical adult populations. *Health Psychology Review, 9*(3), 366-378. doi: 10.1080/17437199.2015.1022901

Reddy, K. M., Meyer, C. E., Palazzo, F. F., Conaghan, P., Blunt, M. C., Stebbings, W. S., . . . Cullen, P. T. (2003). Postoperative stay following colorectal surgery: a study of factors associated with prolonged hospital stay. *Annals of the Royal College of Surgeons of England, 85*(2), 111-114. doi: 10.1308/003588403321219894

Redmond, H. P., Watson, R. W., Houghton, T., Condron C, Watson RG, Bouchier-Hayes D. (1994). Immune function in patietnts undergoing open vs laparoscopic cholecytectomy. *Archives of surgery. 129* (12), 1240-1246.

Rejbi, I. B., Trabelsi, Y., Chouchene, A., Ben Turkia, W., Ben Saad, H., Zbidi, A., . . . Tabka, Z. (2010). Changes in six-minute walking distance during pulmonary rehabilitation in patients with COPD and in healthy subjects. *International Journal of Chronic Obstructive Pulmonary Disease, 5*, 209-215.

Rejeski, W. J., Ettinger, W. H., Jr., Schumaker, S., James, P., Burns, R., & Elam, J. T. (1995). Assessing performance-related disability in patients with knee osteoarthritis. *Osteoarthritis Cartilage, 3*(3), 157-167.

Rejeski, W. J., Foy, C. G., Brawley, L. R., Brubaker, P. H., Focht, B. C., Norris, J. L., 3rd, & Smith, M. L. (2002). Older adults in cardiac rehabilitation: a new strategy for enhancing physical function. *Medicine & Science in Sports & Exercise, 34*(11), 1705-1713. doi: 10.1249/01.mss.0000035045.50914.24

Richard, V., Kaeffer, N., & Thuillez, C. (1996). Delayed protection of the ischemic heart--from pathophysiology to therapeutic applications. *Fundamental & Clinical Pharmacology, 10*(5), 409-415.

Ridda, I., MacIntyre, C. R., Lindley, R. I., & Tan, T. C. (2010). Difficulties in recruiting older people in clinical trials: An examination of barriers and solutions. *Vaccine, 28*(4), 901-906. doi: http://dx.doi.org/10.1016/j.vaccine.2009.10.081

Ries, J. D., Echternach, J. L., Nof, L., & Gagnon Blodgett, M. (2009). Test-retest reliability and minimal detectable change scores for the timed "up & go" test, the six-minute walk test, and gait speed in people with Alzheimer disease. *Physical Therapy, 89*(6), 569-579. doi: 10.2522/ptj.20080258

Ritossa, F. (1962). A new puffing pattern induced by temperature shock and DNP in drosophila. *Experientia, 18*(12), 571-573. doi: 10.1007/BF02172188

Rodas, G., Ventura, J. L., Cadefau, J. A., Cusso, R., & Parra, J. (2000). A short training programme for the rapid improvement of both aerobic and anaerobic metabolism. *European Journal of Applied Physiology, 82*(5-6), 480-486. doi: 10.1007/s004210000223

Rooks, D. S., Huang, J., Bierbaum, B. E., Bolus, S. A., Rubano, J., Connolly, C. E., . . . Katz, J. N. (2006). Effect of preoperative exercise on measures of functional status in men and women undergoing total hip and knee arthroplasty. *Arthritis & Rheumatology, 55*(5), 700-708. doi: 10.1002/art.22223

Rosenfeldt, F., Braun, L., Spitzer, O., Bradley, S., Shepherd, J., Bailey, M., . . . Esmore, D. (2011). Physical conditioning and mental stress reduction--a randomised trial in patients undergoing cardiac surgery. *BMC Complementary & Alternative Medicine, 11*, 20. doi: 10.1186/1472-6882-11-20

Rosenfeldt, F., Wilson, M., Lee, G., Kure, C., Ou, R., Braun, L., & de Hann, J. (2013). Oxidative stress in surgery in an ageing population: Pathophysiology and therapy. *Experimental Gerontology, 48* (1), 45-54. doi: 10.1016/j.exger.2012.03.010.

Ross, R. M., Murthy, J. N., Wollak, I. D., & Jackson, A. S. (2010). The six minute walk test accurately estimates mean peak oxygen uptake. *BMC Pulmonary Medicine, 10*, 31. doi: 10.1186/1471-2466-10-31

Ruchalski, K., Mao, H., Singh, S. K., Wang, Y., Mosser, D. D., Li, F., . . . Borkan, S. C. (2003). HSP72 inhibits apoptosis-inducing factor release in ATP-depleted renal epithelial cells. *American Journal of Physiology: Cell Physiology, 285*(6), C1483-1493. doi: 10.1152/ajpcell.00049.2003

Sachdeva, A., Dalton, M., Amaragiri, S. V., & Lees, T. (2014). Graduated compression stockings for prevention of deep vein thrombosis. *Cochrane Database of Systematic Reviews*, 12, Cd001484. doi: 10.1002/14651858.CD001484.pub3

Sahrmann, S. (2002). *Diagnosis and Treatment of Movement Impairment Syndromes*. St Louis: Mosby.

Saleh, A., Srinivasula, S. M., Balkir, L., Robbins, P. D., & Alnemri, E. S. (2000). Negative regulation of the Apaf-1 apoptosome by Hsp70. *Nature Cell Biology, 2*(8), 476-483. doi: 10.1038/35019510

Samson, M. M., Meeuwsen, I. B., Crowe, A., Dessens, J. A., Duursma, S. A., & Verhaar, H. J. (2000). Relationships between physical performance measures, age, height and body weight in healthy adults. *Age & Ageing, 29*(3), 235-242.

Sandhu, S. K., & Kaur, G. (2002). Alterations in oxidative stress scavenger system in aging rat brain and lymphocytes. *Biogerontology, 3*(3), 161-173.

Sandstrom, M. E., Madden, L. A., Taylor, L., Siegler, J. C., Lovell, R. J., Midgley, A., & McNaughton, L. (2009). Variation in basal heat shock protein 70 is correlated to core temperature in human subjects. *Amino Acids, 37*(2), 279-284. doi: 10.1007/s00726-008-0144-4

Sandstrom, M. E., Siegler, J. C., Lovell, R. J., Madden, L. A., & McNaughton, L. (2008). The effect of 15 consecutive days of heat-exercise acclimation on heat shock protein 70. *Cell Stress & Chaperones, 13*(2), 169-175. doi: 10.1007/s12192-008-0022-8

Santa Mina, D., Clarke, H., Ritvo, P., Leung, Y. W., Matthew, A. G., Katz, J., . . . Alibhai, S. M. (2014). Effect of total-body prehabilitation on postoperative outcomes: a systematic review and meta-analysis. *Physiotherapy, 100*(3), 196-207. doi: 10.1016/j.physio.2013.08.008

Santarelli, R. L., Pierre, F., & Corpet, D. E. (2008). Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. *Nutrition & Cancer, 60*(2), 131-144. doi: 10.1080/01635580701684872

Sawatzky, J. A., Kehler, D. S., Ready, A. E., Lerner, N., Boreskie, S., Lamont, D., . . . Duhamel, T. A. (2014). Prehabilitation program for elective coronary artery bypass graft surgery patients: a pilot randomized controlled study. *Clinical Rehabilitation, 28*(7), 648-657. doi: 10.1177/0269215513516475

Schaubert, K., & Bohannon, R. W. (2005a). Reliability of the sit-to-stand test over dispersed test sessions. *Isokinetics & Exercise Science, 13*(2), 119-122.

Schaubert, K. L., & Bohannon, R. W. (2005b). Reliability and validity of three strength measures obtained from community-dwelling elderly persons. *Journal of Strength & Conditioning Research, 19*(3), 717-720. doi: 10.1519/R-15954.1

Schlotz, W., Hellhammer, J., Schulz, P., & Stone, A. A. (2004). Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response. *PsychosomaticMedicine, 66*(2), 207-214.

Schmidt, K., Vogt, L., Thiel, C., Jager, E., & Banzer, W. (2013). Validity of the six-minute walk test in cancer patients. *International Journal of Sports Medicine, 34*(7), 631-636. doi: 10.1055/s-0032-1323746

Schneider, U., Schwenk, H. U., & Bornkamm, G. (1977). Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. *International Journal of Cancer, 19*(5), 621-626.

Schoetz, D. J., Jr., Bockler, M., Rosenblatt, M. S., Malhotra, S., Roberts, P. L., Murray, J. J., . . . Rusin, L. C. (1997). "Ideal" length of stay after colectomy: whose ideal? *Diseases of the Colon & Rectum, 40*(7), 806-810.

Schultz, A. B. (1992). Mobility impairment in the elderly: challenges for biomechanics research. *Journal of Biomechanics, 25*(5), 519-528.

Schweiger, T., Nikolowsky, C., Starlinger, P., Traxler, D., Zimmermann, M., Birner, P., . . . Ankersmit, H. J. (2015). Stromal expression of heat-shock protein 27 is associated with worse clinical outcome in patients with colorectal cancer lung metastases. *PLoS One, 10*(3), e0120724. doi: 10.1371/journal.pone.0120724

Segura-Orti, E., & Martinez-Olmos, F. J. (2011). Test-retest reliability and minimal detectable change scores for sit-to-stand-to-sit tests, the six-minute walk test, the one-leg heel-rise test, and handgrip strength in people undergoing hemodialysis. *Physical Therapy, 91*(8), 1244-1252. doi: 10.2522/ptj.20100141

Serru, V., Baudin, B., Ziegler, F., David, J. P., Cals, M. J., Vaubourdolle, M., & Mario, N. (2001). Quantification of reduced and oxidized glutathione in whole blood samples by capillary electrophoresis. *Clinical Chemistry, 47*(7), 1321-1324.

Shastry, S., Toft, D. O., & Joyner, M. J. (2002). HSP70 and HSP90 expression in leucocytes after exercise in moderately trained humans. *Acta Physiologica Scandinavica, 175*(2), 139-146. doi: 10.1046/j.1365-201X.2002.00979.x

Shin, Y.-O., Oh, J.-K., Sohn, H.-S., Bae, J.-S., Lee, M.-Y., Lee, J.-B., . . . Matsumoto, T. (2004). Expression of exercise-induced HSP70 in long-distance runner's leukocytes. *Journal of Thermal Biology, 29*(7–8), 769-774. doi: http://dx.doi.org/10.1016/j.jtherbio.2004.08.053

Shotar, A. M. (2005). P53 and heat shock protein 70 expressions in colorectal adenocarcinoma. *Saudi Medical Journal, 26*(10), 1602-1606.

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability. *Psychological Bulletin, 86*(2), 420-428.

Shrubsole, M. J., Wu, H., Ness, R. M., Shyr, Y., Smalley, W. E., & Zheng, W. (2008). Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps. *American Journal of Epidemiology, 167*(9), 1050-1058. doi: 10.1093/aje/kwm400

Shumway-Cook, A., Brauer, S., & Woollacott, M. (2000). Predicting the probability for falls in community-dwelling older adults using the Timed Up & Go Test. *Physical Therapy, 80*(9), 896-903.

Simar, D., Malatesta, D., Badiou, S., Dupuy, A. M., & Caillaud, C. (2007). Physical activity modulates heat shock protein-72 expression and limits oxidative damage accumulation in a healthy elderly population aged 60 90 years. *Journal of Gerontology: Biological Sciences & Medical Sciences, 62*(12), 1413-1419.

Simar, D., Malatesta, D., Koechlin, C., Cristol, J. P., Vendrell, J. P., & Caillaud, C. (2004). Effect of age on Hsp72 expression in leukocytes of healthy active people. *Experimental Gerontology, 39*(10), 1467-1474. doi: 10.1016/j.exger.2004.08.002

Simar, D., Malatesta, D., Mas, E., Delage, M., & Caillaud, C. (2012). Effect of an 8-weeks aerobic training program in elderly on oxidative stress and HSP72 expression in leukocytes during antioxidant supplementation. *Journal of Nutrition, Health & Aging, 16*(2), 155-161.

Sinclair, R. C., Batterham, A. M., Davies, S., Cawthorn, L., & Danjoux, G. R. (2012). Validity of the 6 min walk test in prediction of the anaerobic threshold before major non-cardiac surgery. *British Journal of Anaesthesia, 108*(1), 30-35. doi: 10.1093/bja/aer322

Singh, F., Newton, R. U., Galvao, D. A., Spry, N., & Baker, M. K. (2013). A systematic review of pre-surgical exercise intervention studies with cancer patients. *Surgical Oncology, 22*(2), 92-104. doi: 10.1016/j.suronc.2013.01.004

Singh, R., Kolvraa, S., Bross, P., Jensen, U. B., Gregersen, N., Tan, Q., . . . Rattan, S. I. (2006). Reduced heat shock response in human mononuclear cells during aging and its association with polymorphisms in HSP70 genes. *Cell Stress & Chaperones, 11*(3), 208-215.

Singleton, K. D., & Wischmeyer, P. E. (2007). Glutamine's protection against sepsis and lung injury is dependent on heat shock protein 70 expression. *American Journal of*

Physiology: Regulatory, Integrative & Comparative Physiology, 292(5), R1839-1845. doi: 10.1152/ajpregu.00755.2006
Smith, J. A., King, P. M., Lane, R. H., & Thompson, M. R. (2003). Evidence of the effect of 'specialization' on the management, surgical outcome and survival from colorectal cancer in Wessex. *British Journal of Surgery, 90*(5), 583-592. doi: 10.1002/bjs.4085
Snowden, C. P., Prentis, J., Jacques, B., Anderson, H., Manas, D., Jones, D., & Trenell, M. (2013). Cardiorespiratory fitness predicts mortality and hospital length of stay after major elective surgery in older people. *Annals of Surgery, 257*(6), 999-1004. doi: 10.1097/SLA.0b013e31828dbac2
Soares, M. P., & Bach, F. H. (2009). Heme oxygenase-1: from biology to therapeutic potential. *Trends in Molecular Medicine, 15*(2), 50-58. doi: 10.1016/j.molmed.2008.12.004
Solaini, G., & Harris, D. A. (2005). Biochemical dysfunction in heart mitochondria exposed to ischaemia and reperfusion. *Biochemical Journal, 390*(Pt 2), 377-394. doi: 10.1042/BJ20042006
Sorensen, L. T., Jorgensen, T., Kirkeby, L. T., Skovdal, J., Vennits, B., & Wille-Jorgensen, P. (1999). Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. *British Journal of Surgery, 86*(7), 927-931. doi: 10.1046/j.1365-2168.1999.01165.x
Sparkes, A. C., & Smith, B. (2013). *Qualitative Research Methods in Sport, Exercise and Health: From Process to Product*. London: Routledge.
Stankiewicz, A. R., Lachapelle, G., Foo, C. P., Radicioni, S. M., & Mosser, D. D. (2005). Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. *Journal of Biological Chemistry, 280*(46), 38729-38739. doi: 10.1074/jbc.M509497200
Steel, R., Doherty, J. P., Buzzard, K., Clemons, N., Hawkins, C. J., & Anderson, R. L. (2004). Hsp72 inhibits apoptosis upstream of the mitochondria and not through interactions with Apaf-1. *Journal of Biological Chemistry, 279*(49), 51490-51499. doi: 10.1074/jbc.M401314200
Steenhagen, E. (2016). Enhanced Recovery After Surgery: It's Time to Change Practice! *Nutrition in Clinical Practice, 31*(1), 18-29. doi: 10.1177/0884533615622640
Steffen, T. M., Hacker, T. A., & Mollinger, L. (2002). Age- and gender-related test performance in community-dwelling elderly people: Six-Minute Walk Test, Berg Balance Scale, Timed Up & Go Test, and gait speeds. *Physical Therapy, 82*(2), 128-137.
Stratford, P. W., & Goldsmith, C. H. (1997). Use of the standard error as a reliability index of interest: an applied example using elbow flexor strength data. *Physical Therapy, 77*(7), 745-750.
Strober, W. (2001). Trypan blue exclusion test of cell viability. *Current Protocols in Immunology, Appendix 3*. doi: 10.1002/0471142735.ima03bs21
Struthers, R., Erasmus, P., Holmes, K., Warman, P., Collingwood, A., & Sneyd, J. R. (2008). Assessing fitness for surgery: a comparison of questionnaire, incremental shuttle walk, and cardiopulmonary exercise testing in general surgical patients. *British Journal of Anaesthesia, 101*(6), 774-780. doi: 10.1093/bja/aen310
Su'a, B. U., Pollock, T. T., Lemanu, D. P., MacCormick, A. D., Connolly, A. B., & Hill, A. G. (2015). Chewing gum and postoperative ileus in adults: a systematic literature review and meta-analysis. *International Journal of Surgery, 14*, 49-55. doi: 10.1016/j.ijsu.2014.12.032
Swain, D. P., & Franklin, B. A. (2002). VO(2) reserve and the minimal intensity for improving cardiorespiratory fitness. *Medicine & Science in Sports & Exercise, 34*(1), 152-157.
Swan, E. (2006). *Colorectal Cancer*: Wiley.
Swank, A. M., Kachelman, J. B., Bibeau, W., Quesada, P. M., Nyland, J., Malkani, A., & Topp, R. V. (2011). Prehabilitation before total knee arthroplasty increases strength and function in older adults with severe osteoarthritis. *Journal of Strength & Conditioning Research, 25*(2), 318-325. doi: 10.1519/JSC.0b013e318202e431
Szatrowski, T. P., & Nathan, C. F. (1991). Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Research, 51*(3), 794-798.
Takahashi, M., Miyashita, M., Kawanishi, N., Park, J. H., Hayashida, H., Kim, H. S., . . . Suzuki, K. (2013). Low-volume exercise training attenuates oxidative stress and

neutrophils activation in older adults. *European Journal of Applied Physiology, 113*(5), 1117-1126. doi: 10.1007/s00421-012-2531-5

Tang, R., Chen, H. H., Wang, Y. L., Changchien, C. R., Chen, J. S., Hsu, K. C., . . . Wang, J. Y. (2001). Risk factors for surgical site infection after elective resection of the colon and rectum: a single-center prospective study of 2,809 consecutive patients. *Annals of Surgery, 234*(2), 181-189.

Tang, S., Chen, H., Cheng, Y., Nasir, M. A., Kemper, N., & Bao, E. (2016). The interactive association between heat shock factor 1 and heat shock proteins in primary myocardial cells subjected to heat stress. *International Journal of Molecular Medicine, 37*(1), 56-62. doi: 10.3892/ijmm.2015.2414

Taylor, I., Garcia-Aguilar, J., & Goldberg, S. M. (2002). *Colorectal Cancer* (2nd ed.). Oxford: Health Press Limited.

Taylor, L., Hillman, A. R., Midgley, A. W., Peart, D. J., Chrismas, B., & McNaughton, L. R. (2012). Hypoxia-mediated prior induction of monocyte-expressed HSP72 and HSP32 provides protection to the disturbances to redox balance associated with human sub-maximal aerobic exercise. *Amino Acids, 43*(5), 1933-1944. doi: 10.1007/s00726-012-1265-3

Taylor, L., Midgley, A. W., Chrismas, B., Hilman, A. R., Madden, L. A., Vince, R. V., & McNaughton, L. R. (2011). Daily hypoxia increases basal monocyte HSP72 expression in healthy human subjects. *Amino Acids, 40*(2), 393-401. doi: 10.1007/s00726-010-0644-x

Taylor, L., Midgley, A. W., Chrismas, B., Madden, L. A., Vince, R. V., & McNaughton, L. R. (2010a). Daily quadratic trend in basal monocyte expressed HSP72 in healthy human subjects. *Amino Acids, 38*(5), 1483-1488. doi: 10.1007/s00726-009-0360-6

Taylor, L., Midgley, A. W., Chrismas, B., Madden, L. A., Vince, R. V., & McNaughton, L. R. (2010b). The effect of acute hypoxia on heat shock protein 72 expression and oxidative stress in vivo. *European Journal of Applied Physiology, 109*(5), 849-855. doi: 10.1007/s00421-010-1430-x

Terluin, B., Brouwers, E. P., van Marwijk, H. W., Verhaak, P., & van der Horst, H. E. (2009). Detecting depressive and anxiety disorders in distressed patients in primary care; comparative diagnostic accuracy of the Four-Dimensional Symptom Questionnaire (4DSQ) and the Hospital Anxiety and Depression Scale (HADS). *BMC Family Practice, 10*, 58. doi: 10.1186/1471-2296-10-58

Thomas, S., & Balasubramanian, K. A. (2004). Role of intestine in postsurgical complications: involvement of free radicals. *Free Radical Biology & Medicine, 36*(6), 745-756. doi: 10.1016/j.freeradbiomed.2003.11.027

Thomas, S., Pulimood, A., & Balasubramanian, K. A. (2003). Heat preconditioning prevents oxidative stress-induced damage in the intestine and lung following surgical manipulation. *British Journal of Surgery, 90*(4), 473-481. doi: 10.1002/bjs.4062

Thompson, H. S., Scordilis, S. P., Clarkson, P. M., & Lohrer, W. A. (2001). A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiologica Scandinavica, 171*(2), 187-193. doi: 10.1046/j.1365-201x.2001.00795.x

Thorell, A., Nygren, J., Hirshman, M. F., Hayashi, T., Nair, K. S., Horton, E. S., . . . Ljungqvist, O. (1999a). Surgery-induced insulin resistance in human patients: relation to glucose transport and utilization. *American Journal of Physiology, 276*(4 Pt 1), E754-761.

Thorell, A., Nygren, J., & Ljungqvist, O. (1999b). Insulin resistance: a marker of surgical stress. *Current Opinion in Clinical Nutrition & Metabolic Care , 2*(1), 69-78.

Thornton, M., Joshi, H., Vimalachandran, C., Heath, R., Carter, P., Gur, U., & Rooney, P. (2011). Management and outcome of colorectal anastomotic leaks. *International Journal of Colorectal Disease, 26*(3), 313-320. doi: 10.1007/s00384-010-1094-3

Timmerman, H., de Groot, J. F., Hulzebos, H. J., de Knikker, R., Kerkkamp, H. E., & van Meeteren, N. L. (2011). Feasibility and preliminary effectiveness of preoperative therapeutic exercise in patients with cancer: a pragmatic study. *Physiotheraphy Theory & Practice, 27*(2), 117-124. doi: 10.3109/09593981003761509

Townsend, N., Wickramasinghe, K., Williams, J., Bhatnagar, P., & Rayner, M. (2015). *Physical Activity Statistics 2015*. Retrieved from https://www.bhf.org.uk/publications/statistics/physical-activity-statistics-2015

Townsley, C. A., Selby, R., & Siu, L. L. (2005). Systematic review of barriers to the recruitment of older patients with cancer onto clinical trials. *Journal of Clinical Oncology, 23*(13), 3112-3124. doi: 10.1200/jco.2005.00.141

Troosters, T., Gosselink, R., & Decramer, M. (1999). Six minute walking distance in healthy elderly subjects. *European Respiratory Journal, 14*(2), 270-274.

Tschentscher, M., Eichinger, J., Egger, A., Droese, S., Schonfelder, M., & Niebauer, J. (2016). High-intensity interval training is not superior to other forms of endurance training during cardiac rehabilitation. *European Journal of Preventive Cardiology, 23*(1), 14-20. doi: 10.1177/2047487314560100

Tweedle, E. M., Khattak, I., Ang, C. W., Nedjadi, T., Jenkins, R., Park, B. K., . . . Costello, E. (2010). Low molecular weight heat shock protein HSP27 is a prognostic indicator in rectal cancer but not colon cancer. *Gut, 59*(11), 1501-1510. doi: 10.1136/gut.2009.196626

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry & Cell Biology, 39*(1), 44-84. doi: 10.1016/j.biocel.2006.07.001

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 160*(1), 1-40. doi: 10.1016/j.cbi.2005.12.009

van der Wouden, J. C., Blankenstein, A. H., Huibers, M. J., van der Windt, D. A., Stalman, W. A., & Verhagen, A. P. (2007). Survey among 78 studies showed that Lasagna's law holds in Dutch primary care research. *Journal of Clinical Epidemiology, 60*(8), 819-824. doi: 10.1016/j.jclinepi.2006.11.010

Varadhan, K. K., Neal, K. R., Dejong, C. H., Fearon, K. C., Ljungqvist, O., & Lobo, D. N. (2010). The enhanced recovery after surgery (ERAS) pathway for patients undergoing major elective open colorectal surgery: a meta-analysis of randomized controlled trials. *Clinical Nutrtion , 29*(4), 434-440. doi: 10.1016/j.clnu.2010.01.004

Verhagen, A. P., de Vet, H. C. W., de Bie, R. A., Kessels, A. G. H., Boers, M., Bouter, L. M., & Knipschild, P. G. (1998). The Delphi List: A Criteria List for Quality Assessment of Randomized Clinical Trials for Conducting Systematic Reviews Developed by Delphi Consensus. *Journal of Clinical Epidemiology, 51*(12), 1235-1241. doi: 10.1016/S0895-4356(98)00131-0

Vince, R. V., Oliver, K., Midgley, A. W., McNaughton, L. R., & Madden, L. A. (2010). In vitro heat shock of human monocytes results in a proportional increase of inducible Hsp70 expression according to the basal content. *Amino Acids, 38*(5), 1423-1428. doi: 10.1007/s00726-009-0354-4

Voss, M. R., Stallone, J. N., Li, M., Cornelussen, R. N., Knuefermann, P., & Knowlton, A. A. (2003). Gender differences in the expression of heat shock proteins: the effect of estrogen. *American Journal of Physiology: Heart & Circulatory Physiology, 285*(2), H687-692. doi: 10.1152/ajpheart.01000.2002

Wada, I., Otaka, M., Jin, M., Odashima, M., Komatsu, K., Konishi, N., . . . Watanabe, S. (2006). Expression of HSP72 in the gastric mucosa is regulated by gastric acid in rats-correlation of HSP72 expression with mucosal protection. *Biochemical & Biophysical Research Communications, 349*(2), 611-618. doi: 10.1016/j.bbrc.2006.08.088

Wall, C. R., DeHaven, M. J., & Oeffinger, K. C. (2002). Survey methodology for the uninitiated. *Journal of Family Practice, 51*(6), 573.

Walter, C. J., Collin, J., Dumville, J. C., Drew, P. J., & Monson, J. R. (2009). Enhanced recovery in colorectal resections: a systematic review and meta-analysis. *Colorectal Disease, 11*(4), 344-353. doi: 10.1111/j.1463-1318.2009.01789.x

Wang, A. W., Gilbey, H. J., & Ackland, T. R. (2002). Perioperative exercise programs improve early return of ambulatory function after total hip arthroplasty: a randomized, controlled trial. *American Journal of Physical Medicine & Rehabilitation, 81*(11), 801-806. doi: 10.1097/01.PHM.0000030727.23011.9C

Wang, A. Y., Sea, M. M., Ho, Z. S., Lui, S. F., Li, P. K., & Woo, J. (2005). Evaluation of handgrip strength as a nutritional marker and prognostic indicator in peritoneal dialysis patients. *American Journal of Clinical Nutrition, 81*(1), 79-86.

Wang, F., Zhang, P., Shi, C., Yang, Y., & Qin, H. (2012). Immunohistochemical detection of HSP27 and hnRNP K as prognostic and predictive biomarkers for colorectal cancer. *Medical Oncology, 29*(3), 1780-1788. doi: 10.1007/s12032-011-0037-3

Wang, X., Wang, W., Li, L., Perry, G., Lee, H. G., & Zhu, X. (2014). Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochimica et Biophysica Acta, 1842*(8), 1240-1247. doi: 10.1016/j.bbadis.2013.10.015

Wang, X. P., Qiu, F. R., Liu, G. Z., & Chen, R. F. (2005). Correlation between clinicopathology and expression of heat shock protein 70 and glucose-regulated protein 94 in human colonic adenocarcinoma. *World Journal of Gastroenterology, 11*(7), 1056-1059.

West, M. A., Loughney, L., Barben, C. P., Sripadam, R., Kemp, G. J., Grocott, M. P., & Jack, S. (2014a). The effects of neoadjuvant chemoradiotherapy on physical fitness and morbidity in rectal cancer surgery patients. *European Journal of Surgical Oncology*. doi: 10.1016/j.ejso.2014.03.021

West, M. A., Loughney, L., Lythgoe, D., Barben, C. P., Sripadam, R., Kemp, G. J., . . . Jack, S. (2015). Effect of prehabilitation on objectively measured physical fitness after neoadjuvant treatment in preoperative rectal cancer patients: a blinded interventional pilot study. *British Journal of Anaesthesia, 114*(2), 244-251. doi: 10.1093/bja/aeu318

West, M. A., Lythgoe, D., Barben, C. P., Noble, L., Kemp, G. J., Jack, S., & Grocott, M. P. (2014b). Cardiopulmonary exercise variables are associated with postoperative morbidity after major colonic surgery: a prospective blinded observational study. *British Journal of Anaesthesia, 112*(4), 665-671. doi: 10.1093/bja/aet408

West, M. A., Parry, M. G., Lythgoe, D., Barben, C. P., Kemp, G. J., Grocott, M. P., & Jack, S. (2014c). Cardiopulmonary exercise testing for the prediction of morbidity risk after rectal cancer surgery. *British Journal of Surgery, 101*(9), 1166-1172. doi: 10.1002/bjs.9551

Westin, L., Carlsson, R., Israelsson, B., Willenheimer, R., Cline, C., & McNeil, T. F. (1997). Quality of life in patients with ischaemic heart disease: a prospective controlled study. *Journal of Internal Medicine, 242*(3), 239-247.

Weston, M., Weston, K. L., Prentis, J. M., & Snowden, C. P. (2016). High-intensity interval training (HIT) for effective and time-efficient pre-surgical exercise interventions. *Perioperative Medicine, 5*, (2). doi: 10.1186/s13741-015-0026-8

Whitham, M., Walker, G. J., & Bishop, N. C. (2006). Effect of caffeine supplementation on the extracellular heat shock protein 72 response to exercise. *Journal of Applied Physiology, 101*(4), 1222-1227. doi: 10.1152/japplphysiol.00409.2006

Wientjes, F. B., & Segal, A. W. (1995). NADPH oxidase and the respiratory burst. *Seminars in cell biology, 6* (6), 257-265.

Wieske, M., Benndorf, R., Behlke, J., Dolling, R., Grelle, G., Bielka, H., & Lutsch, G. (2001). Defined sequence segments of the small heat shock proteins HSP25 and alphaB-crystallin inhibit actin polymerization. *European Journal of Biochemistry, 268*(7), 2083-2090.

Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wust, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology, 32*(4), 358-366. doi: 10.1016/j.psyneuen.2007.01.008

Wille-Jorgensen, P., Guenaga, K. F., Castro, A. A., & Matos, D. (2003). Clinical value of preoperative mechanical bowel cleansing in elective colorectal surgery: a systematic review. *Diseases of the Colon & Rectum, 46*(8), 1013-1020. doi: 10.1097/01.dcr.0000080151.35300.20

Willoughby, D. S., Priest, J. W., & Nelson, M. (2002). Expression of the stress proteins, ubiquitin, heat shock protein 72, and myofibrillar protein content after 12 weeks of leg cycling in persons with spinal cord injury. *Archives of Physical Medicine & Rehabilitation, 83*(5), 649-654.

Wilmore, D. W., & Kehlet, H. (2001). Management of patients in fast track surgery. *BMJ, 322*(7284), 473-476.

Wilson, R. J., Davies, S., Yates, D., Redman, J., & Stone, M. (2010). Impaired functional capacity is associated with all-cause mortality after major elective intra-abdominal surgery. *British Journal of Anaesthesia, 105*(3), 297-303. doi: 10.1093/bja/aeq128

Wischmeyer, P. E., Kahana, M., Wolfson, R., Ren, H., Musch, M. M., & Chang, E. B. (2001). Glutamine induces heat shock protein and protects against endotoxin shock in the rat. *Journal of Applied Physiology (1985), 90*(6), 2403-2410.

Wisloff, U., Stoylen, A., Loennechen, J. P., Bruvold, M., Rognmo, O., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094. doi: 10.1161/circulationaha.106.675041

Wolin, K. Y., Yan, Y., Colditz, G. A., & Lee, I. M. (2009). Physical activity and colon cancer prevention: a meta-analysis. *British Journal of Cancer, 100*(4), 611-616. doi: 10.1038/sj.bjc.6604917

Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R., & Turner, N. D. (2004). Glutathione metabolism and its implications for health. *Journal of Nutrition, 134*(3), 489-492.

Wust, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response - normal values and confounds. *Noise & Health, 2*(7), 79-88.

Yaglom, J. A., Gabai, V. L., & Sherman, M. Y. (2007). High levels of heat shock protein Hsp72 in cancer cells suppress default senescence pathways. *Cancer Research, 67*(5), 2373-2381. doi: 10.1158/0008-5472.can-06-3796

Yamada, P. M., Amorim, F. T., Moseley, P., Robergs, R., & Schneider, S. M. (2007). Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. *Journal of Applied Physiology, 103*(4), 1196-1204. doi: 10.1152/japplphysiol.00242.2007

Yanbaeva, D. G., Dentener, M. A., Creutzberg, E. C., Wesseling, G., & Wouters, E. F. (2007). Systemic effects of smoking. *Chest, 131*(5), 1557-1566. doi: 10.1378/chest.06-2179

Yang, C. S., Chou, S. T., Liu, L., Tsai, P. J., & Kuo, J. S. (1995). Effect of ageing on human plasma glutathione concentrations as determined by high-performance liquid chromatography with fluorimetric detection. *Journal of Chromatography B: Biomedical Sciences & Applications, 674*(1), 23-30.

Yu, C. M., Li, L. S., Ho, H. H., & Lau, C. P. (2003). Long-term changes in exercise capacity, quality of life, body anthropometry, and lipid profiles after a cardiac rehabilitation program in obese patients with coronary heart disease. *American Journal of Cardiology, 91*(3), 321-325.

Yu, Z., Zhi, J., Peng, X., Zhong, X., & Xu, A. (2010). Clinical significance of HSP27 expression in colorectal cancer. *Molecular Medicine Reports, 3*(6), 953-958. doi: 10.3892/mmr.2010.372

Yümin, E. T., Şimşek, T. T., Sertel, M., Öztürk, A., & Yümin, M. (2011). The effect of functional mobility and balance on health-related quality of life (HRQoL) among elderly people living at home and those living in nursing home. *Archives of Gerontology & Geriatrics, 52*(3), e180-e184. doi: 10.1016/j.archger.2010.10.027

Zeni, J. A., Jr., & Snyder-Mackler, L. (2010). Early postoperative measures predict 1- and 2-year outcomes after unilateral total knee arthroplasty: importance of contralateral limb strength. *Physical Therapy, 90*(1), 43-54. doi: 10.2522/ptj.20090089

Zhang, B., Rong, R., Li, H., Peng, X., Xiong, L., Wang, Y., Yu, X & Mao, H. (2015). Heat shock protein 72 suppresses apoptosis by increasing the stability of X-linked inhibitor of apoptosis protein in renal ischemia/reperfusion injury. *Molecular Medicine Reports, 11*(3), 1793-1799. doi: 10.3892/mmr.2014.2939

Zhao, M., Ding, J. X., Zeng, K., Zhao, J., Shen, F., Yin, Y. X., & Chen, Q. (2014). Heat shock protein 27: a potential biomarker of peritoneal metastasis in epithelial ovarian cancer? *Tumor Biology, 35*(2), 1051-1056. doi: 10.1007/s13277-013-1139-7

Zhao, M., Shen, F., Yin, Y. X., Yang, Y. Y., Xiang, D. J., & Chen, Q. (2012). Increased expression of heat shock protein 27 correlates with peritoneal metastasis in epithelial ovarian cancer. *Reproductive Sciences, 19*(7), 748-753. doi: 10.1177/1933719111432875

Zhuang, C. L., Ye, X. Z., Zhang, X. D., Chen, B. C., & Yu, Z. (2013). Enhanced recovery after surgery programs versus traditional care for colorectal surgery: a meta-analysis of randomized controlled trials. *Diseases of the Colon & Rectum, 56*(5), 667-678. doi: 10.1097/DCR.0b013e3182812842

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica*, *67*(6), 361-370.

Appendix A: Attitudes to Exercise in the Preparation for Surgery Questionnaire (General public version)

What is the purpose of the study?

Undergoing any major surgical procedure such as a hip or knee replacement or resection surgery to remove a tumour can place considerable physical and psychological stress on the individual. Traditionally as part of the recovery process, patients may undertake a period of rehabilitation following surgery with the intention of aiding their return to their pre-surgery physical state. The question is: If performing specific exercises following surgery can aid recovery, are there any benefits to patients undertaking a short period of exercise training prior to surgery?

The intention of this questionnaire therefore is to assess the opinion of the general public from the Hull and East Yorkshire region on the potential use of exercise training as a means of preparing patients for major surgery. This will hopefully inform clinical research in the future.

A. Personal Background

1. Are you male or female?
2. What is your current age?
3. What is your profession
4. Are you based in… **Hull** **East riding**
5. How would you describe your present level of **lifestyle** activity? (Lifestyle activity refers to everyday activities which require an element of physical exertion on you. This may include activities such as walking to the shops, gardening, cleaning the house or physical demands of your job). **Sedentary / Moderately Active / Active / Highly Active**
6. How would you describe your present level of **exercise** activity? (Exercise activity refers to any activity requiring physical effort that is carried out with the intention of sustaining or improving health and fitness. This may include: going to the gym, playing sports such as football and tennis, jogging)

   Sedentary / Moderately Active / Active / Highly Active
7. How many hours of exercise activity do you participate in on average each week?

   None **1-2 hours** **2-3** **3-4** **4+**
8. Please provide details of your typical weekly exercise/lifestyle activity?

   ……

B. Attitude to exercise prehabilitation

The term EXERCISE PREHABILITATION has been given to refer to a period of exercise training that is performed prior to major surgery with the intention of preparing the individual for the stresses surgical treatment brings.

In the following section we would like to know your opinion to the following statements (please tick the answer which applies most to you):

9. I believe the concept of exercise prehabilitation prior to surgery is a good idea in principle

   Strongly disagree / Disagree / Unsure / Agree / Strongly Agree
10. I believe that participating in exercise in the weeks before surgery would help prepare the individual for surgery?

    Strongly disagree / Disagree / Unsure / Agree / Strongly Agree
11. If my doctor recommended exercise prehabilitation I would be more likely to participate?

    Strongly disagree / Disagree / Unsure / Agree / Strongly Agree
12. I would be more likely to participate if the potential benefits were clearly explained?

    Strongly disagree / Disagree / Unsure / Agree / Strongly Agree
13. I would be more likely to participate in exercise prehabilitation if it was…

    Home-based **Fitness facility based (E.g. leisure centre)**

14. I would be more likely to participate in exercise prehabilitation if the activity was…

A. Group based classes (E.g. exercise class style)	**B.** One to one with an instructor	**C.** Performed on their own in a fitness facility but with guidance available if required	**D.** Detailed on a leaflet and/or DVD so it could be performed at home in patients own time

15. I believe the most appropriate number of prehabilitation sessions per week would be…

1-2 **3-4** **5-6** **7+**

16. Which of the following would represent an obstacle to your participation in an exercise prehabilitation programme? (Please tick all that apply)

Lack of time	Lack of interest	Do not feel it would be beneficial
Cost	Travel	Family commitments
Not knowing what it involves	Self confidence	Not feeling physically capable of participating
Already physically active	Find exercise tiring or painful	Work responsibilities
None of the above	Other (Please detail):	

17. What do you think the potential benefits of participating in an exercise prehabilitation programme would be? (Please tick all that apply)

Improved mental well-being	Improved physical fitness	Faster recovery time from surgery
Reduced anxiety	Improved functional mobility	Improved social support
Reduced depression	Reduced fatigue	None of the above
Other: (please detail)		

18. If I was awaiting major surgery I would be interested in participating in an exercise prehabilitation programme

Strongly disagree / Disagree / Unsure / Agree / Strongly Agree

19. Which of the following options would you be most likely to participate in…

Prehabilitation (Pre-surgery)	Rehabiliatation (Post-surgery)	Both Prehabilitation and Rehabilitation	Neither

20. Please share any other thoughts, comments, concerns or questions you may have regarding exercise prehabilitation in the space provided below.

Appendix B: Attitudes to Exercise in the Preparation for Surgery Questionnaire (GP version)

What is the purpose of the study?

Undergoing any major surgical procedure such as a hip or knee replacement or resection surgery to remove a tumour can place considerable physical and psychological stress on the individual. Traditionally as part of the recovery process, patients may undertake a period of rehabilitation following surgery with the intention of aiding their return to their pre-surgery physical state. The question is: If performing specific exercises following surgery can aid recovery, are there any benefits to patients undertaking a short period of exercise training prior to surgery?

The intension of this questionnaire therefore is to assess the opinion of general practitioners from the Hull and East Yorkshire region on the potential use of exercise training as a means of preparing patients for major surgery. This will hopefully inform clinical research in the future.

A. Personal Background

1. Are you male or female?
2. What is your current age?
3. Years as a GP?
4. Are you based in… **Hull** **East riding**
5. How would you describe your present level of **lifestyle** activity? (Lifestyle activity refers to everyday activities which require an element of physical exertion on you. This may include activities such as walking to the shops, gardening, cleaning the house or physical demands of your job). **Sedentary / Moderately Active / Active / Highly Active**
6. How would you describe your present level of **exercise** activity? (Exercise activity refers to any activity requiring physical effort that is carried out with the intention of sustaining or improving health and fitness. This may include: going to the gym, playing sports such as football and tennis, jogging)

   Sedentary / Moderately Active / Active / Highly Active
7. How many hours of exercise activity do you participate in on average each week?

   None **1-2 hours** **2-3** **3-4** **4+**
8. Please provide details of your typical weekly exercise/lifestyle activity?

   ……

B. Attitudes and Perspectives on Exercise Prehabilitation

The term EXERCISE PREHABILITATION has been given to refer to a period of exercise training that is performed prior to major surgery with the intention of preparing the individual for the stresses surgical treatment brings.

In the following section we would like to know your opinion to the following statements (please tick the answer which applies most to you):

9. I believe the concept of exercise prehabilitation prior to surgery is a good idea in principle

   Strongly disagree / Disagree / Unsure / Agree / Strongly Agree
10. If a patient asked my opinion about participating in an exercise prehabilitation clinical trial, I would recommend they participate…

    Strongly disagree / Disagree / Unsure / Agree / Strongly Agree
11. I would be unlikely or reluctant recommended exercise prehabilitation to my patients because…

There is currently a lack of scientific evidence to support its use	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Patients would be unlikely to be interested	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
It would cost too much money to the patient	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
It would be a waste of time	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
It would be too risky in certain clinical populations	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**

12. I believe the concept of exercise prehabilitation would be useful/suitable for…

Male patients	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Female patients	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Young patients	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Elderly patients	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Overweight or obese populations	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Sedentary populations	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Physically unfit patients	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Patients who are interested	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Patients who are anxious/fearful about surgery	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Cancer patients receiving chemoradiotherapy prior to surgery	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Cancer patients NOT receiving chemoradiotherapy prior to surgery	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Orthopedic surgery patients (e.g. Hip replacements)	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Vascular surgery patients (Abdominal aortic aneurysm)	**Strongly Disagree**	**Disagree**	**Unsure**	**Agree**	**Strongly Agree**
Other (Please state): Improved functional mobility/strength					

13. I believe the best format for exercise prehabilitation would be…

A. Group based classes (E.g. exercise class style)

B. One to one with an instructor

C. Performed on their own in a fitness facility but with guidance available if required

D. Detailed on a leaflet and/or DVD so it could be performed at home in patients own time

14. I believe the most appropriate number of prehabilitation sessions per week would be…

1-2 **3-4** **5-6** **7+**

15. What do you feel would be the biggest obstacles to patient participation? (Please tick all that are applicable)

Lack of time (Patients time)	Lack of interest	Self confidence
Cost	Travel	Family commitments
Not knowing what it involves	Not feeling physically capable of participating	Do not feel it would be beneficial
Already physically active	Find exercise tiring or painful	Work responsibilities
Insufficient time before surgery	Other (Please detail)	

16. What do you feel could be the potential benefits of exercise prehabilitation for the patients awaiting surgery

Improved mental well-being	Improved physical fitness	Faster recovery time from surgery
Reduced anxiety	Improved social support	Improved functional mobility/strength
Reduced depression	Reduced fatigue	None of the above
Other: (please detail)		

17. If **<u>you</u>** were awaiting major surgery, **<u>you</u>** would be interested in participating in an exercise prehabilitation programme

Strongly disagree / Disagree / Unsure / Agree / Strongly Agree

18. Please share any other thoughts, comments, concerns or questions regarding exercise prehabilitation in the space provided below.

Appendix C: Hospital Anxiety and Depression Scale

Hospital Anxiety and Depression Score (HADS)

This questionnaire helps your physician to know how you are feeling. Read every sentence. Place an "X" on the answer that best describes how you have been feeling during the LAST WEEK. You do not have to think too much to answer. In this questionnaire, spontaneous answers are more important

A	**I feel tense or 'wound up':**	
	Most of the time	3
	A lot of the time	2
	From time to time (occ.)	1
	Not at all	0
D	**I still enjoy the things I used to enjoy:**	
	Definitely as much	0
	Not quite as much	1
	Only a little	2
	Hardly at all	3
A	**I get a sort of frightened feeling as if something awful is about to happen:**	
	Very definitely and quite badly	3
	Yes, but not too badly	2
	A little, but it doesn't worry me	1
	Not at all	0
D	**I can laugh and see the funny side of things:**	
	As much as I always could	0
	Not quite so much now	1
	Definitely not so much now	2
	Not at all	3
A	**Worrying thoughts go through my mind:**	
	A great deal of the time	3
	A lot of the time	2
	From time to time, but not often	1
	Only occasionally	0
D	**I feel cheerful:**	
	Not at all	3
	Not often	2
	Sometimes	1
	Most of the time	0
A	**I can sit at ease and feel relaxed:**	
	Definitely	0
	Usually	1
	Not often	2
	Not at all	3

D	**I feel as if I am slowed down:**	
	Nearly all the time	3
	Very often	2
	Sometimes	1
	Not at all	0
A	**I get a sort of frightened feeling like "butterflies" in the stomach:**	
	Not at all	0
	Occasionally	1
	Quite often	2
	Very often	3
D	**I have lost interest in my appearance:**	
	Definitely	3
	I don't take as much care as I should	2
	I may not take quite as much care	1
	I take just as much care	0
A	**I feel restless as I have to be on the move:**	
	Very much indeed	3
	Quite a lot	2
	Not very much	1
	Not at all	0
D	**I look forward with enjoyment to things:**	
	As much as I ever did	0
	Rather less than I used to	1
	Definitely less than I used to	2
	Hardly at all	3
A	**I get sudden feelings of panic:**	
	Very often indeed	3
	Quite often	2
	Not very often	1
	Not at all	0
D	**I can enjoy a good book or radio/TV program:**	
	Often	0
	Sometimes	1
	Not often	2
	Very seldom	3

Appendix D: EORTC QLQ-C30

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ⌊_⌊_⌊_⌊_⌋

Your birthdate (Day, Month, Year): ⌊_⌊_⌊_⌊_⌊_⌊_⌊_⌊_⌋

Today's date (Day, Month, Year): 31 ⌊_⌊_⌊_⌊_⌊_⌊_⌊_⌊_⌋

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent